

Development of Xen2174 - a novel γ -conopeptide inhibitor of the norepinephrine transporter with analgesic potential

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Introduction

There is a large clinical unmet need in the pain market where opioids remain the gold standard, despite the many unwanted side effects. Even so, the number of novel mechanisms and new chemical entities (NCEs) in development are limited. Xen2174, is a NCE with a novel mechanism of action on a validated pain target; this being the first allosteric (non-saturable) inhibitor of the norepinephrine transporter (NET). NET belongs to a family of monoamine transporters that mediate the reuptake of catecholamines (NE, serotonin & dopamine) into presynaptic terminals. In the spinal cord, NE is one of the major mechanisms controlling pain transmission to the brain. Compounds that potentiate the release of NE (such as morphine) or prevent the removal of NE (such as Xen2174) at the synapse promote antinociception. The pathway to the selection of Xen2174 is described, along with selected pre-clinical and clinical data supporting continued clinical development.



Figure 1. Shell from *Conus marmoreus*: sourced from the Great Barrier Reef

Materials and methods

Peptides were assembled by solid phase peptide synthesis. Potency to inhibit [³H]-NE uptake through human NET was assessed directly at the transporter expressed in Cos-7 cells [2]. Analogues of interest were characterised for stability, target specificity and ability to reverse allodynia in animal models of neuropathic pain. Xen2174 was selected as the preferred peptide candidate to enter pre-clinical development. Pharmacology, pre-clinical and toxicology studies of Xen2174 led to an IND approval for clinical development.

Results

Discovery

A ch γ -conotoxin was originally discovered from the venom of *Conus marmoreus* (Figure 1) and identified as a norepinephrine (NE) uptake inhibitor from effects seen on the biphasic contractile response of the electrically stimulated rat prostatic smooth muscle [1]. This peptide was defined as γ -MriA (NGVCCGYKLCCHOC) which proved to be chemically unstable due to the asparagine (N) at the N-terminus of the sequence which degrades to aspartimide. An analogue program was initiated to find a more stable clinical candidate.

Key outcomes from the analogue program to improve MriA were:

- Over 300 γ -conotoxin analogues were synthesized
- γ -conotoxins act competitively with [³H]-norepinephrine displacement but are non-competitive with [³H]-NE uptake
- Amino acids critical to activity were identified in loop 2 (GYKL) [2]
- Analogue potency on NET ranged from 80-fold increased to 1,000-fold reduced
- Xen2174 (UGVCCGYKLCCHOC) displayed adequate stability for further development
- Selected peptides were tested in CCI model for spinal level pain relief
- Several analogues had reduced efficacy or caused side effects
- Xen2174 was selected as lead candidate to enter pre-clinical development

Pre-clinical

Xen2174 was tested in animal models of neuropathic pain - namely the chronic constriction injury (CCI) rat model and the L5/L6 spinal nerve injured rat model.

Figures 2 and 3 show selected data from the rat CCI model. Key findings:

- Pronounced ceiling effect for morphine but not Xen2174
- Xen2174 displayed extended duration of action as compared with morphine
- Xen2174 produced dose-dependent relief of mechanical allodynia in CCI model and the spinal nerve ligated rats (data not shown)

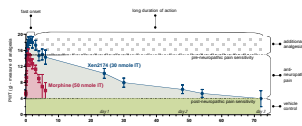


Figure 2. Intrathecal (IT) Xen2174 produces long-lasting relief from signs of allodynia in a CCI model of neuropathic pain. IT morphine shown for comparison [3].

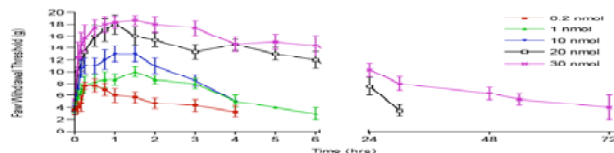


Figure 3. IT Xen2174 (ipsilateral paw) produces dose-dependent relief of mechanical allodynia in CCI rats.

