Mechanistic and Therapeutic Evaluations of Chronic Cough

A Thesis Submitted to the University of Manchester for the Degree of Doctor of Philosophy in the Faculty of Medical and Human Sciences.

2014

Dr Rayid Abdulqawi

School of Medicine

List of Contents

List of Tables

List of Figures

List of Abbreviations

CXR	Chest X Ray
ACEI	Angiotensin Converting Enzyme Inhibitor
COPD	Chronic Obstructive Pulmonary Disease
NTS	Nucleus Tractus Solitarius
RAR	Rapidly Adapting Receptor
SAR	Slowly Adapting Receptor
SP	Substance P
CGRP	Calcitonin Gene Related Peptide
ATP	Adenosine Triphosphate
5-HT	5-hydroxytriptamine
TRP	Transient Receptor Potential
ASIC	Acid Sensing Ion Channel
ADP	Adenosine Diphosphate
PGE2	Prostaglandin E2
siRNA	Silencing Ribonucleic Acid
mRNA	Messenger Ribonucleic Acid
DRG	Dorsal Root Ganglia
CFA	Complete Freunds Adjuvant
4-HNE	4-hydroxy-2-nonenal
NaV	Voltage-gated Sodium
CNS	Central Nervous System
TTX	Tetrodotoxin
shRNA	small hairpin Ribonucleic Acid
NGF	Nerve Growth Factor
BDNF	Brain-derived Neurotrophic Factor
TrK	Tropomyosin-related Kinase
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate Receptor
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
EPSP	Excitatory Postsynaptic Potentials
COX	Cyclooxygenase
HDM	House Dust Mite
ETS	Environmental Tobacco Smoke
MRI	Magnetic Resonance Imaging

EMG	Electromyography/Electromyogram
VAS	Visual Analogue Scale
CQLQ	Cough Quality of Life Questionnaire
MID	Minimal Important Difference
LCQ	Leicester Cough Questionnaire
VGSC	Voltage Gated Sodium Channel
ECG	Electrocardiogram
SD	Standard Deviation
GEE	Generalised Estimating Equations
CI	Confidence Interval
UTC	Urge to Cough
IQR	Interquartile Range
BMI	Body Mass Index
eGFR	Estimated Glomerular Filtration Rate
HIV	Human Immunodeficiency Virus
FBC	Full Blood Count
U&E	Urea & Electrolytes
LFT	Liver Function Test
GROCS	Global Rating of Change Scale
ITT	Intention To Treat
PP	Per Protocol
BBB	Blood-brain Barrier
BAL	Bronchoalveolar Lavage

Abstract

Mechanistic and Therapeutic Evaluations of Chronic Cough
The University of Manchester
Dr Rayid Abdulqawi
Submitted for the Degree of Doctor of Philosophy
January 2014

Introduction: Patients with chronic cough suffer significantly from impaired quality of life. However, safe and well-tolerated effective treatments remain a major unmet clinical need. Afferent pathways of the cough reflex are almost entirely mediated via the vagus nerve. The vagal afferents relay information to second-order neurons in the nucleus tractus solitaries (NTS) in the brainstem. Hyper excitability of the cough reflex pathways is thought to be the main mechanism in chronic cough. Various ion channels are involved in the transduction of cough signals and generation of action potentials. The potential mechanistic and therapeutic role of neuronal ion channels warrants clinical evaluation.

Methods: I recruited patients with refractory chronic cough into three separate clinical trials of ion channel antagonists (NaV, P2X3, and NMDA). The primary outcome measure was objectively recorded cough frequency using the ambulatory acoustic recording device, VitaloJAK™. In the first trial, I investigated the effect of the pan NaV blocker, lidocaine, administered via nebulization compared to placebo. To enhance blinding, I also included treatment with lidocaine throat spray. In the second trial, I enrolled patients into a randomised study of the P2X3 antagonist, oral AF-219, vs. placebo. In the third trial, I explored the feasibility of evaluating memantine (use-dependent NMDAR antagonist) in chronic cough.

Results

- Objective cough frequency after treatment with nebulised lidocaine was not significantly different from placebo. A small (-19%, p=0.026) difference in cough rate was seen after lidocaine throat spray compared with placebo.
- The P2X3 antagonist, AF-219, compared with placebo markedly and significant improved both daytime objective cough frequency (-75%, p<0.001) and patient reported outcomes.
- Memantine was associated with significant intolerable side effects and there was no meaningful reduction in either cough frequency or CQLQ scores.

Conclusions: Nebulised lidocaine is not an effective anti-tussive. This could be because of the deposition site within the airways. Nebulised particles are of small sizes that allow them to be deposited primarily within the distal small airways. Stimulation of bronchial rather than pulmonary C-fibres has been shown to initiate cough. More potent and sensory neurons-selective blockers of NaV may prove to be effective and safe in improving cough. P2X3 channels appear to contribute to the hyper excitability of the afferent pathways mediating cough and their antagonists represent a promising new class of anti-tussives. NMDARs-mediated central sensitisation does not seem to play an important role in chronic cough and NMDAR antagonists are poorly tolerated.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

All the work contained in this thesis is my own, unless otherwise specified.

