MRGPRX1 Positive Allosteric Modulator (PAM) program

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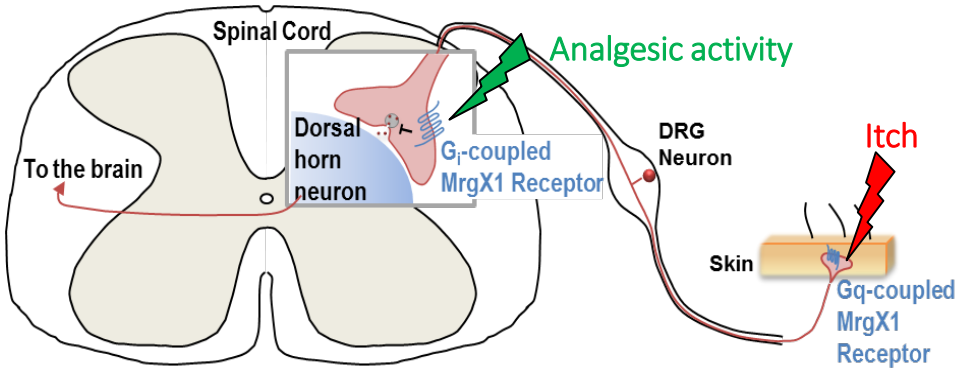
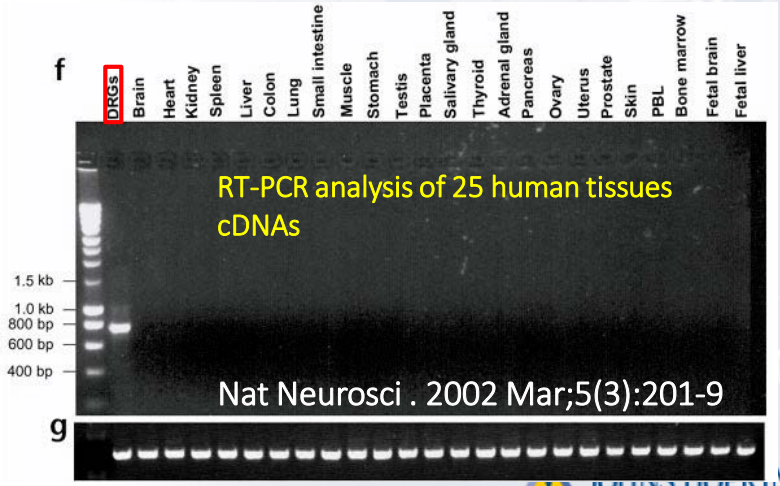
MRGPRX1: Human Sensory Neuron-Specific G-Protein-Coupled Receptors

- Human MrgprX1 is a GPCR expressed specifically in nociceptive DRG neurons but not in the brain or other tissues (minimizing side effects)
- MrgprX1 is expressed at both peripheral axons (e.g., skin), soma, and centrally projecting axons (in the spinal cord) of DRG neurons
- Activation of MrgprX1 and MrgprC (the rodent homologue) at the central terminals inhibits synaptic transmission and pain, but activation at the peripheral terminals in the skin can produce itch





BAM8-22
Endogenous MRGPRX1 Agonist

BAM8-22: Val-Gly-Arg-Pro-Glu-Trp-Trp-Met-Asp-Tyr-Gln-Lys-Arg-Tyr-Gly

- First isolated from **b**ovine **a**drenal **m**edulla (hence, BAM).
- Derived from proenkephalin A
- Displays no affinity for opioid receptors
- Displays potent agonist activity towards MrgprX1
- Intrathecal injection of BAM8-22 was found to attenuate both mechanical and thermal hypersensitivities in chronic pain models in mice and rats
- Skin administration of BAM8-22 was found to cause itch in human and mice due to the peripheral MrgprX1 activation

Adv Exp Med Biol. 2016;904:87-103

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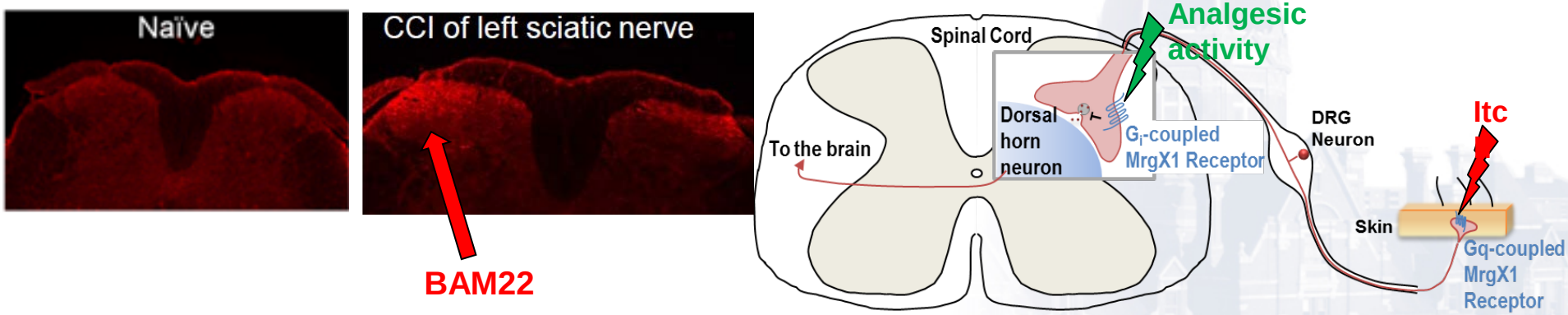
Limitations of MrgprX1 agonists

- BAM8-22, an endogenous peptide-based MrgprX1 agonist, is not orally available, nor can it inhibit pain via systemic administration (i.p., i.v.). It induces pain inhibition only by intrathecal injection
- No orally available small molecule MrgprX1 agonists have been identified
- Systemic exposure to a MrgprX1 agonist may induce itch side effects

