BLU-5937: A Selective P2X3 Antagonist With Potent Anti-Tussive Effect and No Taste Alteration

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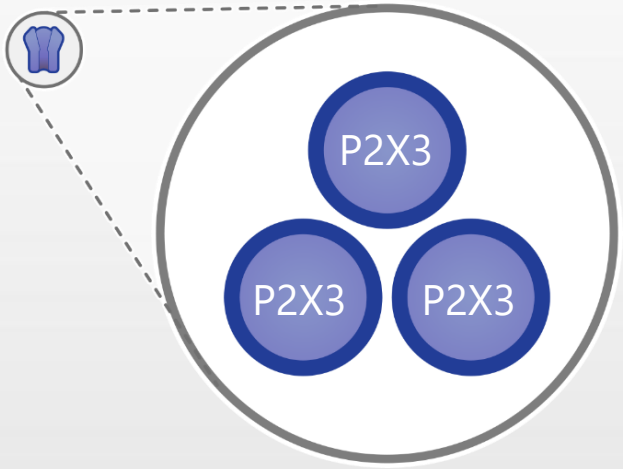
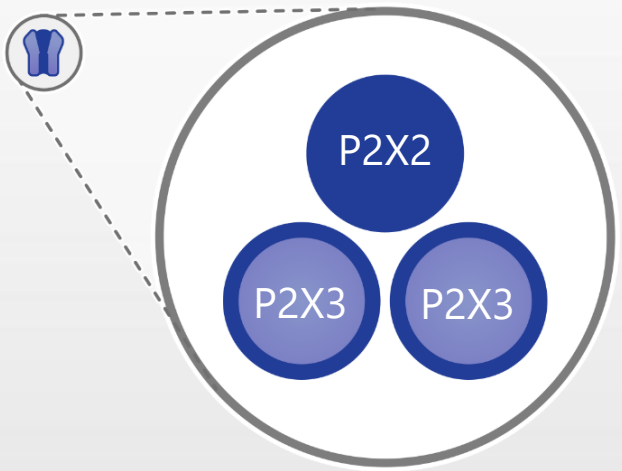
Targeting P2X3 to Treat Chronic Cough with Potential No Taste Effect



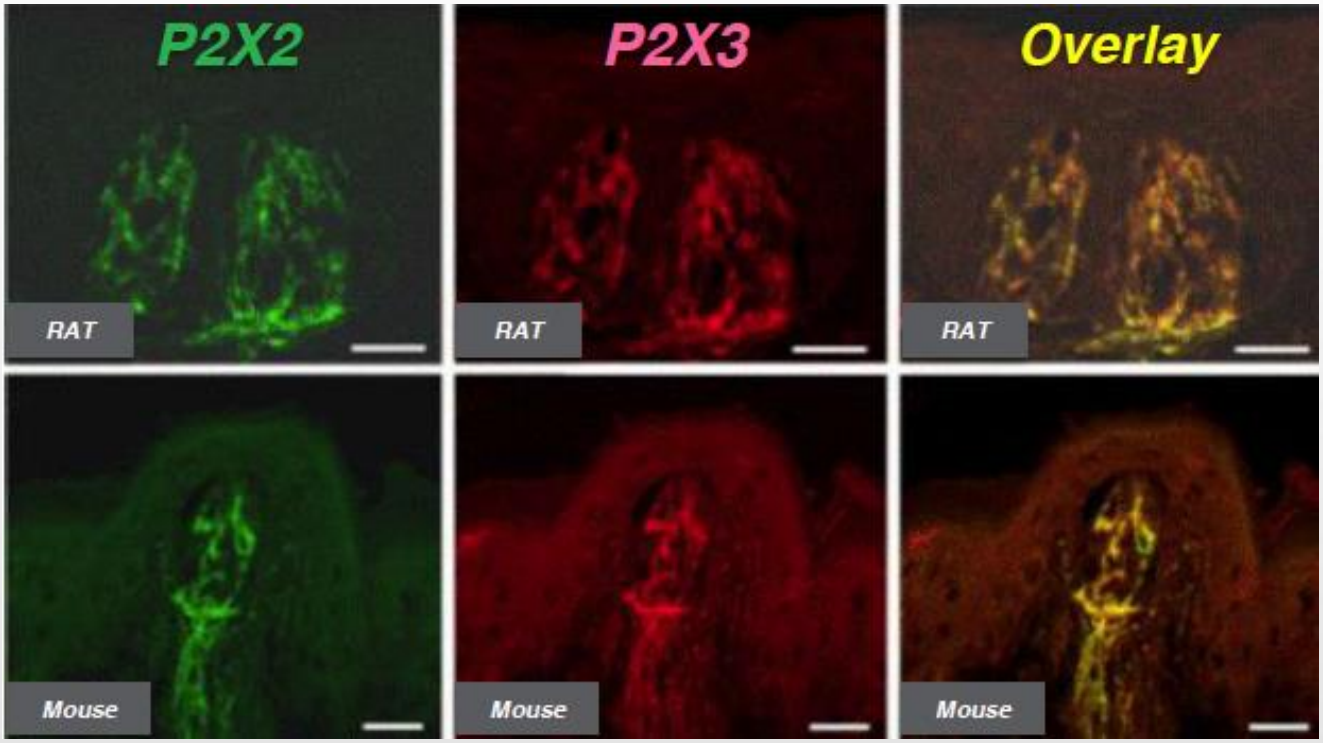
P2X3 homotrimeric receptors are linked to cough hypersensitivity



