Dose-Ranging of The Selective GluN2B Allosteric Inhibitor Ifenprodil Illustrates Near-Complete Cough Suppression Within The Therapeutic Index

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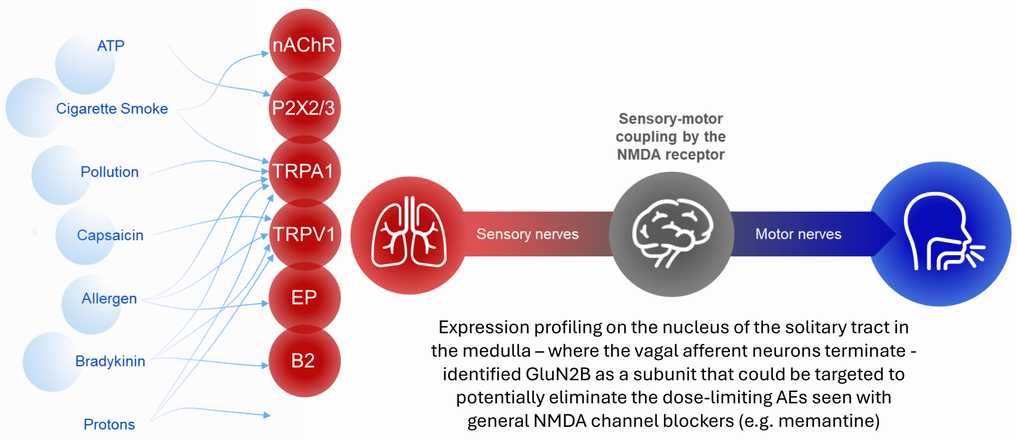
Introduction

Irrespective of the receptors in the lungs or trachea that are engaged by triggers of cough, all these peripheral events result in depolarization of the vagal afferent neurons sending a signal to the brain. Neuronal tracing and functional imaging studies have located the of these sensory netermination sites urons to the nucleus of the solitary tract (nTS) and the paratrigeminal nucleus (Pa5) in the medulla. The uniquely sustained and high-frequency signals from these afferent neurons are transmitted via glutamate release across synapses to N-methyl-D-aspartate (NMDA) receptors on neurons leading to the cough center in the brain. NMDA receptors are the key node of signal transduction from heterogeneous peripheral inputs, culminating in depolarization of descending efferent motor neurons that cause coughing. To understand which subunits of the hetero-tetrameric NMDA receptor were responsible for this transmission, we microdissected and expression profiled the nTS region of the brain in Guinea pigs (GP) leading to the observation that the GluN2A subunit of the NMDA receptor is not expressed, whereas the remaining GluN2 subunits are expressed. This presents an opportunity to identify selective inhibitors to certain receptor subtypes to attempt to reduce the well described dose-limiting adverse events (AEs) of general non-selective NMDA receptor blockers. We identified ifenprodil as a molecule with >200x selectivity for GluN2B vs. the other GluN2 subtypes. Ifenprodil has a well-established human safety record and well-defined no adverse event levels (NOAEL) in multiple pre-clinical models after long-term repeat dosing. We previously showed that a 1.5 mg/kg oral dose of ifenprodil reduced cough count in the GP model (1M citric acid exposure) by 40% ($p<0.01$) and resulted in an almost doubling of the time to cough ($p<0.05$). This translates to a human equivalent dose (HED) of ~20 mg. Based on these promising preclinical results, an open-label human Phase 2a trial was performed at this dose (20 mg orally three times a day) in 20 patients with idiopathic pulmonary fibrosis (IPF) and associated refractory chronic cough (RCC). 24-hour cough count was measured using the VitaloJAK® device at 12 weeks. There was a geometric mean cough count reduction of ~40% ($p<0.01$), a 37.4% improvement in the visual analog score (VAS; $p<0.001$), and improvements on the Leicester Cough Questionnaire ($p<0.05$). For reference, the single largest and longest running IPF-RCC placebo arm (in a gefapixant trial) had an ~8% geometric mean cough count reduction. A post-hoc responder analysis using integrated historical placebo controls from all prior IPF-RCC trials illustrates significance across all response groups (Figure 1a). The drug had no safety concerns and was well tolerated in our study. This profile promises the known anti-tussive effects of the non-selective NMDA receptor blockers without the neuropsychiatric side effects that have been shown to be dose limiting with these compounds. We performed a dose-ranging study in GPs to identify the maximal suppression of the cough reflex within the therapeutic index and to inform the clinic dose arms of our upcoming Phase 2b clinical trials in RCC.