Copyright Statement

Dedication

To my parents who raised me up.

To my amazing wife, Randa, and my lovely kids, Rafa, Omar and Maice.

Acknowledgements

I would like to thank my supervisors Dr Jaclyn Smith and Professor Ashley Woodcock for their ongoing support and guidance during the three years of my PhD study.

I am grateful for:

Danielle Birchall (nee Yuill) for her help with submitting approvals for the regulatory authorities.

Kimberley Holt and Rachel Dockry for providing assistance in conducting the thesis studies.

Members of the Manchester Cough Research team for processing and counting the cough recordings.

Dr Julie Morris and Gary Layton for their guidance in conducting the statistical analysis in chapters 2 and 3, respectively.

And finally, I am indebted to all the patients who volunteered to participate in the studies.

Funding Disclosure:

My PhD was funded through funds from GlaxoSmithKline, Afferent Pharmaceuticals and profits from Dr Jacky Smith's other commercial trials.

The lidocaine study (chapter 2) was an investigator-led study funded by GlaxoSmithKline.

Afferent Pharmaceuticals funded and sponsored the study of P2X3 antagonism (Chapter 3).

The fund for the pilot study of Memantine (Chapter 4) was obtained from the Medical Research Council. This was part of a fellowship award to Dr Emma Hilton who finished her PhD in 2012 before being able to initiate the study.

The Author

I graduated from Marmara University Medical School (Istanbul, Turkey) in 2003. Following that I moved to the UK to pursue my postgraduate training. I completed my Foundation Year 2 and Core Medical Training in the West Midland Deanery. In 2008, I started my Higher Specialty Training in Respiratory and General Internal Medicine. In the period of 2008 to 2010, I completed 2 years of training and successfully passed the Specialty Certificate Examination in Respiratory Medicine. In August 2010, I took time out of my training programme and I registered with the University of Manchester to study for a three-year PhD degree (Sept 2010 – Sept 2013). I am now back in the West Midland and I am working in the University Hospitals Birmingham NHS Foundation Trust to complete my Respiratory and General Internal Medicine training.

CHAPTER 1

Introduction and Literature Review

1.1 Background

Cough is a vital reflex mechanism defending the airways and lungs against noxious and unpleasant stimuli such as foreign bodies and secretions. It is characterised by a deep inspiration followed by closure of the glottis and expiration; forceful opening of the glottis gives rise to the explosive sound of cough [1]. Patients present to their doctors with a complaint of cough more than with any other symptom [2]. Although most coughs are self-limiting and follow upper respiratory infections, cough is a common symptom of many, if not all, respiratory illnesses such as lung cancer, chronic obstructive pulmonary disease (COPD), asthma, and lung fibrosis.

Diagnosis and management of chronic cough (> 8 weeks) can be challenging. Cough variant asthma, eosinophilic bronchitis, rhinosinusitis, and gastro-oesophageal reflux are long believed to be the causes of chronic cough in the majority of patients [1, 3-5]. Such patients are typically non-smokers, not on angiotensin converting enzyme inhibitors (ACEI) and have normal chest x-ray (CXR) and spirometry. However, despite extensive investigations and treatment trials following nationally and internationally published protocols [1, 6], a significant proportion of chronic cough patients attending specialist clinics remain without identifiable aetiology or refractory to treatments targeted at cause(s) of cough [7, 8].

Patients attending chronic cough clinics are predominantly female (~65-75%) [4, 7, 9]. Healthy females have a more sensitive cough reflex compared to males [10]. Likewise, women with chronic cough have a lower threshold for experimentally induced cough [11, 12] and have higher spontaneous cough counts compared to men [12]. The exact mechanistic explanation for this gender effect remains undetermined.

12% of people who responded to a postal questionnaire in Yorkshire were suffering from weekly to daily cough for at least 2 months and cough was troublesome in 7% [13]. Chronic cough impacts significantly on quality of life; psychosocial aspects are impaired the most [14, 15]. A significant proportion of sufferers report anxiety, depression, frustration, fatigue, sleep disturbance and stress incontinence [15]. Successful treatment of cough results in improvement of associated depression [16].

Treatment for cough is a significant unmet clinical need. A huge amount of money (£101.7 million in 2011 in the UK [17]) is spent on buying over the counter cough medicines with little, if any, evidence of their efficacy and safety [18]. Dextromethorphan was the last treatment licensed for cough more than 50 years ago.

It has been withdrawn in children <5 years old in the USA, due to lack of evidence of efficacy and safety concerns. Treatment options for chronic cough are limited. Morphine and gabapentin therapy have been associated with improvements in quality of life, but there is no objective evidence of their efficacy in reducing cough in terms of 24 hour ambulatory cough monitoring [19, 20]. The long term efficacy, tolerability and safety of both is also questionable. There is a lack of specific treatments targeted against afferent nerves mediating the cough reflex. If such treatments prove to be effective, they may be safer and better tolerated compared to centrally acting agents.

