Poster Number: #40

Human Efficacy of FLX-787, a Dual Activator of TRPV1 and TRPA1 Ion Channels, Reducing Muscle Cramp Frequency and Pain in Randomized Controlled Studies

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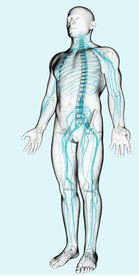
Novel Treatments for Neuromuscular Conditions



Summary

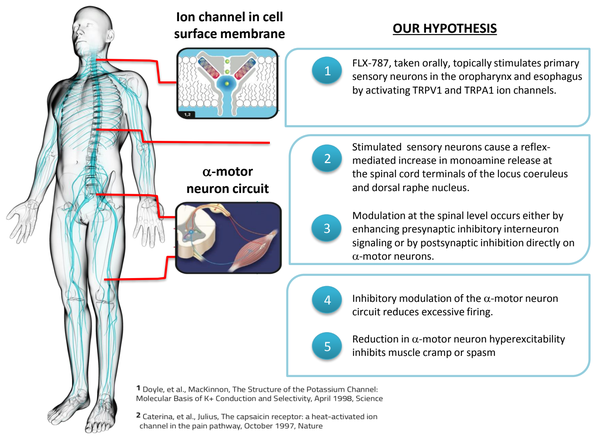
Objectives: Night leg cramps (NLC) are painful supramaximal contractions caused by alpha-motor neuron hyperexcitability and lead to sleep disruptions and reduced quality of life. We have previously demonstrated that activation of TRPV1 and TRPA1 ion channels in the oropharynx and esophagus significantly decrease electrically-induced cramps in healthy adults. We investigated whether this effect would translate to clinical improvements in subjects suffering from NLC. Using a GMP synthesized single molecule that is a naturally-occurring dual TRPV1/TRPA1 activator, FLX-787, we conducted an exploratory cross-over study in subjects with NLC to examine the impact on muscle cramp frequency and pain.

Methods: We investigated two doses of FLX-787 (17 mg and 25 mg) in an orally-disintegrating tablet (ODT) formulation in a randomized, blinded, placebo-controlled two-part cross-over study in otherwise healthy subjects who claimed to experience at least 4 NLCs per week (n=72). At the conclusion of the study, a questionnaire was administered post-hoc to assess the likelihood (not-likely, possible, probable) that participants actually suffered from NLC relative to other conditions such as restless leg syndrome (RLS).

Results: FLX-787 ODT was safe and well-tolerated. Analyses restricted to the first cross-over period of each study part, together with subject selection based on NLC likelihood, demonstrated efficacy. By "possible and probable" NLC criteria, combined active treatments demonstrated a 23% decrease in the weekly mean cramp frequency ($p< 0.05$) and a 31% decrease in weekly mean cramp pain ($p<0.01$). As expected for subjects with likely RLS, no difference in cramp frequency or pain was observed.

Conclusion: These results demonstrate that an ODT formulation of FLX-787 may be effective at reducing both cramp frequency and pain associated with NLC, but may not be useful in subjects with RLS. Careful adjudication of self-reported NLC must be performed prior to randomization to avoid confounding data from subjects with other sleep disorders. These hypothesis-generating conclusions, in conjunction with restricting future studies to parallel design to avoid carry-over effects, will allow for improved clinical studies in NLC as well as in neurological indications (ALS, MS, and Charcot-Marie-Tooth neuropathy) where cramping is prevalent.

Topical Chemical Neuro Stimulation



OUR HYPOTHESIS

1. FLX-787, taken orally, topically stimulates primary sensory neurons in the oropharynx and esophagus by activating TRPV1 and TRPA1 ion channels.
2. Stimulated sensory neurons cause a reflex-mediated increase in monoamine release at the spinal cord terminals of the locus coeruleus and dorsal raphe nucleus.
3. Modulation at the spinal level occurs either by enhancing presynaptic inhibitory interneuron signaling or by postsynaptic inhibition directly on α-motor neurons.
4. Inhibitory modulation of the α-motor neuron circuit reduces excessive firing.
5. Reduction in α-motor neuron hyperexcitability inhibits muscle cramp or spasm

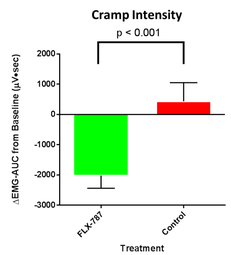
1 Doyle, et al., MacKinnon, The Structure of the Potassium Channel: Molecular Basis of K+ Conduction and Selectivity, April 1998, Science