Figure 4 shows the results of Xen2174 administered both post- (4a) and pre- (4b) surgery in an incisional pain model [4]. Key findings from this model were:

- Xen2174 produced dose-dependent relief of mechanical allodynia
- Xen2174 reduced hypersensitivity when administered post-operatively or pre-operatively

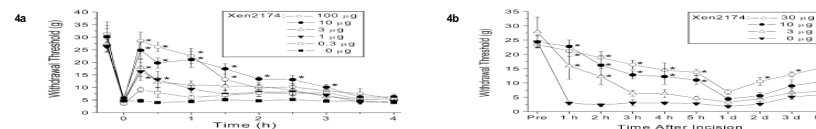


Figure 4. IT Xen2174 produces dose-dependent relief of hypersensitivity in an incisional pain model when administered 1 day post surgery (a) or 15 min before surgery (b).

Pre-clinical toxicology

A series of single and repeat bolus pre-clinical toxicology studies performed in rats and dogs, together with genotoxicity, hemolysis and flocculation studies were undertaken to assess the safety profile of Xen2174. Key findings:

- Xen2174 was well tolerated in rats and dogs, with no observable adverse effects levels (NOAELs) in repeat bolus studies in rats (15 mg/kg/day IV; 5 µg/day IT) and dogs (1 mg/kg/day IV; 0.5 mg/day IT)
- Xen2174 produced dose-dependent relief of mechanical allodynia
- Xen2174 reduced hypersensitivity when administered post-operatively or pre-operatively

Clinical

Phase I (XC0104)

Study title: A Phase I, Double blind, Randomised, Dose Escalation Study to Assess the Safety, Tolerability, Anti-nociceptive Effects and Pharmacokinetics of a Single Intravenous Dose of Xen2174. Key findings were:

- Xen2174 was safe and well tolerated in single bolus doses ranging from 10 µg/kg - 200 µg/kg, with no SAEs or patient withdrawals during the conduct of the study
- Linear PK - dose-proportional increases of C_{max} with dose, with a half-life ranging from 2hrs 58mins to 4hrs 54mins.

Phase II (XC0205)

Study title: A Phase II Open Label, Single Blind, Multicenter, Single-Dose, Dose-Escalating Safety and Tolerability Study of Intrathecal Xen2174 in Oncology Patients. Key findings:

- A single bolus IT injection of Xen2174 in a mixed oncology patient population was both safe and tolerated in a dose range from 0.025 mg to 30.0 mg
- Adverse event analysis identified no consistent trends in side effects
- 78% of all drug-related, treatment-emergent adverse events were considered either mild or moderate in severity
- Of the 12 SAEs experienced, 7(6%) SAEs from 3(8%) of patients were considered related to the study drug
- PK analysis revealed that both C_{max} and AUC₀₋₂₄ values increased in a dose-proportional manner
- Efficacy results were indicative of both early onset and extended duration of action.

Figure 5 shows a self-assessment of pain by two patients after a single treatment with Xen2174.

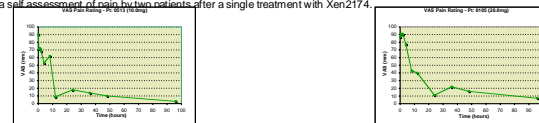


Figure 5. Two patient visual analog scale (VAS) assessment results (100 point scale), when asked the question: How would you assess your current level of cancer pain?

Conclusion

Based on preclinical and early clinical results, Xen2174 is an excellent candidate for continued clinical development.

A Phase II post-surgical (bunionectomy) pain study has been initiated. Study design: placebo-controlled, single-blinded, single IT bolus administration of Xen2174. The primary study objective for this trial is to evaluate the effect of Xen2174 on rescue analgesia consumption. Secondary objectives include: to assess safety and tolerability in an acute pain setting, explore dose-effect relationship, evaluate single-dose pharmacokinetics profiles and estimate duration of efficacy.

Literature cited

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For further information

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