P2X2/3 heterotrimeric receptors are
linked to taste function

Hypothesis:
Selective inhibition of P2X3 homotrimeric receptors would reduce cough without impact on taste perception

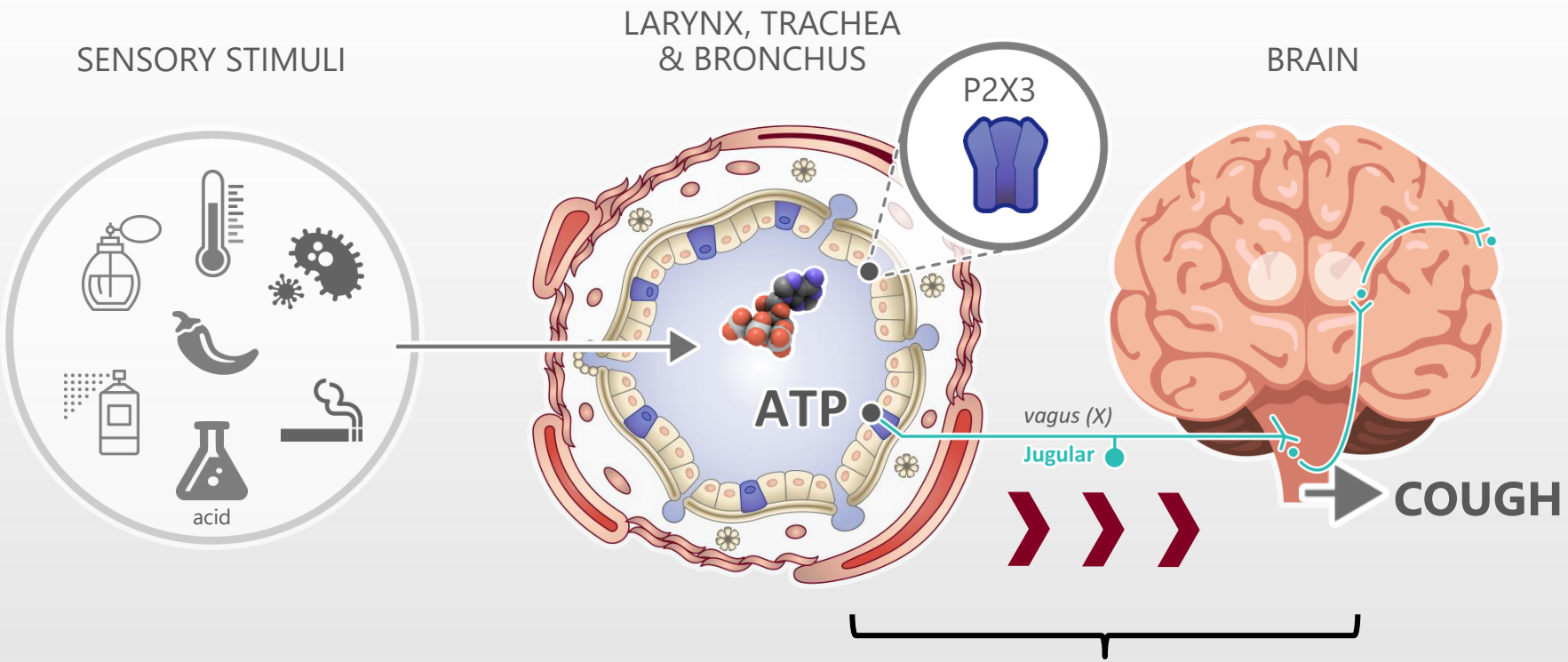
P2X2/3 is Linked to Taste Function



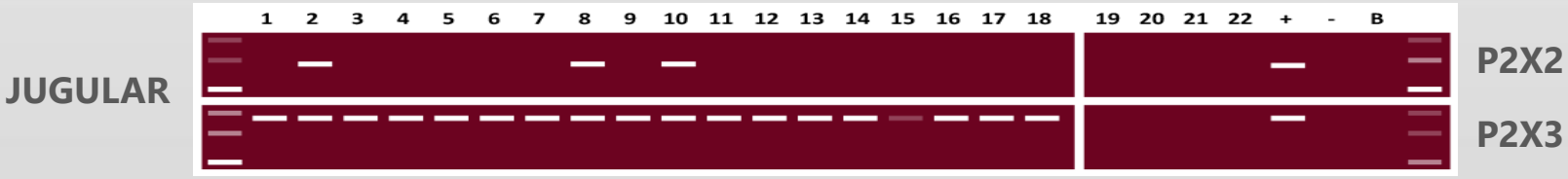
- Both P2X2 and P2X3 channels are expressed in taste buds[1]
- Almost all nerve in the fungiform papillae are double stained for P2X2 and P2X3[1]
- Knockout of both P2X2 and P2X3 is required for taste loss in KO mouse[2]

[1]Ishida et al 2009; [2]Finger et al 2005

P2X3 is Linked to Cough Hypersensitivity



Jugular C-fibers (expressing predominantly P2X3) innervating upper airways transmit cough sensitization signals to CNS



Kwong et al 2008: Single-cell RT-PCR analysis of 22 lung specific jugular neurons

BLU-5937: High Selectivity for P2X3 Provides Proof of Concept in Preclinical Models