2 Caterina, et al., Julius, The capsaicin receptor: a heat-activated ion channel in the pain pathway, October 1997, Nature

Figure 1. Overview of suspected mechanism of muscle cramps and methods of cramp inhibition by activation of TRP ion channels. Muscle cramping is caused by the uncontrolled and repetitive firing of α-motor neurons in the spinal cord (1), resulting in maintained contraction of the muscle. FLX-787 is thought to exploit a general principle of neural circuits whereby strong excitatory sensory input from one source enhances overall inhibitory tone by increased recruitment of inhibitory neurons, thereby reducing excitability in other parts of the circuit (3). FLX-787 stimulates primary sensory neurons in the mouth, esophagus and stomach by activating TRPV1 and TRPA1 ion channels (4,5). When activated, these sensory neurons, which project both directly and indirectly to the spinal cord, enhance the inhibitory tone in spinal cord circuits to reduce repetitive firing of α-motor neurons which prevents or reduces the frequency and intensity of muscle cramps and spasms.

EIC Efficacy of FLX-787

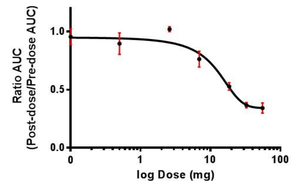
Figure 2.



Exemplary dataset demonstrating that FLX-787 (29 mg), a single molecule that co-activates TRPA1 and TRPV1, affords a decrease in electrically-induced muscle cramp intensity. The treatment effect was calculated based upon the area under the curve (AUC) of a surface EMG measurement of cramping relative to a pre-treatment baseline cramp (n=9). FLX-787 treatment led to a 5-fold reduction in delta-AUC compared to a study specific vehicle control. FLX-787 treatment demonstrated a significant difference from vehicle control (ANOVA, $p < 0.001$).

Figure 3.

FLX-787 Inhibition of electrically-Induced cramps in healthy subjects is dose-responsive

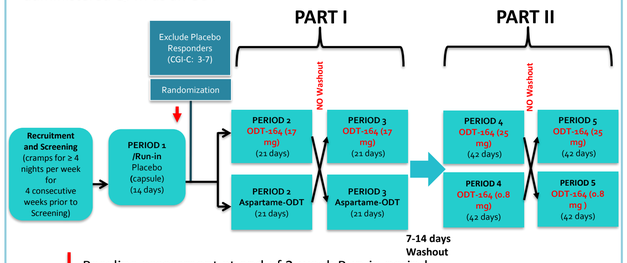


- Six doses of FLX-787 ODT yield classic sigmoidal dose curve (n=5, $p<0.05$)
- Efficacy saturates at ~32 mg FLX-787-ODT
- Initial human PK (n=4) indicates no measurable system exposure of parent drug in plasma at potential therapeutic dose

NLC Study Design

Figure 4.

A randomized, double-blind, placebo-controlled, cross-over study to evaluate the effects of a FLX-787 (named ODT-164 in study) on NLC cramp frequency when self administered QPM as an ODT



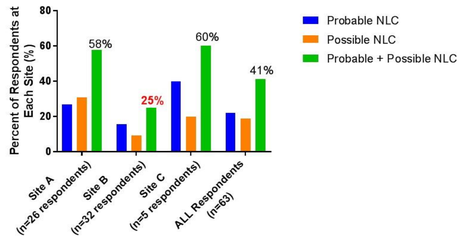
- Topline analysis did not demonstrate efficacy for either cramp frequency or pain (n=72)
- Analysis demonstrated carry-over between cross-over periods
- Analysis suggested that study site data from one site (n=37) was discordant with other sites

NLC Questionnaire & Adjudication

- Post-hoc questionnaire was administered after study completion to identify if enrolled subjects displayed common NLC characteristics
  - Assessment of recalled cramp frequency, associated pain and cramp characterization
  - Questionnaire was independently administered and reviewed in a blinded fashion and subjects adjudicated as having possible/probable/no NLC
- Subjects not meeting diagnostic criteria:
  - Cramp frequency too low, <4 per week: 31 out of 63 Subjects
  - Typical description of painful cramps but probable comorbidity of RLS: 4 out of 63 Subjects
  - Probable diagnosis of RLS without evidence of painful cramps (pain <4 on a 0-10 scale) 10 out of 63 Subjects
  - Pain episodes without evidence of muscle contraction 2 out of 63 Subjects
  - Inconsistencies between cramp description, pain and impact on sleep 1 out of 63 Subjects

Figure 5.