1.2 Neurophysiology of Cough

To date, the neurophysiology of cough has mainly been studied in experimental animal models. These have shown some notable differences between species in both anesthetised and conscious animals. Afferent pathways of cough are almost entirely mediated via the vagus nerve [21]. Consistent with this, heart-lung transplant patients have no vagal innervation in the tracheobronchial tree below the anastomosis site, and in the majority of subjects, cough cannot be experimentally evoked by inhalation of low chloride solution [22]. Furthermore, irritant induced cough is not lost in patients with cervical spinal cord injury [23]. Pulmonary vagal afferents relay sensory information to second-order neurons in the nucleus of the solitary tract (NTS) in the brain stem [24]. Efferent nerves then activate muscles of the diaphragm, larynx and chest wall resulting in a cough motor response. In man, airway irritant stimuli are also accompanied by the perceived sensation of urge to cough, which happens as a result of the sensory information being relayed to the cortical areas of the brain [25] (see section on urge to cough 1.5.1).

1.2.1 Airway Afferent Nerves and their Role in Cough

The different subtypes of vagal afferent nerves in the airways and their contribution to cough are summarised below.

Intrapulmonary Rapidly Adapting Receptors (RARs)

RARs are best described as intrapulmonary stretch sensors that adapt quickly to sustained lung inflations [26, 27]. Their conduction velocity is in the range of Aβ fibres (14-23 m/sec), which suggests that they are myelinated. In guinea pigs, their cell bodies have been shown to be located in the nodose ganglia [28]. Peripheral nerve endings of RARs are thought to be within or just underneath the epithelium [26, 27]. Intra-pulmonary RARs do not play a direct role in cough as during normal breathing, they have baseline activity, and yet despite this there is no coughing [26]. Stimuli resulting in activation of these fibres include lung collapse/deflation, pulmonary embolism/oedema and airway smooth muscle contraction [29-31]. Upon activation, responses such as bronchoconstriction and mucus production have been reported [27, 32].

There is no conclusive evidence of RARs inducing cough, but they might play a role in facilitating cough initiated by other pathways [33]. On the one hand, vagal cooling (7-8 °C), which abolishes the activation of RARs but maintains the responsiveness of C-fibres, inhibits the cough reflex in animals [34]. On the other hand, direct stimuli of RARs with agents such as methacholine and leukotriene C4 have not been shown to significantly affect the cough reflex or evoke cough in humans [35, 36]. Chemical stimuli such as capsaicin and bradykinin, which are well recognised to evoke coughs in animals and humans, do not directly activate the RARs, but indirectly result in their activation through their tissue effects such as bronchospasm and hypersecretion of mucus [26, 37].

Slowly Adapting Receptors (SARs)

SARs are myelinated intrapulmonary stretch sensors located in the smooth muscle and conduct action potentials in the range of Aβ fibres (14-23 m/sec) [21]. Their activity peaks at the end of inspiration and as a result has been suggested to lead to the termination of inhalation and start of exhalation (Hering-Breuer reflex) [38]. In contrast to RARs, they slowly adapt to lung inflation and cause bronchodilation rather than bronchospasm by attenuating the parasympathetic drive of the airways [38].

The role for SARs in cough is doubtful [33]. Ammonia-induced cough in rabbits was not associated with enhanced activity of SARs [39]. However, in an experimental study in rabbits, sulphur dioxide inhibited the SARs mediated reflex (Hering-Breuer reflex) and significantly inhibited coughs elicited by mechanical stimulation of the airways and ammonia inhalation, suggesting a role for SARs in modulating cough [40].

Bronchopulmonary C-Fibres

C-fibres are the most abundant type of airway vagal afferents [31, 41, 42], distributed widely in the epithelium of both large and small airways [26, 31, 43]. Axons of C-fibres are unmyelinated, and have a conduction velocity of <2 m/sec [31]. They are directly activated by a variety of noxious, irritant chemical stimuli such as capsaicin and bradykinin, and therefore best defined as “nociceptors” [31, 42, 44]. Unlike RARs and SARs, C-fibres are not activated by low-threshold mechanical stimuli [28] . Both peripheral and central terminals of C-fibres have been shown to stain positive for neurochemicals such as substance P (SP), neurokinins, and calcitonin gene-related peptide (CGRP) in some animal species [45, 46].

Two subtypes of C-fibres have been described: pulmonary and bronchial. This subdivision is based on differences in the location of their peripheral terminals, cell body location, neuropeptide expression, sensitivity to different stimuli and elicited responses [28]. Peripheral terminals of bronchial C-fibres receive blood supply from the systemic circulation via bronchial arteries (bronchial circulation), whereas peripheral terminals of pulmonary C-fibres are supplied by “pulmonary circulation” [47, 48]. The ganglionic origin of C-fibre cell bodies from jugular or nodose ganglia is also an important characteristic of this subdivision of C-fibres into pulmonary and bronchial subtypes in guinea pigs [49]. Jugular and nodose ganglia have different embryonic origin; jugular ganglia originate from neural crest tissue (as the dorsal root ganglia), whereas nodose ganglia originate from placodal tissue. Jugular derived C-fibres are located both within the main extrapulmonary airways and the small airways within the lung parenchyma. In contrast, C-fibres arising from the nodose ganglia are distributed mainly in the intrapulmonary small airways and lung parenchyma. Both types (jugular and nodose C-fibres) are activated by capsaicin, bradykinin and acid, but only nodose C-fibres are responsive to adenosine, adenosine triphosphate (ATP), and 5-hydroxytriptamine (5-HT) [49]. Furthermore, the majority of jugular C-fibres contain neuropeptides such as SP, CGRP, and neurokinin A, unlike the majority of nodose C-fibres [49]. Interestingly, in the anaesthetised cat, stimulation of pulmonary C-fibres by IV capsaicin or phenylbiguanide (agonist of 5HT3 receptors) has been shown to abolish the cough reflex [50].