Rationale for Exploring Positive Allosteric Modulators (PAMs) of MrgprX1

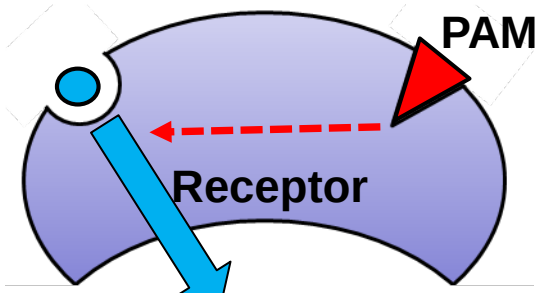


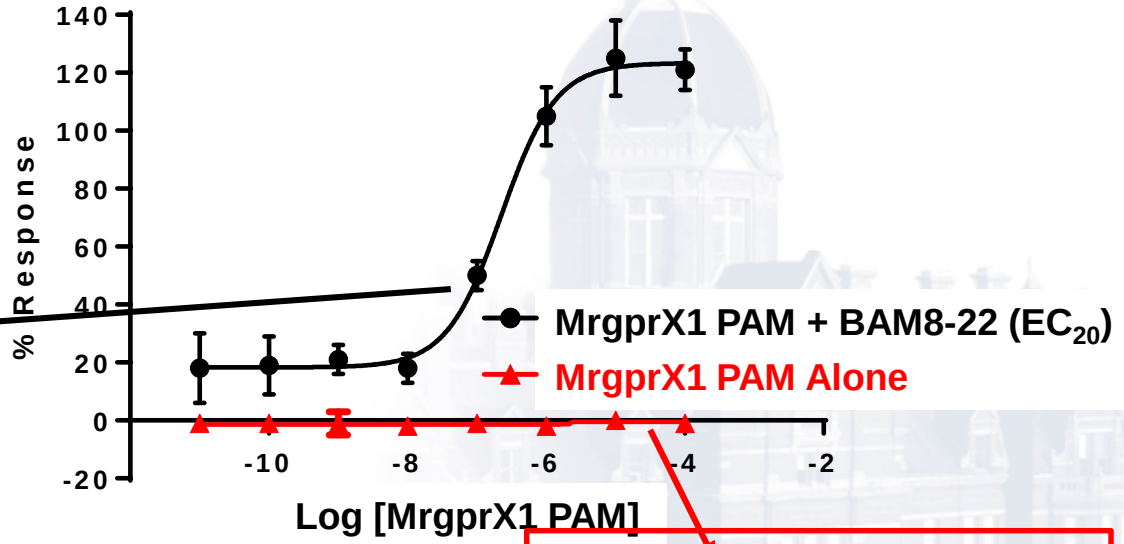
- BAM22 immunoreactivity appeared at superficial layers (laminae I–II) of dorsal horn where nociceptive fibers mostly terminate, and was elevated on the injured side in both CFA- and CCI-subjected mice
- Its level is below the limit of detection in the skin
- MrgprX1 PAMs may provide a unique opportunity to treat pain by
  - Enhancing endogenous MrgprX1 agonist-induced activation of MrgprX1 preferentially at the central terminals
  - without producing itch side effects due their inability to activate the peripheral receptors

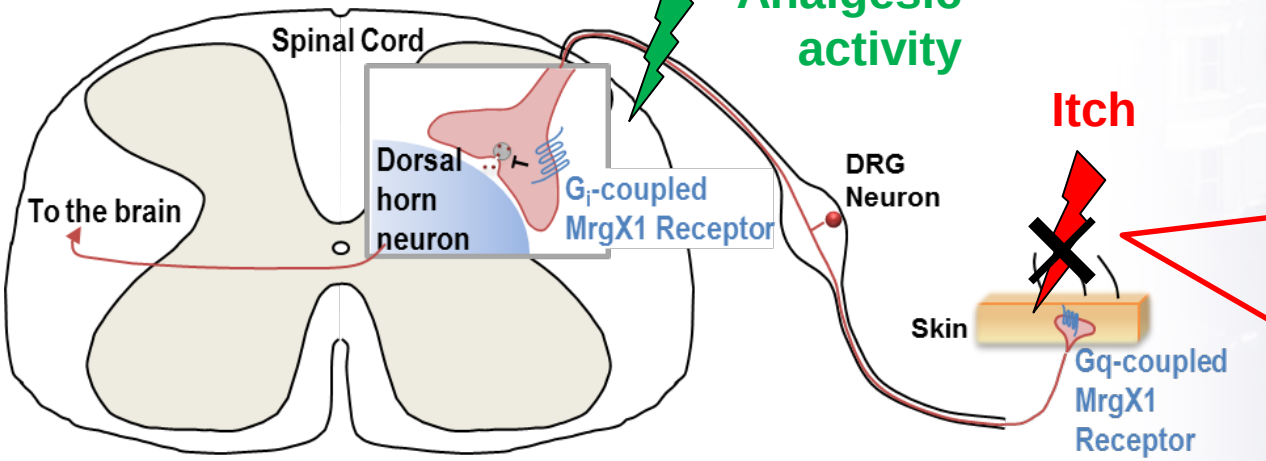
Proc Natl Acad Sci U S A. 2017;114(10):E1996-E2005