Overview of Key Preclinical Studies with BLU-5937

Cell-based FLIPR assay	DRG neuron sensitization	Guinea pig cough model	Rat taste model
>2000 more selective for hP2X3 vs. hP2X2/3	Blocks ATP mediated neuronal sensitization	Reduces cough at concentration blocking P2X3 but not P2X2/3	No effect on taste

BLU-5937:

Potential to reduce cough with no taste effect at targeted therapeutic dose

BLU-5937: Highly Selective for Human P2X3 Receptors

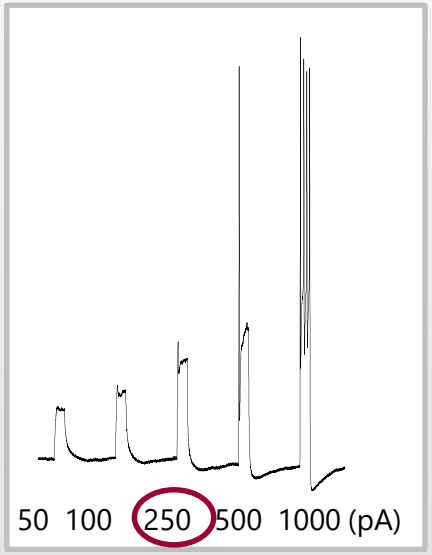
BLU-5937	hP2X3 (IC_{50})	hP2X2/3 (IC_{50})	Selectivity ratio
α,β-me ATP 3 μM	11 nM	>30 μM	**>2000**
α,β-me ATP 30 μM	13 nM	>30 μM	**>2000**

Cloned hP2X3 and hP2X2/3 channels expressed in HEK295 cells; (Ca^{2+} FLIPR)

- Potent antagonist of hP2X3
- Highly selective for hP2X3 vs hP2X2/3
- Not ATP-competitive
- No inhibition of other P2X channels
- Not agonist/antagonist of 159 targets tested