There is good evidence that C-fibres play an important role in mediating cough. Airway C-fibres express TRPV1 receptors [51] and bradykinin receptors [52]. Stimuli such as capsaicin, through its agonist action on TRPV1 receptors [51], and bradykinin readily result in cough when inhaled in both humans [53] and conscious animals [28, 31, 54]. In guinea pigs at least, airway C-fibres have been shown to be positive for neurokinins on immunohistochemical tests [55]. Neurokinin antagonists for NK1 and NK2 receptors [56], and TRPV1 antagonist capsazepine have demonstrated anti-tussive effect in animals [57], but no efficacy has been shown in humans, either healthy [58] or with cough [59, 60]. Patients with chronic cough have heightened cough reflex sensitivity to inhaled capsaicin [61], and have been shown to have upregulated TRPV1 receptors in the airways [51].

On the contrary, in anaesthetized guinea pigs, topical application of capsaicin and bradykinin to the larynx and trachea fails to evoke coughing [30]. Possible explanations for this are: (1) C-fibres induced coughs are under the influence of cortical pathways

which are suppressed during general anaesthesia, (2) general anaesthesia augments the inhibitory effect of pulmonary C-fibres [26].

The "Cough Receptor"

The existence of a subtype of airway vagal afferents that are important in mediating the cough reflex and distinct from those previously described has been proposed by Canning et al, based on models of cough in anaesthetized guinea pigs [30]. Topical application of capsaicin or bradykinin, selective stimulants of C-fibres, onto the larynx or trachea did not elicit coughing. However, mechanical probing, electrical stimulation, and citric acid application on the same area resulted in cough. Following characterisation by electrophysiological recordings, it was concluded that these are thinly myelinated (Aδ) fibres, have an intermediate conduction velocity of 5 m/sec (between the unmyelinated C-fibres and the myelinated RARs) and have cell bodies located in the nodose ganglia.

These "polymodal" afferents are activated by low threshold mechanical stimuli, dust inhalation and acid [28, 30, 44]. Acid-induced activation of cough receptors is not altered by administration of the TRPV1 antagonist capsazepine [62], indicating that TRPV1 receptors play no significant role here. Acid sensing ion channels (ASICs) are believed to be primarily mediating the responsiveness to these stimuli [63]. Unlike C-fibres or RARS, cough receptors do not express TRPV1 or tachykinins [46, 64] and are unresponsive to capsaicin, bradykinin, smooth muscle contraction, or changes in airway pressure [28, 30]. A subtype of a Na-K-ATPase pump is thought to play a role in regulating the sensitivity of these afferents, which could provide a potential anti-tussive target in disease [65].

Morphological studies indicate that their peripheral terminals lie between the smooth muscle and the epithelium in the mucosa of larynx, trachea and major bronchi [65]. The exact location of the central terminals of the cough receptor afferents in the NTS of anaesthetized guinea pigs were identified by Canning et al. Glutamate antagonists microinjected to an area rostral and lateral to the obex inhibited the cough reflex evoked by citric acid applied to the trachea, but without an effect on any other respiratory reflex [66]. Subsequent retrograde labelling by injecting DiI dye into the trachea has stained the previously identified NTS area.

1.3 Transduction of Cough Stimuli

Ion channels on the nerve endings of cough vagal afferents result in membrane depolarisation through “generator potentials”, which develop when these fibres are exposed to different stimuli [67]. If the generator potential is large enough and reaches an amplitude threshold, it allows the opening of voltage-gated sodium channels (VGSCs), which play a fundamental role in the generation and conduction of action potentials [67]. The action potentials then travel along the nerve axons to the second order neurons in the brain stem where the cough centre is believed to be located.

In this section, I will provide a summary of the main airway nerve receptors relevant to this thesis and how targeting these receptors may be of therapeutic benefit in the treatment of chronic cough.

1.3.1 P2X Receptors

Structure and Function

P2X receptors belong to a family of ligand-gated purinergic membrane receptors [68, 69]. Purinergic receptors, first described in 1972 [70], are of two types: P1 and P2. P1 receptors are gated by adenosine molecules, whereas P2 are gated by ATP and its metabolite ADP [69]. P2 receptors are further divided into P2X cation channels and P2Y metabotropic G-protein coupled receptors, with several subunits in each (P2X1-7 and P2Y1, 2, 4, 6, 8, 12, 13, 14). The ion channel P2X receptors are gated only by extracellular ATP, while the P2Y receptors are gated by either extracellular ATP or ADP (Figure 1 and Figure 2). Functional P2X channels form subunit trimers (assembly of three subunits), either homotrimeric or heterotrimeric of different subunits [71, 72]. P2X3 receptors are either homotrimeric P2X3 or heteromeric P2X2/3 (containing both subunits 2 and 3). P2X3-containing receptors refer to P2X3 and/or P2X2/3 channels.