Therapeutic Strategy to Allosterically Modulating MrgprX1

PAM can enhance potency and/or efficacy of orthosteric agonist



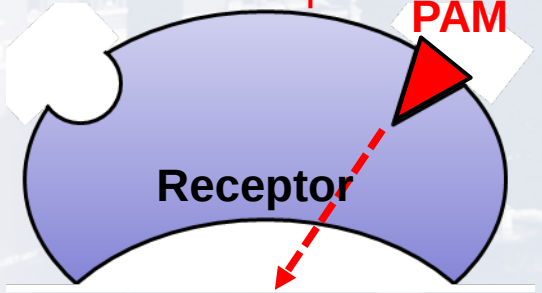




Analgesic activity

Itch

PAM alone cannot activate receptors



No Activation

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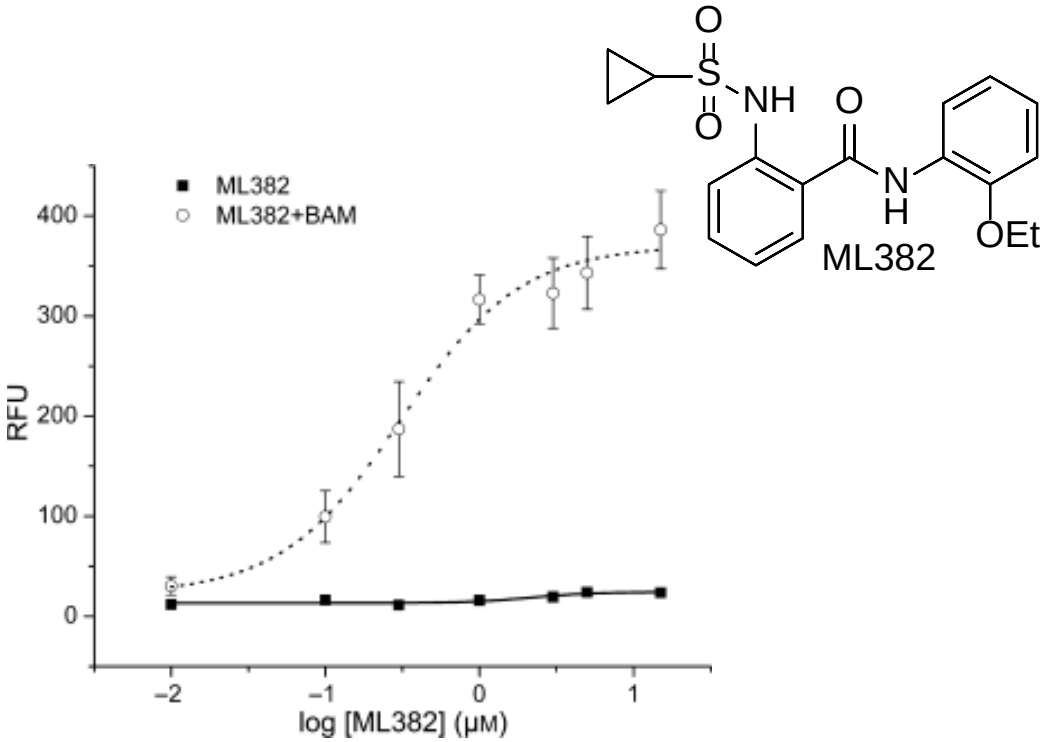
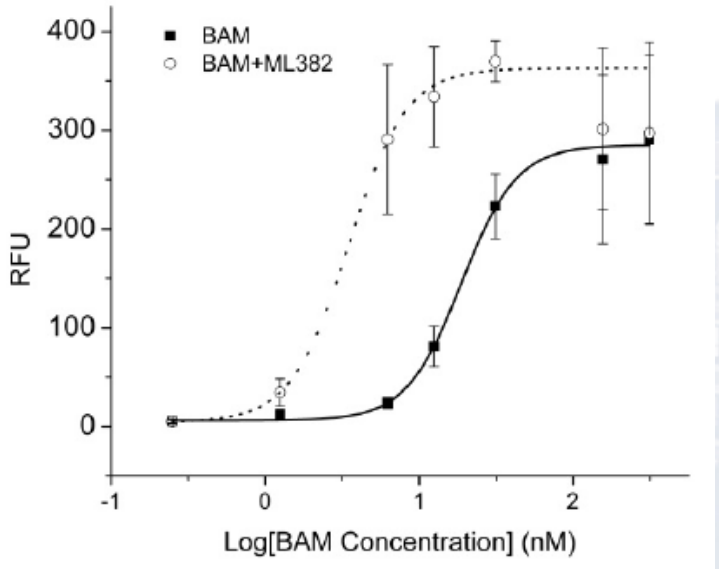
ML382: CNS non-penetrant prototype MrgprX1 PAM





ML382 PAM Potency

w/ BAM8-22 (10 nM): EC_{50} = 190 nM

w/o BAM8-22: no activity

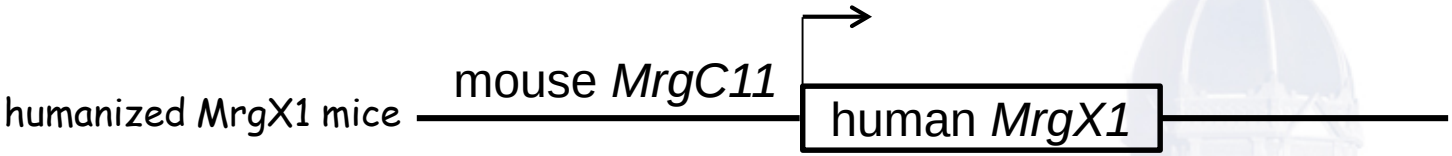
BAM Agonist Potency

w/o ML382: EC_{50} = 19 nM

w/ 5 μM ML382: EC_{50} = 2.9 nM

ChemMedChem. 2015 Jan;10(1):57-61

MrgX1;Mrg$^{-/-}$ Mice



- Dr. Xinzhong Dong generated a BAC (bacterial artificial chromosome)-transgenic mouse line in which human MrgprX1 is expressed under the control of mouse MrgprC11 promoter
- This strain was crossed into Mrg-clusterΔ-/- background to generate BAC-MrgX1;Mrg-clusterΔ$^{-/-}$ (MrgX1;Mrg$^{-/-}$) mouse line.
- In this way, human MrgprX1 agonists/PAMs/antagonists can be tested in mouse pain models.

Proc Natl Acad Sci U S A. 2017;114(10):E1996-E2005

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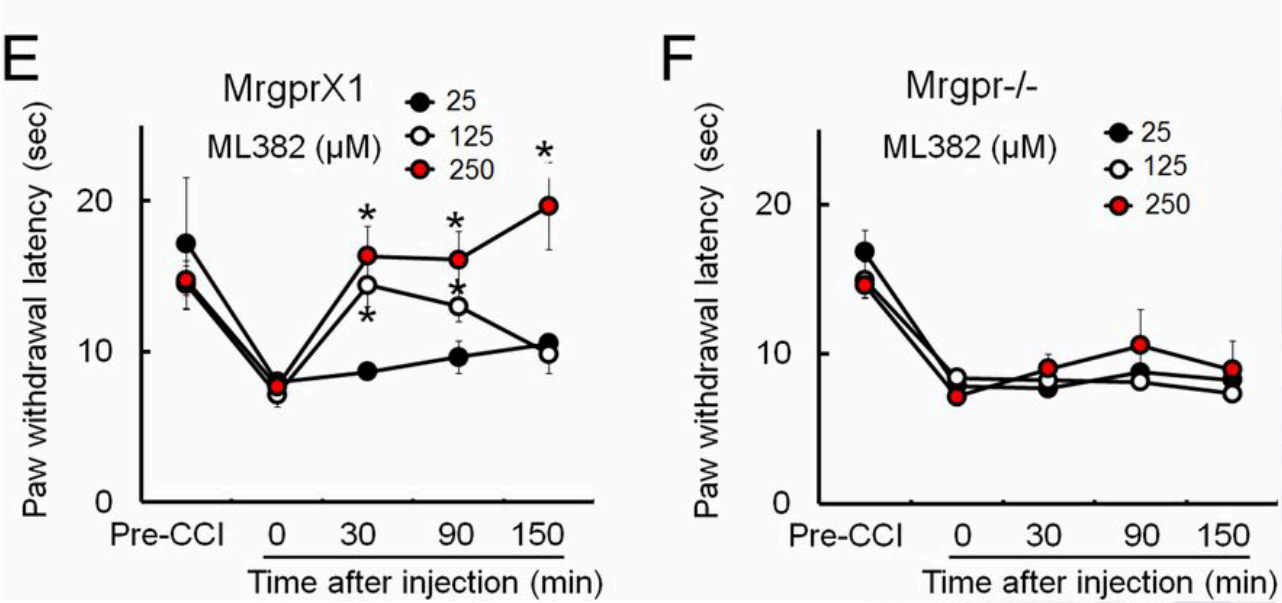
ML382 alone attenuates evoked pain hypersensitivity in MrgprX1 mice