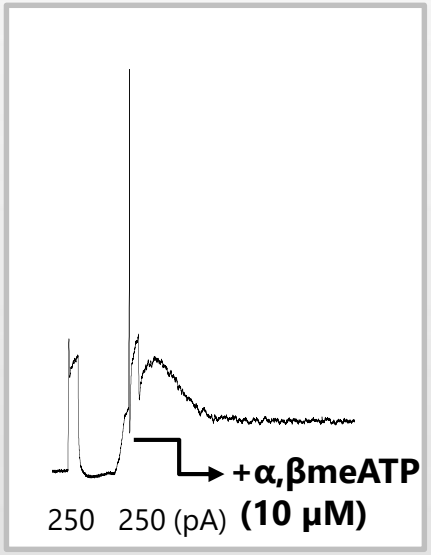
BLU-5937 Blocks ATP-Mediated DRG Neuron Sensitization

1. Inject current



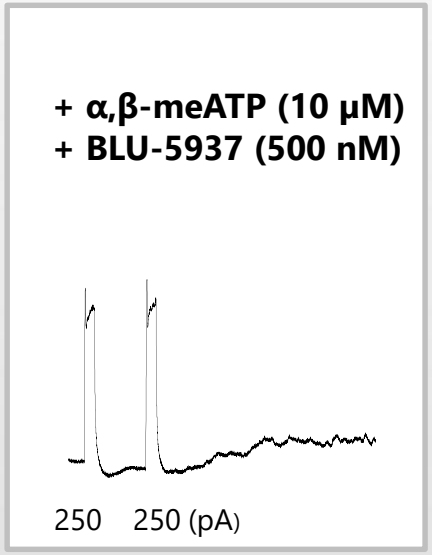
Sub-threshold current

2. Sensitize with ATP



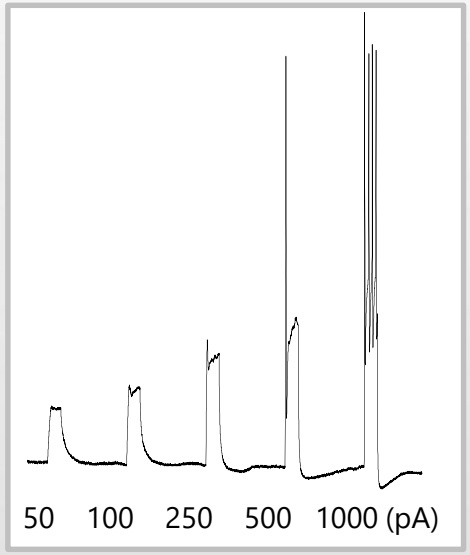