All Cough Triggers Signal Through the NMDA Receptor in the Brain

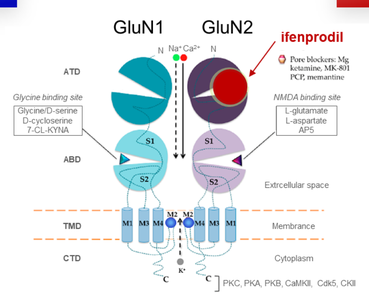


Expression profiling on the nucleus of the solitary tract in the medulla – where the vagal afferent neurons terminate - identified GluN2B as a subunit that could be targeted to potentially eliminate the dose-limiting AEs seen with general NMDA channel blockers (e.g. memantine)

Ifenprodil is GluN2B Subunit-Selective and Reduces High-Frequency Firing

Selective for GluN2B

- An allosteric GluN2B selective inhibitor not a general channel pore blocker
- >200x selectivity for GluN2B over other NMDA receptor members



Reduction of High-Frequency Firing

- The neuronal firing in cough is intense and long-lasting
- Blocks channel function just enough to stop this sustained high-frequency signal transmission

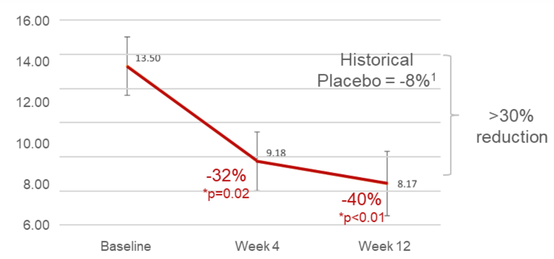
Results

Increasing the dose of ifenprodil reduced coughing in the GP model in a dose-responsive manner. The median cough count in controls (± interquartile range or IQR) is 14 (11, 17) vs. after 30 mg/kg ifenprodil treatment being 1 (0.25, 5.5) with $p=0.036$. The cumulative number of coughs was 0, 0, 1 and 1 in 4 of 6 animals treated with 30 mg/kg ifenprodil. Statistically significant reductions in coughing were also seen at 10 mg/kg. There was no respiratory depression observed, a concern clinically, and an issue seen preclinically with both codeine and baclofen (Figure 1b). The GP equivalent NOAEL dose is 37 mg/kg (based on 52-week rat GLP toxicology data) translating to an HED of ~8 mg/kg or a total single daily dose of ~480 mg.

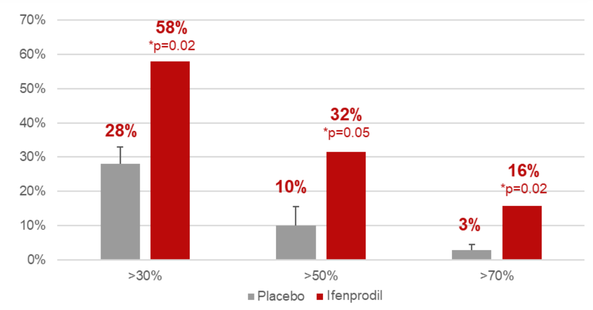
Our Phase 2a Results in IPF-RCC and Historical Control Responder Analysis

Separation Between Ifenprodil and Integrated Placebo Data from All Historical IPF-RCC Trials