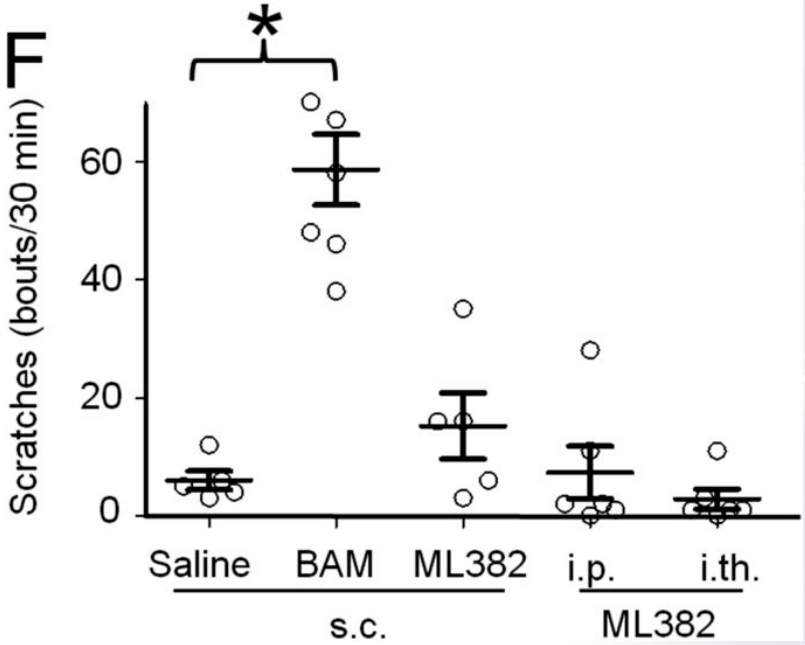
CCI of sciatic nerve-induced heat hypersensitivity of the ipsilateral hind paw
The hypersensitivity was dose-dependently attenuated by i.th. ML382 (25, 125, or 250 μM, 5 μL)* in MrgprX1 mice (E), but not in Mrgpr$^{-/-}$ mice (F)

*ML382 does NOT penetrate CNS and showed no analgesic effects following intraperitoneal injection

Proc Natl Acad Sci U S A. 2017;114(10):E1996-E2005

Unlike BAM8-22, ML382 does not cause itch in MrgprX1 mice

- ML382 did not cause itch in MrgprX1 mice when applied s.c. (1.0 mM, 5 µL) into the back, i.p. (5 mM, 10 µL), or i.th. (25 µM, 5 µL).
- In contrast, s.c. injection of BAM8–22 (BAM; 1.0 mM, 5 µL) caused rigorous scratching behavior



Proc Natl Acad Sci U S A. 2017;114(10):E1996-E2005

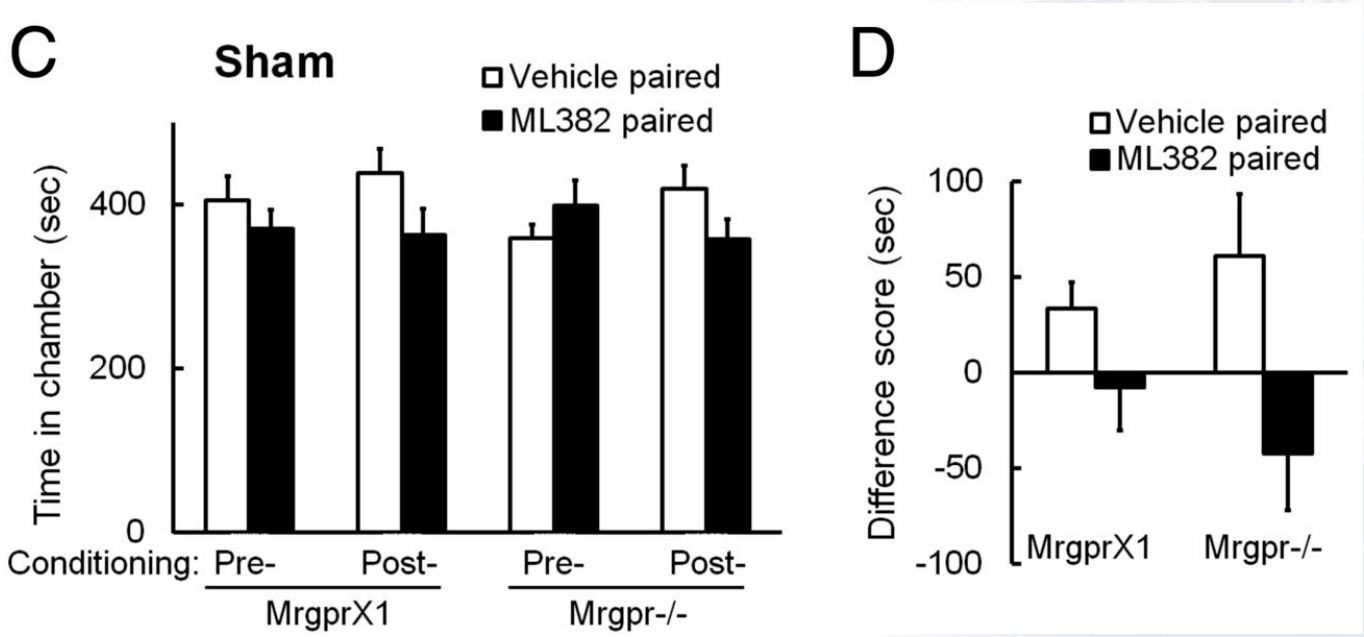
ML382 Induces Pain Relief As Measured By Conditioned Place Preference (CPP)

ML382 increased post-conditioning time spent in the ML382-paired chamber in MrgprX1 mice with CCI, suggesting it may alleviate the affective component of neuropathic pain and ongoing pain.

Proc Natl Acad Sci U S A. 2017;114(10):E1996-E2005

ML382 Does Not Activate Reward Circuitry Suggesting No Abuse Potential

ML382 did not induce CPP in sham-operated MrgprX1 mice, suggesting that the drug itself does not activate innate reward circuitry in the absence of pain and thus has minimal risk of inducing addiction.