Although P2X receptors are widely distributed in different cells and tissues in the body, the homomeric P2X3 and the heteromeric P2X2/3 receptors are found to be expressed predominately on sensory afferent neurons, specifically on C and A delta types, transmitting noxious chemical and mechanical stimuli from somatosensory and visceral tissues [68, 71, 73]. In addition to the expression of P2X3 receptors in nerve terminals, presynaptic terminals also contain P2X3 receptors. Stimulation of presynaptic P2X3

receptors was shown to result in the release of excitatory neurotransmitters, including glutamate and neuromodulators, such as substance P [68].

Figure 1: Purinergic receptors classification

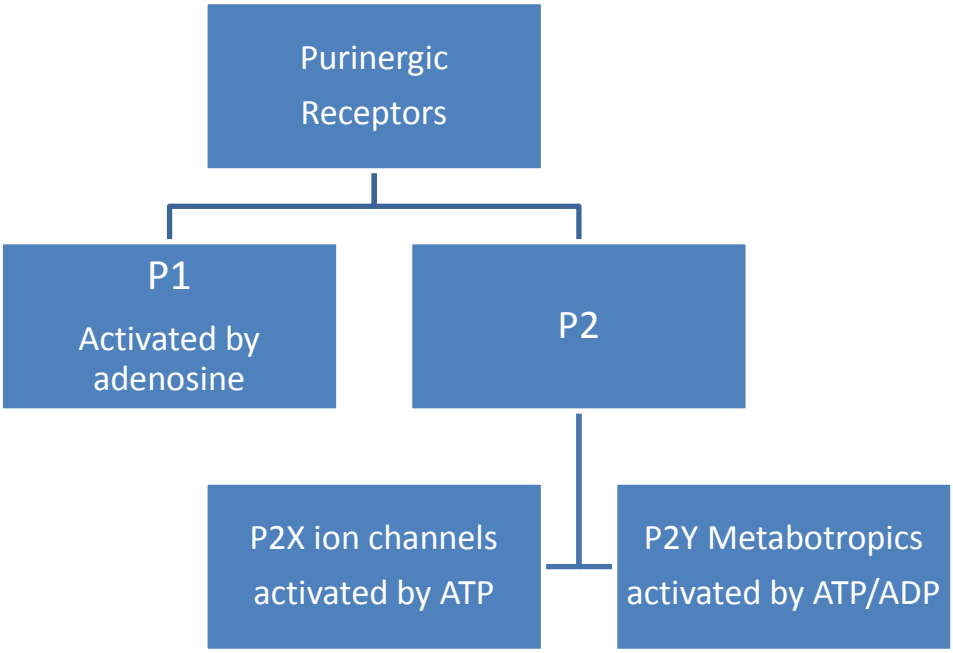
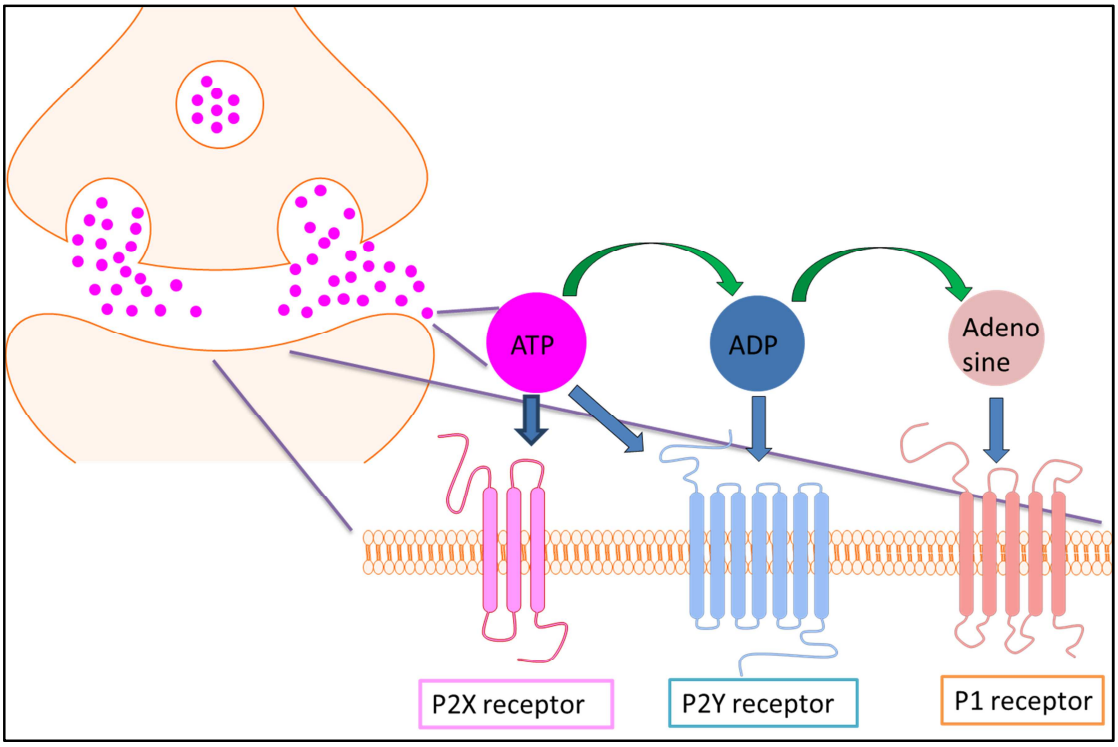