Neuron sensitized

3. BLU-5937



ATP-mediated sensitization blocked

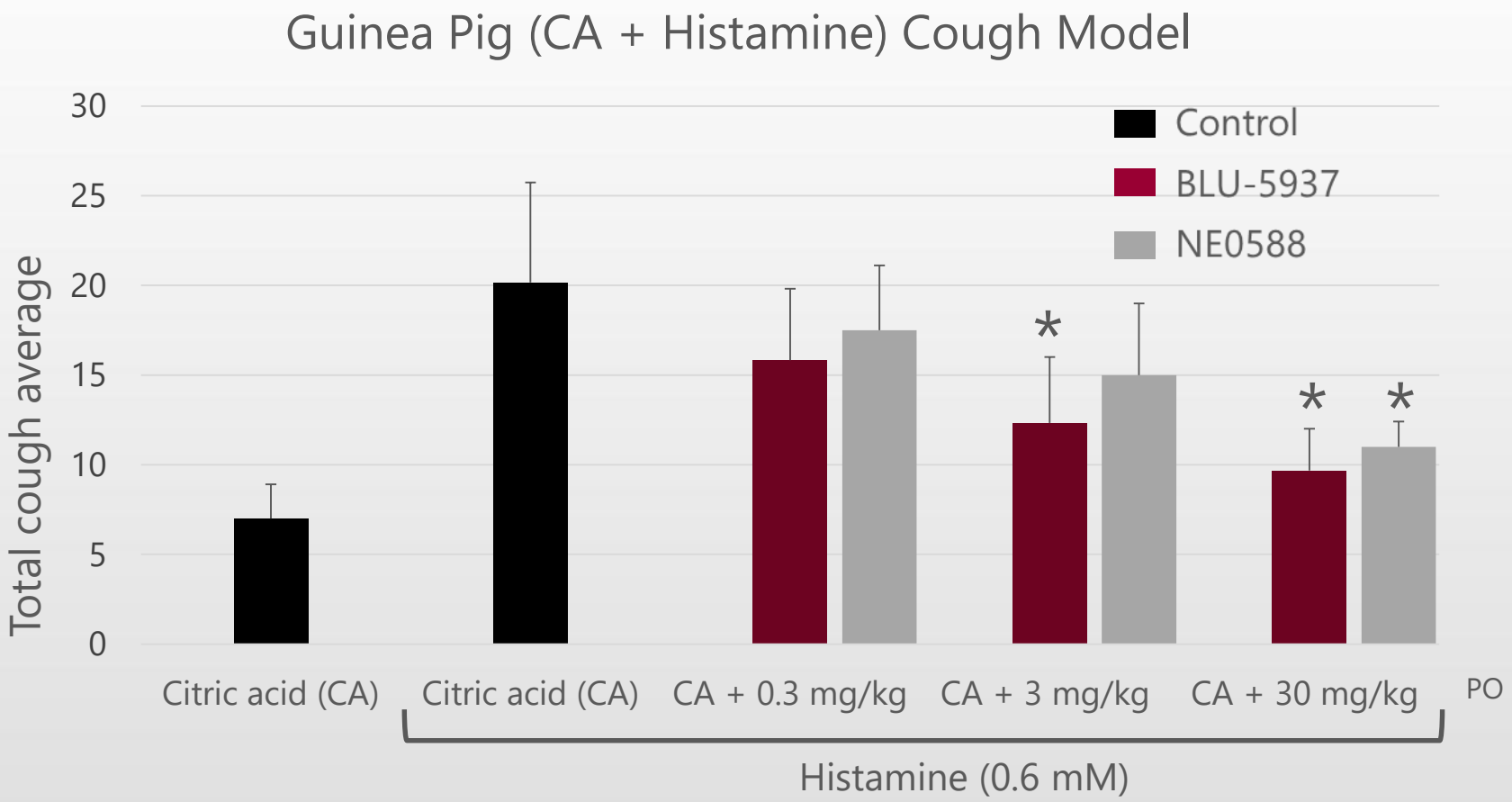
4. Control washout



Reversibility of inhibition

Using current mode, patch-clamp recording in selected P2X3 expressing DRG neurons from rat