Proc Natl Acad Sci U S A. 2017;114(10):E1996-E2005

Summary of findings with ML382

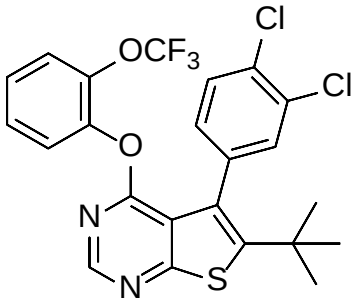
Key Findings

- MrgX1;Mrg$^{-/-}$ enabled us to examine the therapeutic utility of human MrgX1 PAMs in rodent models of neuropathic pain.
- Proof-of-concept studies with ML382 demonstrated allosteric activation of MrgprX1 at central terminals of DRG neurons leads to analgesic effects without producing itch side effects.
- Conditioned Place Preference (CPP) tests indicate that ML382 induces pain relief in CCI mice without activating reward circuitry suggesting no abuse potential

Challenge

- CNS-nonpenetrant ML382 requires intrathecal injection to reach site of action for analgesic effects in MrgX1;Mrg$^{-/-}$ mice

Thienopyrimidine-based MrgprX1 PAM Compound 1t

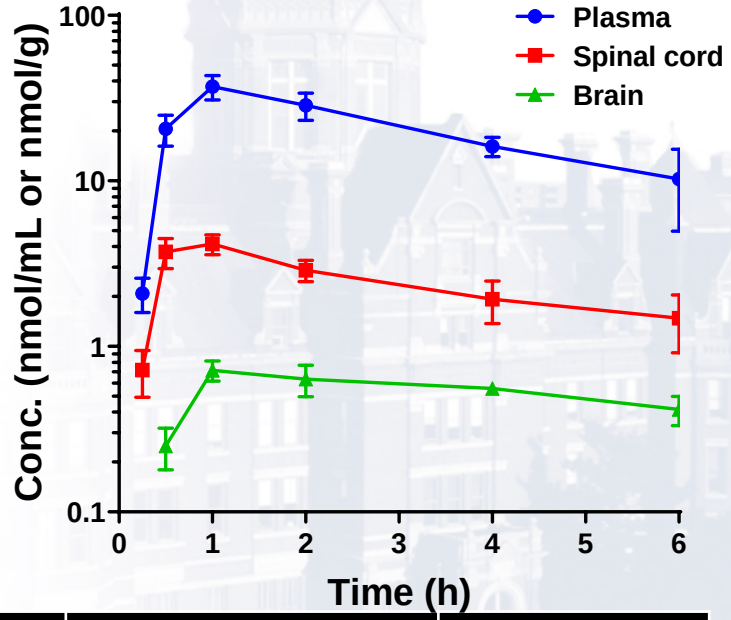


1t (EC_{50} = 0.1 **μ**M)

Liver Microsomes Stability (%remaining)			
Mouse		Human	
30 min	60 min	30 min	60 min
58	25	>95	>95

- Compound **1t** was distributed to plasma and spinal cord following oral administration at 100 mg/kg in mice
- Exposures ($AUC_{0\text{-last}}$) in brain and spinal cord are 2.18% and 12.71% of plasma, respectively

Mouse oral pharmacokinetics of compound **1t**

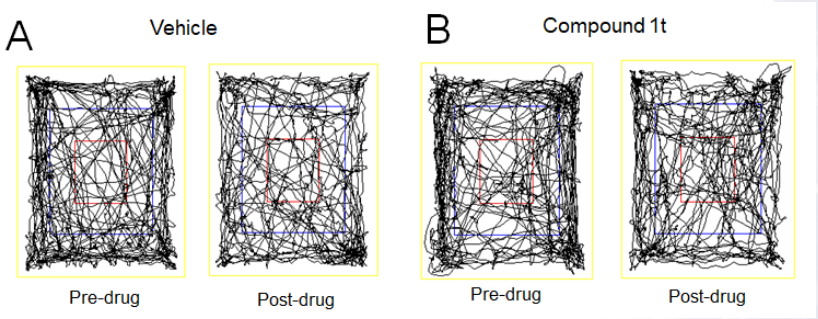


Matrix	Tmax (h)	Cmax (nmol/mL)	$AUC_{0\text{-last}}$ (nmol*h/mL)	AUC Brain (or)Spinal cord to Plasma ratio	% of $AUC_{0\text{-last}}$ Plasma
Plasma	1.67 ± 0.33	38.98 ± 5.06	108.53 ± 1.18	-	-
Brain	1.67 ± 0.33	0.82 ± 0.05	2.37 ± 0.03	0.02	2.18
Spinal cord	1.17 ± 0.44	4.60 ± 0.60	13.79 ± 0.14	0.13	12.71

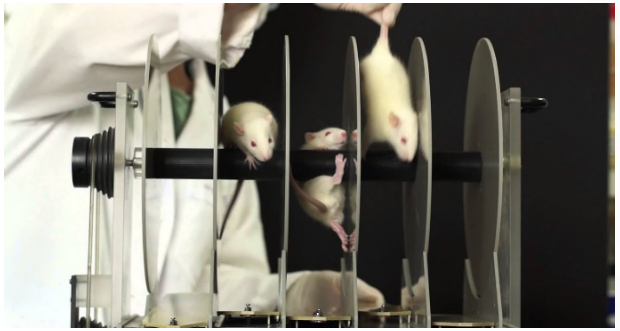
J Med Chem. 2022 Feb 24;65(4):3218-3228

Preliminary in vivo safety studies of Compound 1t (100 mg/kg po) in naïve MgprX1 mice

- No changes in exploratory activity of naïve MrgprX1 mice in open field test



- No changes in the performance of MrgprX1 mice in rotarod test.



- No overt behavioral effects (no scratching behavior) in naïve MrgprX1 mice
- Selective over opioid receptors and MrgprX2 (as agonist/PAM for C48/80)