Figure 2: Purinergic transmission: receptor types and their ligands

(Adapted from [74])



Role of ATP

ATP is an endogenous, both intracellular and extracellular, nucleotide present in all tissues and cells [69]. In the presence of tissue damage and inflammation, levels of ATP are elevated [68]. ATP is shown to be a mediator of pain; this effect is enhanced in inflammation. When intraplantar ATP was administered into the hind paw of rats, pain related behaviours were noted, which was augmented by tissue inflammation induced by ultraviolet radiation and PGE2 [75]. ATP applied to a blister base in human volunteers elicited pain [76]. Likewise, compared to placebo, forearm skin of healthy volunteers became painful when ATP was applied using iontophoresis (a method of inoculating ATP into the skin without the use of a needle) [77]. This effect was attenuated when C-fibres were desensitised by prolonged local capsaicin application, suggesting that capsaicin sensitive afferent fibres are mediating the ATP-induced pain response. Inflammation of the forearm skin through exposure to ultraviolet radiation resulted in hyperalgesia and potentiation of the ATP induced pain [77].

Pain, Neuronal Hyper Excitability and P2X3

Various methods such as knockout mice, antisense oligonucleotides, silencing ribonucleic acid (siRNA), and selective antagonists have been used in experimental animal models of inflammation and nerve injury to study the contribution of P2X3 channels to neuronal hyper excitability. Evidence from pre-clinical studies supports a role for P2X3-containing receptors in mediating chemically and mechanically induced nociception and contributing to hyper excitability of primary afferent nerves. Intraplantar injection of formalin in animals such as mice results in pain by causing inflammation and this is observed as nocifensive behaviour, such as licking, biting or lifting of paw. This behaviour was noted to be markedly diminished in P2X3 knockout mice [78]. In the same study, it was observed that the threshold volume required to elicit bladder contractions was increased. Antisense oligonucleotide directed against P2X3 receptors (to reduce the translation of mRNA into proteins), given intrathecally to rats, inhibited the development and maintenance of hyperalgesia associated with chronic inflammation and nerve injury [79]. P2X3 siRNA, which blocks gene expression, also had anti-nociceptive properties in a rat model of neuropathic pain [80].

The expression of P2X3 receptors is subject to modulation by inflammation and nerve injury. The proportion of P2X3-immunoreactive dorsal root ganglia (DRG) neurons markedly increased after chronic nerve injury in an animal model [81] implicating a role

for P2X3 receptors in chronic sensitisation associated with conditions such as neuropathic pain [82]. Similarly, P2X3 & P2X2/3 proteins, assessed by western blot, were enhanced in a Complete Freund's adjuvant (CFA)-induced chronic inflammation model in rats [83]. In an experimental rat model of chronic neuropathic and inflammatory pain, a potent and selective P2X3, P2X2/3 antagonist, both via local and systemic administration, was successful in reducing pain sensitivity to both noxious and innocuous stimuli [84, 85]. The same antagonist did not alter the sensitivity in acute pain models, suggesting that different pathophysiological processes are involved in chronic sensitisation compared with acute nociception, and that modulation of P2X3 containing receptors is more pertinent to chronic neuronal hyper excitability [84].

P2X3 in the Airways

ATP has been shown to activate airway vagal afferent fibres. In 1996, Pelleg experimented with injecting ATP into the pulmonary circulation of anaesthetised dogs [86]. In this study, it was shown that pulmonary vagal fibres that were capsaicin sensitive and had a conduction velocity of 0.85 +/- 0.13 m/s (i.e. C-fibres) were activated by ATP [86]. In support of the role of P2X receptors in mediating the C-fibres activation by ATP, a P2X antagonist (PPADS) significantly decreased the response. Later work by Undem et al (2004) and Kwong et al (2008) similarly demonstrated that peripheral terminals of airway vagal afferent C-fibres fire action potentials in response to ATP and its non-hydrolysable form αβ-methylene ATP (P2X selective agonist). This effect was blocked by a P2X3, P2X2/3 selective antagonist [87]. Interestingly, the activation was shown only in the nodose C-fibres, but not the jugular C-fibres [49, 87]. While nodose C-fibres express P2X2/3 receptors, jugular C-fibres only express P2X3 receptors [87]. Stimulation of P2X3 channels results in small rapidly inactivating current whereas P2X2/3 channels are associated with a more sustained and larger depolarisation response [87]. This probably explains the lack of activation of jugular C-fibres by ATP as the membrane depolarisation here would fall below the action potential threshold.

