Suppression of Growth Hormone and Insulin-Like Growth Factor 1 in Rats After Oral Administration of CRN00808, a Small Molecule, sst2 Selective Somatostatin Biased Agonist

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Somatostatin analogs (SSAs) are routinely used to treat growth hormone (GH) secreting adenomas that cause acromegaly and pancreatic neuroendocrine tumors (NETs). The most widely used analog, octreotide, primarily targets the sst2 somatostatin receptor subtype to activate G_i signaling that inhibits cAMP production and subsequent hormone secretion. However, octreotide also potently induces receptor phosphorylation, internalization, and desensitization responses that are thought to limit its therapeutic benefits, and many acromegaly patients fail to achieve normalization of insulin-like growth factor 1 (IGF-1) levels and/or experience a return of symptoms near the end of their monthly injection cycle.

We hypothesized that small molecule sst2 selective agonists could be optimized to reduce counter-regulatory activities such as internalization and desensitization to improve efficacy while at the same time introducing drug-like characteristics that enable more convenient once-daily oral delivery. Using an iterative medicinal chemistry-based approach, many highly potent sst2 agonists were obtained with varying degrees of bias for G_i signaling over internalization. In some cases, G_i signaling bias was >300-fold. Surprisingly, agonists biased for internalization over G_i signaling were also obtained. Simultaneous optimization for drug-like characteristics yielded CRN00808. In vitro, CRN00808 is a potent and selective sst2 agonist (hsst2 EC_{50}= 0.25 nM; rsst2 EC_{50}= 1.2 nM) and possesses good oral bioavailability in rats (F= 9-25%) and dogs (F= 48%). In vivo, single oral administration of CRN00808 dose-dependently inhibited GHRH-stimulated GH secretion in rats with maximum suppression equivalent to octreotide and an EC_{50}= 11 ng/mL. In repeat dosing rat studies, continuous octreotide infusion showed suppression of body weight gain and transient suppression of circulating IGF-1 levels indicating potential tachyphylaxis. In contrast, daily oral administration of CRN00808 suppressed growth and maintained suppression of IGF-1 levels suggesting that the tachyphylaxis observed with octreotide was largely avoided. CRN00808 was evaluated for nonclinical safety in rats and dogs, and entered First-In-Human Phase 1 clinical trials in late 2017.

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CRN00808 Is a Potent, Selective, Biased sst2 Agonist In Vitro

Compound	Human sst EC_{50} (nM)					Rat sst EC_{50} (nM)
	sst1	sst2	sst3	sst4	sst5	sst2
CRN00808	>10000	0.25	3300	1100	>10000	1.2
octreotide	>10000	0.058	7.9	470	2.5	0.091
ss14	0.83	0.14	0.16	0.20	0.065	0.18

Table I. Potency and selectivity of CRN00808 compared to octreotide and somatostatin (ss14). All data are from >3 determinations, with the exception of CRN00808 at sst1 and sst5, which were n = 2. SEM values for pEC_{50} determinations are < 0.15 for all data.

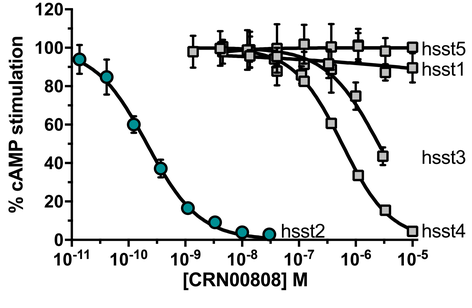

Figure 1. Activity of CRN00808 at human somatostatin receptor subtypes. *Inhibition of stimulated cAMP production by sst receptor activation:* Four days prior to the assay, CHO-K1 cells stably expressing the desired receptor subtype were plated with appropriate media and antibiotic selection. On the day of the assay, media was discarded and cells treated with NKH477 plus a dose-response of compound in assay buffer. Cells were incubated for 20 minutes at 37 ˚C. Cells were lysed and cAMP quantified.