J Med Chem. 2022 Feb 24;65(4):3218-3228

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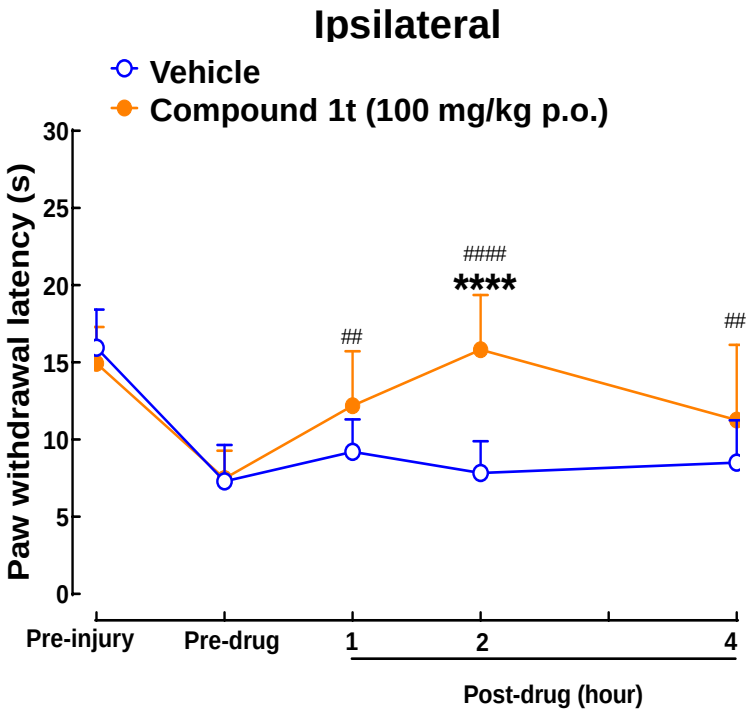
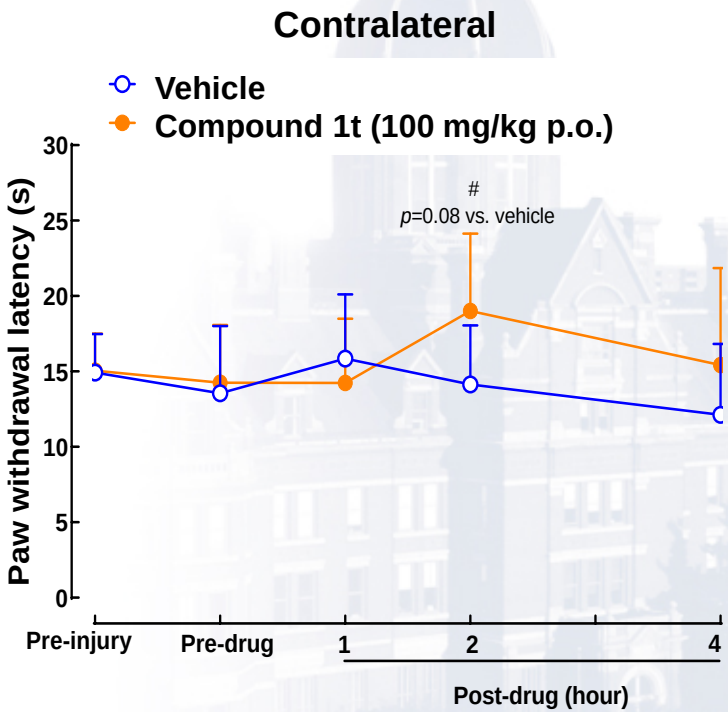
Compound 1t (P.O.) attenuated CCI-induced heat hypersensitivity in MrgprX1 mice





Strong anti-heat hyperalgesia, minimal/no anti-nociceptive effect at this dose
Peak effect: 2 h post-drug; Duration: > 4hr;
No obvious adverse effect (also shown in previous safety study)

N=9/group. Data are expressed as mean + SD.
****P<0.05 vs. vehicle; #P<0.05. ##P<0.01, ####P<0.0001 vs. pre-drug
Two-way mixed-model ANOVA (post Bonferroni test)

Summary and Current Activities

- Potent MrgprX1 PAM (**1t**) were identified through structural optimization of HTS hits
- MrgprX1 PAM (**1t**) was found to be metabolically stable and orally available in mice.
- Oral administration of MrgprX1 PAM (**1t**) attenuated CCI-induced heat hypersensitivity in MrgprX1 mice without producing itch side effects
- Further structural optimization supported by the **NIH HEAL Initiative** led to the discovery of two lead development candidates with improved *in vitro* PAM potency (EC_{50} = 0.01 μM) and in vivo efficacy (ED_{50} ≈ 10 mg/kg)

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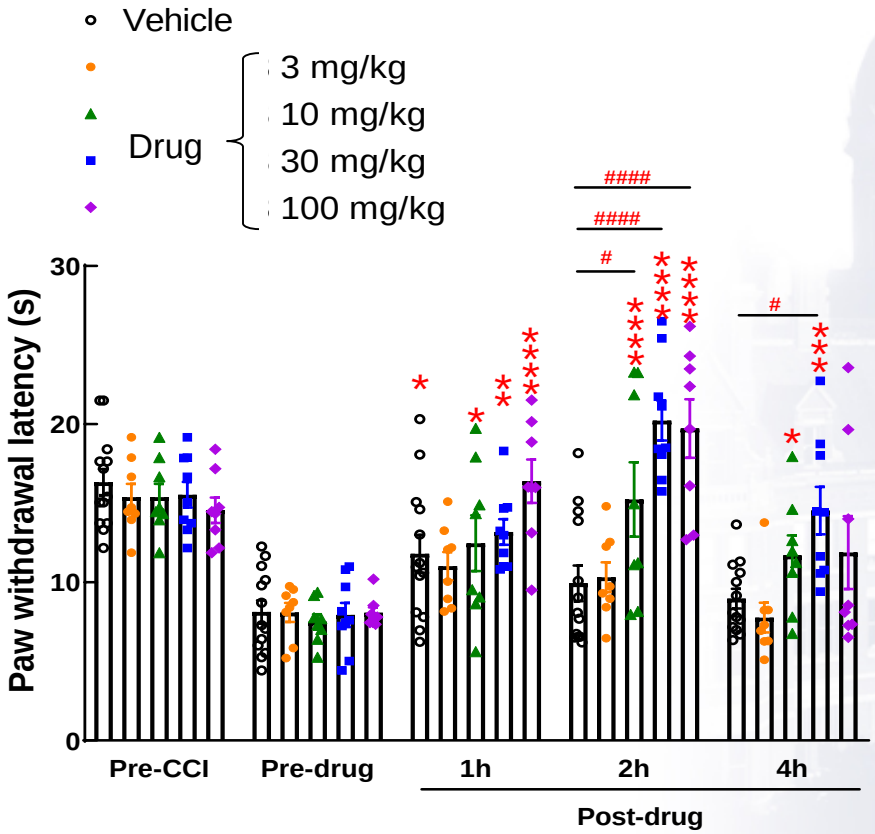
New development candidate dose-dependently inhibited heat hypersensitivity in MrgprX1 mice after sciatic CCI following oral administration