Geometric Mean Cough Count Reduction
24hr Cough Frequency

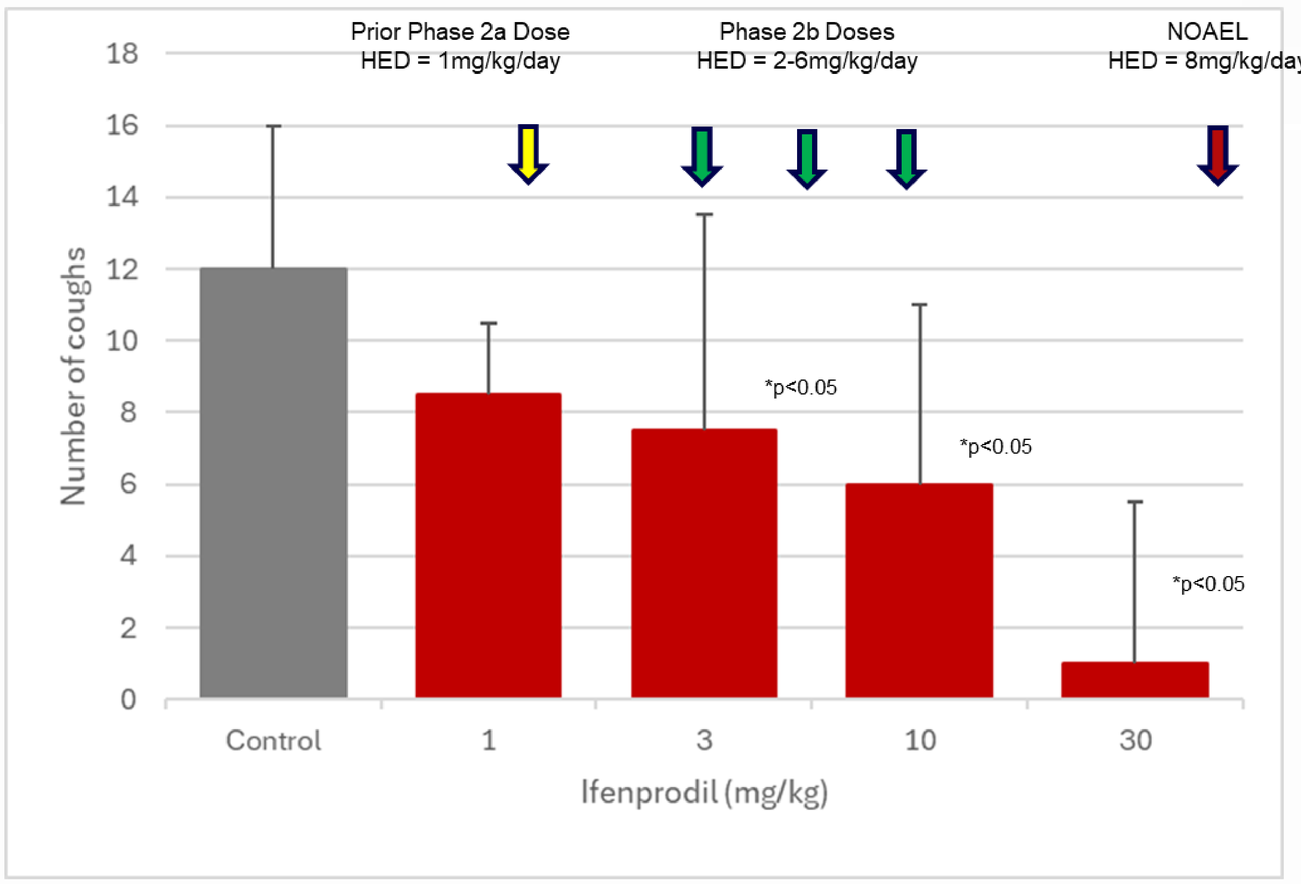


Post-Hoc Integrated Responder Analysis[2-4]
Patients Achieving Threshold Reductions in 24-hour Cough Frequency



[1]Geometric mean cough count reduction in largest available placebo data set in IPF-RCC (n=38) at latest time-point post-dosing (14-days) from Merck Gefipixant trial by Martinez JM *et al. Pulm Ther*. 2021;7(2):471-486.; [2]Martinez JM *et al. Pulm Ther*. 2021;7(2):471-486; [3]Birring SS *et al. Lancet Respir Med*. 2017;5(10):806-815; [4]Trevi Corporate Presentation, April 2023.

Pre-Clinical Dose-Ranging Establishes GluN2B as the Major Subunit Actor and Establishes Phase 2b Dosing Regimen



Phase 2b Clinical Development Plan

Placebo n=60 RCC patients

Low Dose (2 m/kg/day) n=60 RCC patients
(4-8x safety margin)

Middle Dose (4mg/kg/day) n=60 RCC patients
(2-4x safety margin)

High Dose (6mg/kg/day TID) n=60 RCC patients
(1.5-3x safety margin)

Methods

GPs were dosed intraperitoneally with ifenprodil at 0, 1, 3, 10 and 30 mg/kg followed 30' later by 0.1M aerosolized citric acid for 10' and 5' later by 0.3M citric acid for 10' followed by a 5' washout. Cumulative coughs were measured using a Biopac acquisition system and Acknowledge software.

Conclusions

NMDA receptor engagement is essential to the initiation of cough, and additionally, that the antitussive mechanism will be agnostic to peripheral stimulus. Increasing the doses of ifenprodil in our Phase 2b clinical trials in RCC will likely dramatically improve the efficacy while staying well within the therapeutic index.

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