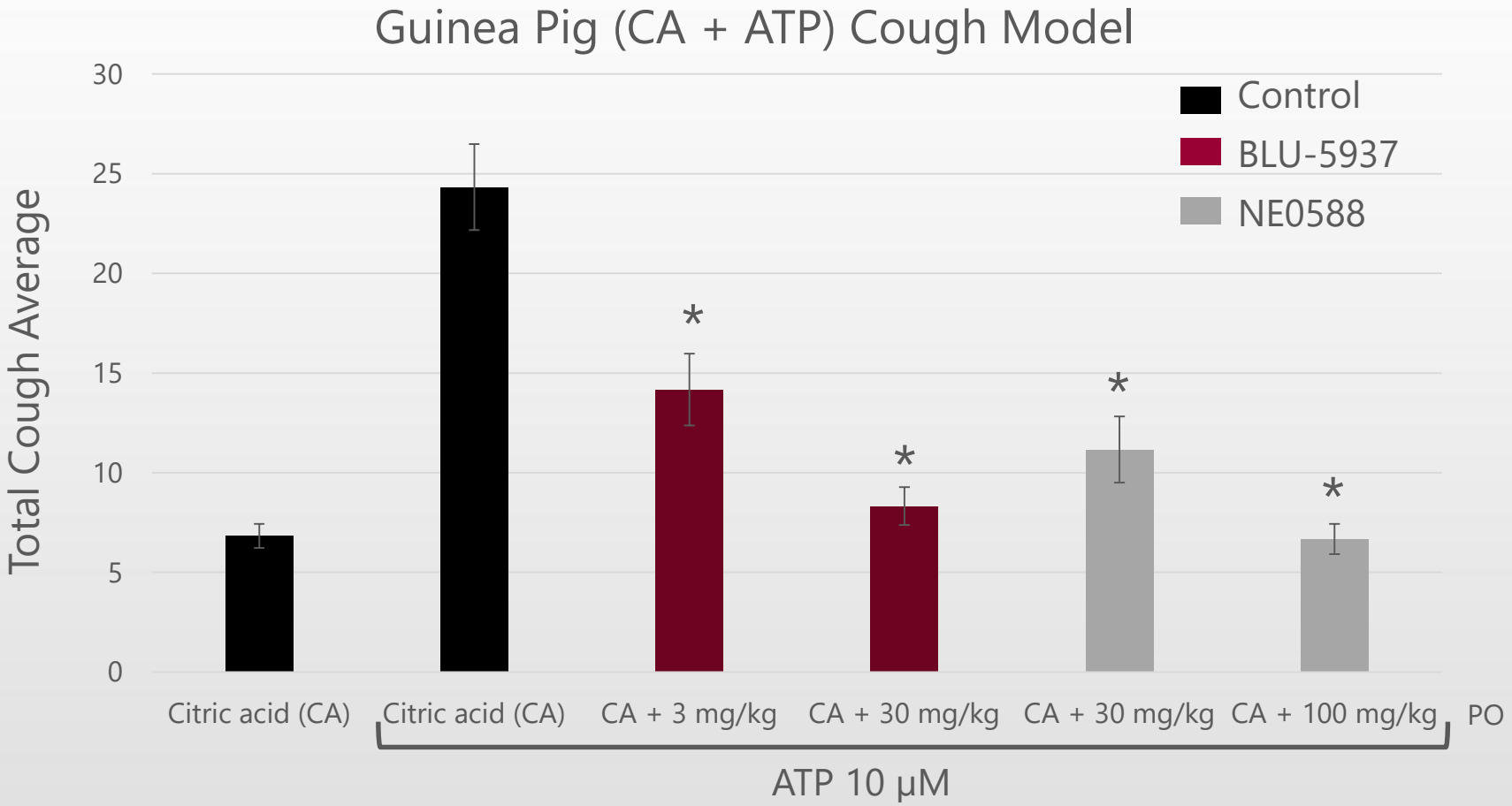
BLU-5937 Reduces Histamine Induced Cough Hypersensitivity



Treatments (control, BLU-5937, NEO588) were administered orally 2 hours prior to tussive agent exposure: citric acid (0.1 M, aerosol) and histamine (0.6 mM, aerosol); n=6 / group; *p<0.05 vs (CA + histamine) ; NEO588 (gefapixant)

BLU-5937 shows similar anti-tussive effect as NE0588, a non-selective gpP2X3 antagonist, at concentration that blocks P2X3 but not P2X2/3