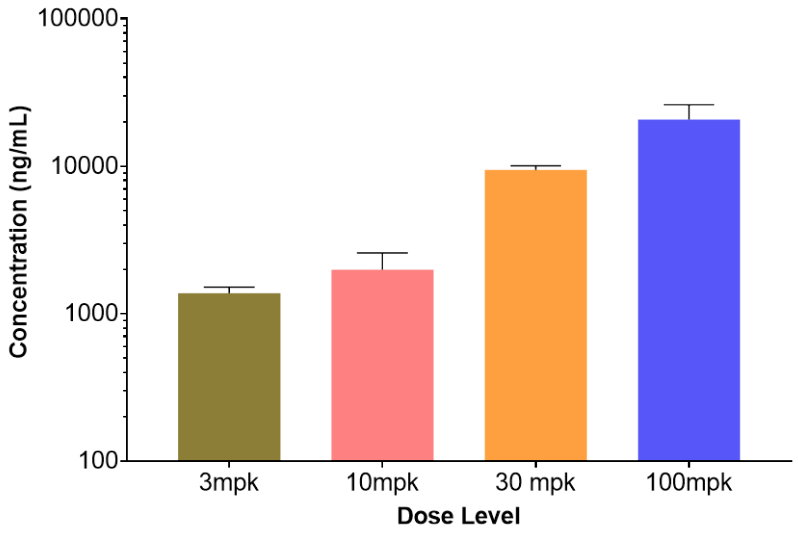


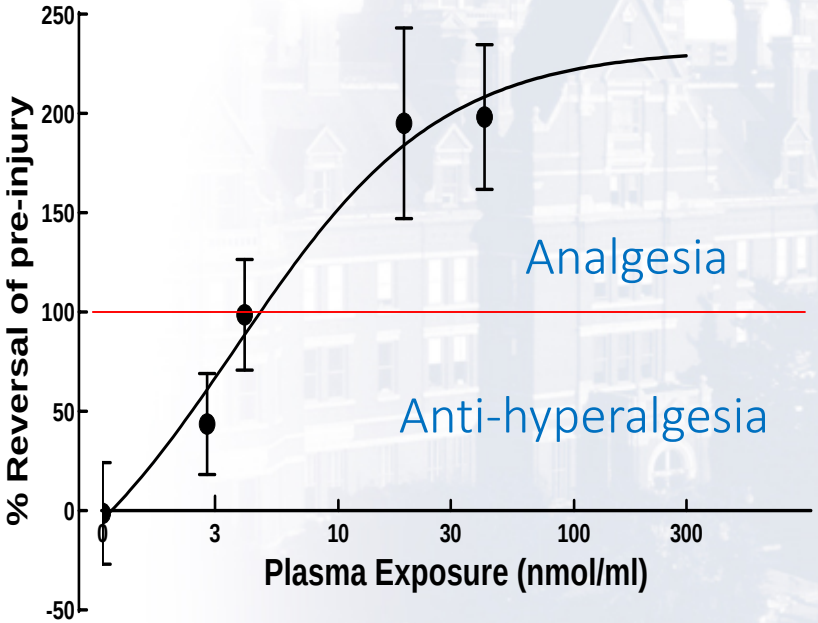
New development candidate (0.3, 1, 3 or 10 mg/mL in vehicle) **P.O.** N=8-13/group, both sexes, day 14-20 post-CCI (peak of neuropathic pain), *p<0.05 vs. pre-drug, # P<0.05 vs. vehicle. Two -way ANOVA (Bonferroni post-hoc test).

PK-PD relationship of the new development candidate

% Reversal of pre-injury (@2 h following oral administration):
(post-drug PWL- Pre-drug PWL)/(pre-injury PWL-pre-drug PWL)*100

Dose Linearity – drug levels in mouse plasma
@ 2h following oral administration

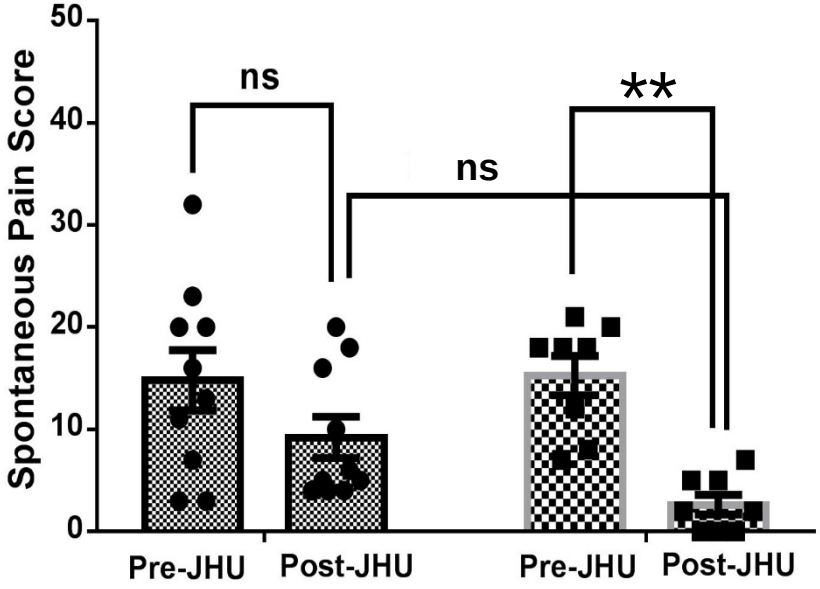


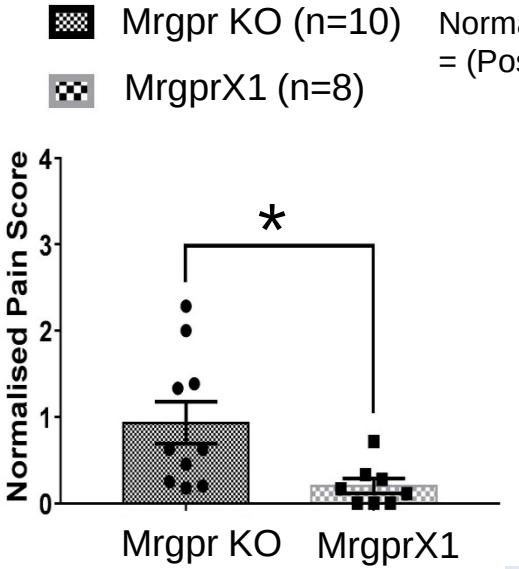


Based on interspecies allometric scaling, anti-hyperalgesia effects are expected at ~1 mg/kg in humans

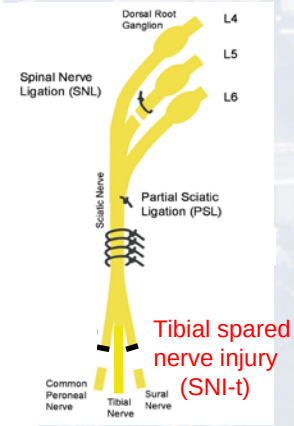
New development candidate attenuated spontaneous pain in MrgprX1 mice after sciatic SNI

Spontaneous pain behavior (flinching; lifting; shaking) was more prominent in SNI model than in CCI model, and was measured in MrgprX1 and Mrgpr KO mice at 14 days post-SNI; Video recording and scoring for 1 hour before and from 2 hour post-drug (100 mg/kg, P.O.).





Normalized pain score
= (Post-JHU)/(Pre-JHU)



Conclusion: SNI induced a significant and comparable level of spontaneous pain behavior in both genotypes. The pain score was significantly decreased by new development candidate (100 mg/kg. P.O.) in an MrgprX1-dependent manner. *P<0.05, **, P<0.01. Mean ± SEM. Blinded Test.