An *in vivo* study of anaesthetised guinea pigs has shown that ATP and αβ-methylene ATP also activated intra-pulmonary RARs [30]. In contrast with the mechanism of histamine/methacholine induced activation of RARs (through smooth muscle contraction), the ATP effect was blocked by pre-treatment with the P2X receptor antagonist PPAD, but not by the smooth muscle relaxant isoproterenol, indicating a role for the P2X receptors in ATP induced excitation of RARs. However, extra-

pulmonary nodose-derived Aδ fibres (cough receptors) are unresponsive to ATP [30]. Therefore, the two main fibre types know to be involved in the initiation of cough, the jugular C-fibres and Aδ fibres are not activated by ATP.

The role played by P2X3 receptors in the regulation of cough has so far been rarely studied, with only a small number of investigations in animals and humans. Inhalation of ATP and αβ-methylene blue ATP failed to evoke cough in conscious guinea pigs in several studies [30, 88, 89]. Two human studies have investigated bronchoconstriction responses to inhaled ATP. One study observed coughing (although the amount of coughing was not quantified) induced by aerosolized ATP in both asthmatics and healthy volunteers [90]. The other study made no mention of ATP induced cough in the same patient groups [91]. To date, there has been good translation between the guinea pig model of cough and the agents that evoke coughing in healthy humans. However, if results from Basoglu's study are reproducible, this would suggest a fundamental difference in cough response in humans compared with guinea pig models of evoked cough, and draw into question the validity of current knowledge of cough obtained from experimental animal studies of purinergic mechanisms. Nonetheless, in guinea pigs, ATP augmented the cough response induced by citric acid, but not capsaicin, indicating a possible sensitising effect of ATP on Aδ fibres [88]. Furthermore, the sensitisation effect of histamine on citric acid induced cough in guinea pigs was mediated by endogenous ATP via its action on P2X receptors [92]. This is supported by the finding that P2X antagonists abolished the histamine induced sensitisation of the cough reflex.

1.3.2 TRPV1 Receptors

The TRPV1 receptor, which was first cloned from rats in 1997 [93], is an ion channel. It belongs to the vanilloid subfamily of the transient receptor potential (TRP) channels. Other described subfamilies of TRP channels are TRPA, TRPC, TRPML, TRPP and TRPM [94]. TRPV1 receptors are found in both Aδ- and C-fibre nociceptive sensory afferents [95] and are stimulated by a wide variety of noxious stimuli including heat (> 42°C), low pH and the chemical irritant capsaicin [93, 95, 96]. In cases of elevated hydrogen ions in the tissue, which could be associated with inflammation, these receptors are active at body temperature [96], indicating that TRPV1 receptors may contribute the heightened sensory afferents activity seen in some inflammatory diseases. Upon activation of the TRPV1 channel, cations (mainly calcium and sodium) flow down its concentration gradient into the cell [94].

A role for TRPV1 channels in mediating noxious stimuli in both physiological and pathological conditions has been shown in animal models [97]. Furthermore, the activity of TRPV1 channels has been shown to be enhanced in the presence of inflammatory substances such as bradykinin and prostaglandin E, via protein kinase dependent phosphorylation of the receptors [95, 98], indicating a role for TRPV1 in mediating heightened neuronal sensitivity seen with inflammation.

Several lines of evidence support a key role of TRPV1 receptors in the pathophysiology of chronic cough. Firstly, in humans, inhalation of capsaicin, a selective activator of TRPV1, is well established in provocation of cough [53, 99]. Secondly, in patients with chronic cough, the capsaicin threshold for eliciting cough is lower than in healthy volunteers [61, 100]. Thirdly, TRPV1 channels are expressed primarily on airway C-fibre nociceptive vagal afferents [49, 87] and their expression has been shown to be enhanced in chronic cough [51]. Finally, known TRPV1 antagonists, capsazepine and iodoresiniferatoxin, have inhibited capsaicin- and citric acid-induced coughs in guinea pigs [57, 101].

Various compounds have been developed as clinical antagonists of TRPV1 receptors [102]. Our group recently conducted a phase 2 proof-of-concept clinical trial investigating the effect of a novel, selective, oral TRPV1 antagonist, SB-705498, in patients with chronic cough. The study demonstrated that there was no difference in objective 24 hour cough frequency, but there was just over one doubling dose increase in log C5 capsaicin concentration in comparison to placebo [59]. It is possible that a greater inhibition of TRPV1 through use of more potent compounds may result in a reduction in cough frequency as well.