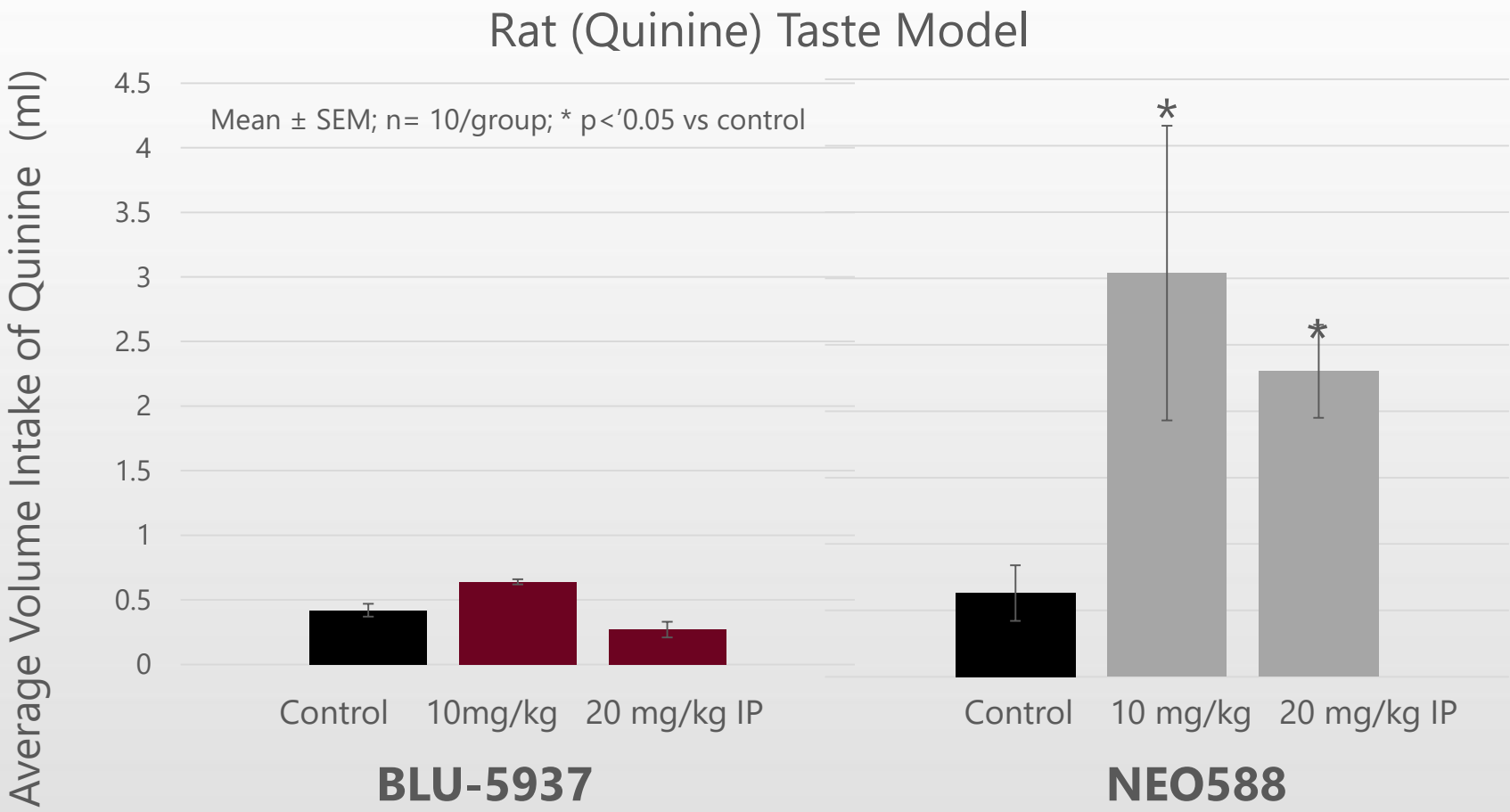
BLU-5937 Reduces ATP Induced Cough Hypersensitivity



Treatments (control, BLU-5937, NEO588) were administered orally 2 hours prior to tussive agent exposure: number of coughs measured for 15 min post-challenge; citric acid (0.1 M, aerosol) and ATP (10 µM, aerosol); n=6 /group; * p=0.01 vs (CA + ATP)

BLU-5937 shows similar anti-tussive effect as NE0588, a non-selective gpP2X3 antagonist, at concentration that blocks P2X3 but not P2X2/3