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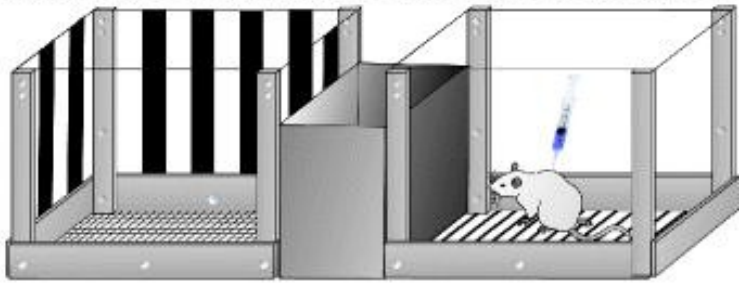
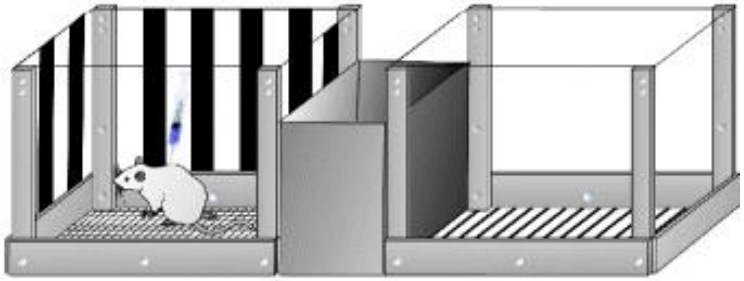
Unlike morphine, current lead candidate does not induce conditioned place preference (CPP) in naïve MrgprX1

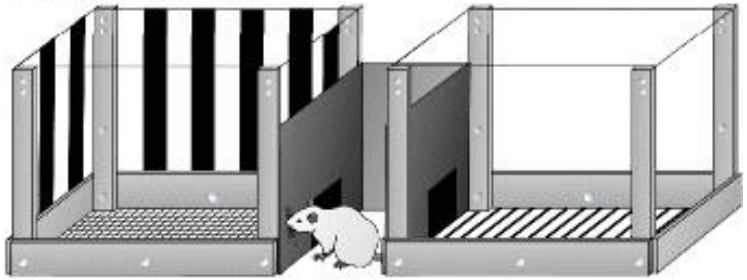
Conditioning with neutral substance (saline) in one compartment

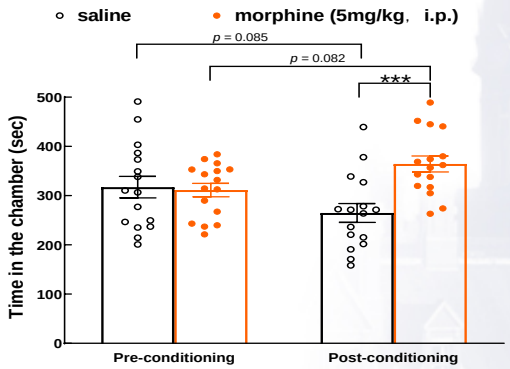


Conditioning with drug in opposite compartment

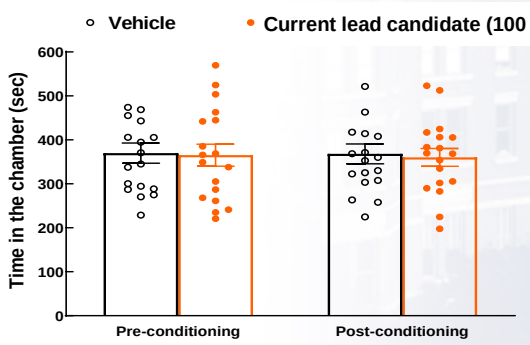


Place preference testing





N=16/group（8 males and 8 females, naïve wildtype C57bl/6J mice). Two-way repeated measures model ANOVA with Bonferroni post-hoc test.
Saline vs. morphine, F(1, 30)=6.475.
Data are expressed as mean ± SEM.
***p<0.001.



N=18/group（8 males and 10 females, MrgprX1 mice).

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Clinical Correlates for Translational Studies: Potential Neuropathic Pain Indications

	Postamputation Pain (nerve transection)	**Chronic Post-surgical Neuropathic Pain** (entrapment, stretch injury, transection)
Etiology	Nerve injury associated with amputation-residual limb and phantom pains	Nerve injury associated with surgery, e.g., thoracotomy, mastectomy, herniorrhaphy
Mechanism	Spontaneous & ectopic activity at injury site and DRG; central sensitization	Spontaneous & ectopic activity at injury site & DRG; central sensitization
Ongoing Pain	Paroxysmal Limits prosthetic use	continuous, increased by activity
Hypersensitivity	Pressure-evoked pain (interference with prosthesis); Thermal (cold and heat)	Static and dynamic mechanical allodynia / hyperalgesia
Unmet needs	30-85%, 5-10% severe; LE amputations /yr: 185,000 Population(US) - 2 million	10-65%, 5-10% severe US: >100 million surgeries/yr > 20 million patients

Schug SA et al. Pain 2019;160:45. Richebe P et al. Anesthesiology 2018;129, 590.

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Current Project Status

- Further lead optimization within this new series is currently funded (non-dilutive) by NIH HEAL Initiative (UH3NS115718)
- Preclinical development including IND-enabling studies of a development candidate will be also funded (non-dilutive) by NIH HEAL Initiative (UH3NS115718)
- A patent application claiming the new series of MrgprX1 PAMs has been published
- Open for strategic alliance to move the project forward with the ultimate objective of out-licensing the optimized MrgprX1 PAMs

Competitive advantages of working with us

- Unlike other targets, activation of MrgprX1 may cause less side effects because of its strict expression in DRG neurons
- MrgprX1 PAMs may preferentially activate the central receptors and minimize itch side effects
- Access to humanized MrgprX1 mice (exclusively available at JHU) enables efficacy assessment in preclinical models of pain
- A composition-of-matter patent application claiming lead development candidates has been filed
- Preclinical development is being supported by non-dilutive funding from NIH
- JHU School of Medicine can offer extensive clinical expertise in pain

Our Team

Xinzhong Dong Ph.D.

- Howard Hughes Medical Institute investigator
- Developed and possesses MrgX1;$Mrg^{-/-}$ Mice
- Expertise in molecular biology and neuroscience

Yun Guan M.D., Ph.D.

- Capable of conducting various pain models in rodents
- Involved in pharmacological characterization of ML382
- Expertise in electrophysiology and pain medicine

Takashi Tsukamoto PhD

Director of Chemistry at Johns Hopkins Drug Discovery (JHDD, drugdiscovery.jhu.edu) headed by Dr. Barbara Slusher. JHDD complements the strength of academic research by offering expertise/resources in

- Medicinal chemistry
- Assay development
- Drug metabolism and pharmacokinetics
- In vivo Pharmacology