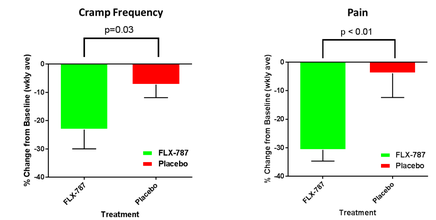
Questionnaire Respondents Adjudicated with NLC



- 41% of questionnaire respondents adjudicated as likely having NLC
- Single site identified previously as being discordant with other sites is the same site with the lowest percentage (25%) of subjects adjudicated with NLC

NLC Efficacy of FLX-787 in Subjects with Confirmed NLC Diagnosis

Figure 6.



- An exploratory sub-analysis was performed limited to subjects with confirmed NLC diagnosis based upon a post hoc questionnaire administered after study completion.
  - To avoid potential influence of carry-over effects observed during cross-over, analysis was restricted to the first cross-over periods (Periods 2 and 4).
  - The analysis is based on the overall active (17 + 25 mg FLX-787) vs placebo (aspartame + 0.8 mg FLX-787) comparison (nonparametric analysis, Wilcoxon Rank-Sum test).
- FLX-787 displayed ~3-fold decrease in cramp frequency ($p=0.03$) and 8-fold decrease in pain ($p<0.01$) relative to Placebo.
- No difference in cramp frequency or pain relative to Placebo was observed for those subjects with probable RLS.

Effect Size Analysis

Effect sizes calculated are on-average larger than those reported in the quinine clinical literature; and greater than many marketed neuro drugs

Endpoint	Treatment/Study	N_{total}	$N_{Eff\ size}$	p-value	Effect Size
Cramp Frequency	TRP-Stim^	50	50	0.08	0.46
	FLX-787 (ODT)/Study 1*	72	37	0.06	0.77
	FLX-787 (liq)/Study 2^	29	29	0.02	0.94
	FLX-787 (ODT)/Study 1‡	72	26	0.03	0.53
Cramp Pain	FLX-787 (ODT)/Study 1‡	72	26	< 0.01	0.83

* The effect size of Study 1 is a sub-analysis (n=37) excluding data from 1 site (n=35).

‡ Sub-analysis of Study 1 is based upon an independent, blinded adjudication of a post-hoc questionnaire to establish the likelihood of NLC. Of the 72 participants, 63 completed the questionnaire and 26 were judged as having likely NLC. The effect size is calculated based upon the overall active vs placebo comparison in this sub-population.

^ Presented at 2016 Society for Neuroscience, Nov 15, 2016.

- Average effect size of cramp frequency derived from quinine literature is **0.12** (95%CI[-3.5,-1.36]). (6)

Conclusions

- Chemical Neuro Stimulation of TRPA1/TRPV1 by FLX-787 is a local, topical phenomenon that does not require systemic bioavailability and may result in indirect inhibition of α-motor neuron hyperexcitability.
- FLX-787 reduces muscle cramp intensity in an EIC-model of the foot.
- FLX-787 is well tolerated, and no treatment-related SAEs have been reported in clinical studies to date.
- Using an NLC questionnaire administered after study completion, a minority of study subjects (26 out of 63) were diagnosed with possible or probable NLC.
- Cross-over studies with FLX-787 in NLC may be hampered by carry-over effects limiting their interpretability.
- Study site that was previously identified as yielding discordant data in comparison to the other study sites enrolled the fewest subjects diagnosed with NLC (8 out of 32 subjects).
- In the diagnosed NLC cohort, FLX-787 reduced both cramp frequency ($p=0.03$) and pain ($p<0.01$) associated with NLC.
- Effect size range of cramp frequency across NLC studies range from 0.46-0.94 in comparison to quinine literature at 0.12.
- Independent adjudication of self-reported NLC should be performed prior to randomization to confirm cramp frequency and the absence of comorbidities such as RLS.
- To minimize the impact of heterogeneous study populations as observed in NLC, upcoming FLX-787 clinical studies will focus in defined disease populations of MND (ALS, PLS, PMA) and Charcot-Marie-Tooth Disease.

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