BLU-5937 has no Effect on Taste in Rat Model



No taste effect

A weakly selective antagonist for rP2X3, inhibits taste

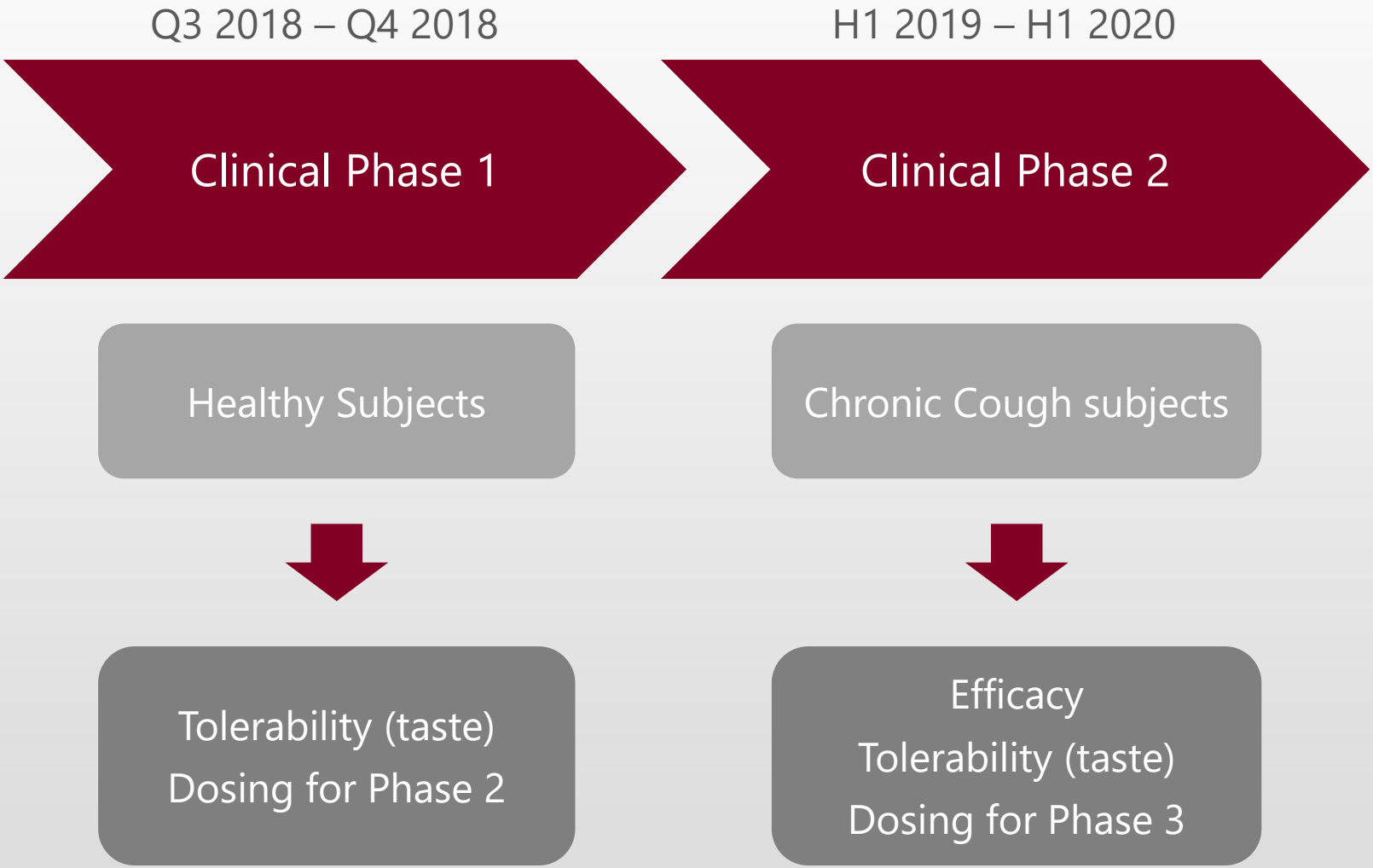
Treatments (control, BLU-5937, NEO588) were administered ip: animals were water-fasted overnight and presented with one bottle water and quinine (0.3mM) at T_{max} ; volume of liquid consumed measured for 15 minutes; n=10 / group

BLU-5937: Drug-like Characteristics

- Good oral bioavailability
- Good metabolic stability in human hepatocytes or liver microsomes
  - Dose regimen predicted in man: twice a day (BID)
- Does not cross blood-brain barrier
  - No adverse effect on general behavior / neurological function in rat
- High safety margin in preclinical toxicity studies (rat & dog)
  - Main clinical sign: emesis observed in dogs at high doses (≥ 300 mg/kg/day)

BLU-5937 characteristics and animal proof of concept support moving into clinical studies

BLU-5937: Clinical Plan to Phase 2 Proof-of-Concept



BLU-5937: Conclusions

- Potent and highly selective hP2X3 homotrimeric receptor antagonist
- Reduces histamine and ATP-induced cough sensitization in the guinea pig cough model through P2X3 homotrimeric receptor inhibition
- No effect on taste perception at concentration that fully block P2X3 homotrimeric receptors in rat behavioral taste model
- It has excellent drug-like characteristics

BLU-5937 has the potential to inhibit cough hypersensitivity without affecting taste perception in chronic cough