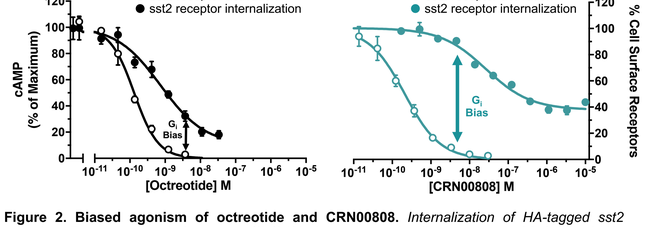

Figure 2. Biased agonism of octreotide and CRN00808. *Internalization of HA-tagged sst2 receptors:* Two days prior to the assay, CHO-K1 stably expressing the human or rat N-terminal-HA-tagged sst2 receptor were plated with appropriate media and cultured at 37 ˚C, 5% CO_2 and 95% humidity. On the day of the assay, dose responses of compounds were incubated for 30 minutes at 37 ˚C, 5% CO_2 and 95% humidity. Cells were fixed and cell surface receptors quantified via ELISA and compared to that of untreated cells.

CRN00808 is Orally Bioavailable and Suppresses GH and IGF-1 in Rats

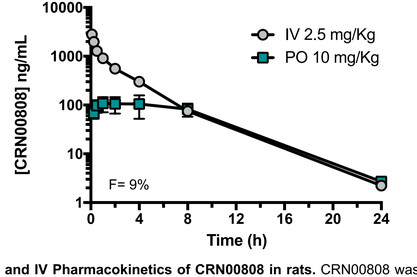

Figure 3. Oral and IV Pharmacokinetics of CRN00808 in rats. CRN00808 was administered to male Sprague Dawley rats by IV bolus in 10% EtOH or by oral gavage in 50% Kolliphor, 25% EtOH and 25% propylene glycol. CRN00808 is rapidly absorbed and exhibits oral bioavailability of 9%. Other formulations improved bioavailability up to 25%. Bioavailability in the dog was 48%.

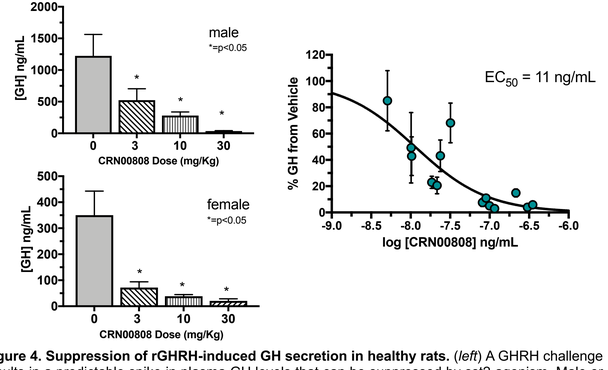

Figure 4. Suppression of rGHRH-induced GH secretion in healthy rats. (*left*) A GHRH challenge results in a predictable spike in plasma GH levels that can be suppressed by sst2 agonism. Male or female Sprague Dawley rats were administered CRN00808 (or vehicle) via oral gavage or sc-injection. 3 µg rGHRH was administered at 1h and/or 3h post-dose. GH levels were quantified via ELISA. Data are presented as mean values ± SEM of 6-8 rats per group. (*right*) Multiple studies in male rats provided a range of exposures and a PK-PD correlation was determined.

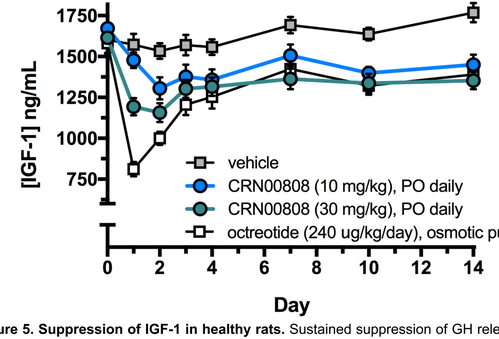

Figure 5. Suppression of IGF-1 in healthy rats. Sustained suppression of GH release results in lowering of plasma IGF-1 levels. However, in contrast to rapid effects on GH response, IGF-1 levels drop gradually and require extended exposure to somatostatin analogs to observe an effect. Male Sprague Dawley rats were administered CRN00808 (or vehicle) via oral gavage daily for 14 days. Octreotide was delivered via osmotic pump. Data are presented as mean values ± SEM of 7-8 rats per group.

Conclusions

CRN00808 is a novel sst2-selective biased agonist currently in clinical development for the treatment of acromegaly:

- **A potent sst2 agonist and selective over other sst receptor subtypes**
- **Biased for cAMP suppression over receptor internalization compared to octreotide**
- **Exhibits good exposure following oral administration to rats**
- **Dose dependently suppresses rGHRH-stimulated GH secretion in rats and dose dependently suppresses IGF-1 in rats with multi-day dosing**

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