Administration of TTX in smaller, more frequent doses may achieve more stable therapeutic effect than a single larger dose. Only those rats with significant mechanical allodynia (Paw Withdrawal Threshold ≤ 4.0 g) were selected for further drug testing.

**Methods & Materials**

*Oxaliplatin-induced neuropathy*

- Oxaliplatin was administered intravenously through the tail vein at 3 mg/kg, twice a week for up to 4 weeks.
- The development of neuropathic pain, characterised by significant mechanical allodynia, was monitored using a series of graduated von Frey hairs applied to the hind-paw to trigger a withdrawal response (Paw Withdrawal Threshold, PWT).
- Only those rats with significant mechanical allodynia (Paw Withdrawal Threshold ≤ 4.0 g) were selected for further drug testing.

*Vincristine-induced neuropathy*

- Vincristine was administered intravenously through the tail vein at 0.1 mg/kg 5 times a week for 2 to 3 weeks.
- The development of neuropathic pain was monitored using a series of graduated von Frey hairs applied to the hind-paw to trigger a PWT.

Treatment with TTX, Duloxetine or placebo

**Oxaliplatin-induced neuropathy**

- TTX 4 µg/kg, BID group; 3 pm and 10 pm for TTX 2.6 µg/kg, TID group. P < 0.05 for D1 1h, D2-0, D3-0, D4-0, D5-0, D7, D10, D14: baseline value for the 2nd, 3rd, 4th, 5th, 7th, 10th, and 14th days after the start of dosing course. *: First dosing on the day (8 am for each group); second/third dosing on the day where applicable (5 pm for TTX 4 µg/kg, BID group; 3 pm and 10 pm for TTX 2.6 µg/kg, TID group). P < 0.05 for D1 1h, D2-0, D3-0, D4-0, D5-0, D7, D10, D14: baseline value for the 2nd, 3rd, 4th, 5th, 7th, 10th, and 14th days after the start of dosing course: 1 h; 2 h; post-dosing value for 1 hour and 2 hours after each dosing. *, **, ***: P < 0.05, 0.01 and 0.001, respectively, compared to the same time points for placebo group, one-way ANOVA.

**Vincristine-induced neuropathy**

- TTX administered SC significantly increased PWT from day 1 to day 7. Greater von Frey filament size (force) was required to elicit paw withdrawal. Peak effect was observed 1h post-dose. For the day before first oxaliplatin injection: W1, W2: 1st and 2nd week after first oxaliplatin injection, respectively; D1, D2, D3, D4, D5, D7, D10, D14: the 1st, 2nd, 3rd, 4th, 5th, 7th, 10th, and 14th days after the start of dosing course.
- *: First dosing on the day (8 am for each group); second/third dosing on the day where applicable (5 pm for TTX 4 µg/kg, BID group; 3 pm and 10 pm for TTX 2.6 µg/kg, TID group). P < 0.05 for D1 1h, D2-0, D3-0, D4-0, D5-0, D7, D10, D14: baseline value for the 2nd, 3rd, 4th, 5th, 7th, 10th, and 14th days after the start of dosing course. **: Peak effect at 1 hour after dosing in each dosing day. ***: Remained until at least day 7 in the 5-day dosing course. ****: Duloxetine increased PWT in rats with oxaliplatin and vincristine-induced neuropathic pain. ▲: TTX provided a more rapid reversal of the PWT decrease than duloxetine and is at the same time points for placebo group, one-way ANOVA.

**Figure 1.** TTX increased PWT in rats with oxaliplatin-induced neuropathy from day 1 to day 7. Greater von Frey filament size (force) was required to elicit paw withdrawal. Peak effect was observed 1h post-dose. For the day before first oxaliplatin injection: W1, W2: 1st and 2nd week after first oxaliplatin injection, respectively; D1, D2, D3, D4, D5, D7, D10, D14: the 1st, 2nd, 3rd, 4th, 5th, 7th, 10th, and 14th days after the start of dosing course.

**Figure 2.** TTX and duloxetine increased baseline PWT in rats with oxaliplatin-induced neuropathy from day 1 to day 7. Greater von Frey filament size (force) was required to elicit paw withdrawal. Pre1, Pre2, Pre3: 1st, 2nd and 3rd days before first oxaliplatin injection; W1, W2: 1st and 2nd week after first oxaliplatin injection, respectively; D1-D3: pre-dosing control; D2-0, D3-0, D4-0, D5-0, D7, D10, D14: baseline value for the 2nd, 3rd, 4th, 5th, 7th, 10th, and 14th days after the start of dosing course. *, **, ***: P < 0.05, 0.01 and 0.001, respectively, compared to the same time points for placebo group, one-way ANOVA.

**Figure 3.** TTX increased PWT in rats with vincristine-induced neuropathy from day 1 to day 7. Greater von Frey filament size (force) was required to elicit paw withdrawal. Peak effect was observed 1h post-dose. Pre1, Pre2, Pre3: 1st, 2nd and 3rd days before first vincristine injection; W1, W2: 1st and 2nd week after first vincristine injection, respectively; D1-D3: pre-dosing control; D2-0, D3-0, D4-0, D5-0, D7, D10, D14: baseline value for the 2nd, 3rd, 4th, 5th, 7th, 10th, and 14th days after the start of dosing course: 1 h: 2 h; post-dosing value for 1 hour and 2 hours after each dosing. *, **, ***: P < 0.05, 0.01 and 0.001, respectively, compared to the same time points for placebo group, one-way ANOVA.

**Summary and Conclusions**

- Oxaliplatin-induced neuropathy in rats.
- TTX administered SC significantly increased PWT from day 1 of dosing.
- Peak effect at 1 hour after dosing in each dosing day.
- Remained until at least day 7 in the 5-day dosing course.
- At 1 and 2 hours post-dosing on days 2 and 3, TTX at 8 µg/kg QD, and 4 µg/kg BID produced significantly higher PWT than that of 2.6 µg/kg TID, but no significant difference was observed on later days and at the baseline time points.
- Vincristine-induced neuropathy in rats.
- TTX administered SC significantly increased PWT from day 1 of dosing.
- Peak effect at 1 hour after dosing in each dosing day.
- Remained until at least day 7 in the 5-day dosing course.
- Duloxetine increased PWT in rats with oxaliplatin and vincristine-induced neuropathic pain.
- TTX provided a more rapid reversal of the PWT decrease than duloxetine and is at least equal to duloxetine in terms of its overall efficacy and duration of action.
- TTX may have therapeutic effects in treating chemotherapy-induced neuropathy.
- Administration of TTX is smaller doses, multiple times may achieve more stable therapeutic effect and generate less adverse side effects than a single larger dose.

The efficacy of TTX for chemotherapy-induced neuropathic pain is being further investigated in a phase III clinical trial.