1.3.3 TRPA1

Another recently identified subfamily of the TRP channels family is TRPA1, which is co-expressed with TRPV1 in airway nociceptive vagal C-fibres [103]. In rats, afferent C-fibres from the lung produce action potentials in response to stimulation of TRPA1 receptors [104]. TRPA1 receptors are activated by noxious cold temperatures (<17°C) [105], which may provide a molecular basis for the anecdotal reports that many patients with chronic cough report cough when exposed to cold air. In addition, pungent ingredients of many products such as mustard oil, garlic, and cinnamon have been shown to activate TRPA1 [106-108]. Of interest, exposure to many of the environmental pollutants such as formaldehyde and acrolein (present in smoke from

cigarettes and car exhausts) has been reported to activate TRPA1 [108-110]. The endogenous ligands for this channel are aldehydes such as 4-hydroxy-2-nonenal (4-HNE) [101, 108], which are products of oxidative stress as a result of inflammation [111], indicating that TRPA1 may contribute to the heightened cough response in inflammation.

Inhalation of TRPA1 agonists such as cinnamaldehyde (active substance of cinnamon) and acrolein induce cough in experimental animals and healthy subjects [110, 112], supporting an involvement of this channel in the cough reflex. In COPD, elevated levels of 4-HNE have been demonstrated [113], which may contribute to cough in this population. The lack of clinically suitable selective and potent TRPA1 antagonists limits our understanding of the contribution of these receptors to cough in respiratory diseases.

1.3.4 Voltage-Gated Sodium Channels

Voltage-gated sodium (NaV) channels are widely distributed in various body tissues including the heart, the peripheral and central nervous system, and skeletal muscle [114]. NaV channels are crucial for the generation and propagation of action potentials [115]. Nine subtypes of NaV (1.1-1.9) have been identified [116]; of which, subtypes 1.7, 1.8 and 1.9 are mainly expressed in primary sensory neurons [115]. A rare mutation of the gene encoding NaV 1.7 has been identified recently which has been linked to a lack of perception of painful stimuli in humans [117]. The selective expression of certain NaV subtypes in sensory neurons provides an opportunity for the development of potentially safe (avoiding cardiac and CNS side effects) and effective voltage-gated sodium channel blockers, which are still unavailable, in the treatment of disorders like pain. NaV channels can be further classified as TTX-sensitive or TTX-resistant based on their susceptibility to inhibition by the neurotoxin tetrodotoxin (TTX) [118]. NaV1.7 is TTX-sensitive, whereas 1.8 and 1.9 are TTX-resistant.

NaV subtypes have some distinct features. NaV 1.7 channels are expressed in both somatosensory nociceptors and sympathetic neurons [115] and are sensitive to low amplitude changes in membrane depolarisation [67]. In dorsal root ganglia, NaV 1.8 and 1.9 are predominantly expressed by small diameter nociceptor neurons [115, 116]. NaV 1.8 contributes to the rapid rise (upstroke) of action potentials and is responsive to persistent depolarising currents, which results in continuous neuronal firing [67, 119].

NaV 1.9 is activated by depolarising currents not far from the resting membrane potential [67].

NaV channels are thought to be involved in neuronal hypersensitivity in conditions such as inflammatory and neuropathic pain [115]. Inflammatory mediators have been shown to modulate the function of NaV 1.8 and 1.9 [120, 121]. A reduced sensitivity to pain due to inflammation was observed in knockout mice for NaV 1.7 [122] and NaV 1.9 [123]. A potent and selective NaV 1.8 antagonist (A-803467) has recently been tested in experimental rat models of neuropathic and inflammatory pain. A-803467 demonstrated a dose-dependent increase in the thresholds for allodynia and hyperalgesia [124].

In the guinea pig, NaV 1.7, 1.8 and 1.9 are predominantly expressed in pulmonary vagal afferent cell bodies, but hardly ever in the heart, brain, or skeletal muscles [114]. On the other hand, subtypes 1.1, 1.2 and 1.3, which are highly distributed in the brain, are very limited in the vagal ganglia [114]. In contrast to dorsal root ganglia, both the nociceptor C-fibres and tracheal low-threshold mechanosensors (Aδ fibres) co-express subtypes 1.7, 1.8 and 1.9 [114]. There are no pharmacologically available NaV subtype-specific blockers to investigate the role of the different subtypes in cough. However, small hairpin RNA (shRNA) can be used to block the expression of certain genes. Application of shRNA against NaV 1.7 into the nodose ganglia of guinea pigs led to almost complete loss of sodium current, inhibition of action potential conduction, and *in vivo*, the abolishment of cough evoked by citric acid, suggesting a predominant role for NaV 1.7 in cough pathways [125]. NaV channels may also contribute to the hyper excitability of afferent nerves mediating the cough reflex. Inflammatory mediators such as prostaglandin E2 have been shown to potentiate the NaV 1.8-like electrical current in cultured airway nociceptive C-fibres [126].

1.4 Neuronal Sensitisation in Chronic Cough

Chronic cough patients have a sensitive cough reflex [61] and produce coughing in response to minimal stimuli. It has been postulated that sensitisation of the cough reflex at the peripheral nerve endings (*peripheral sensitisation*) and/or increased excitability within the CNS (*central sensitisation*) may be responsible for this [127]. This is analogous to the suggested neural mechanisms underlying chronic pain disorder [128].

1.4.1 Peripheral Sensitisation

Sensitisation of peripheral nerve endings and/or increased nerve density in the airways may in part be responsible for the lowered threshold for tussive stimuli and the exaggerated cough responses in chronic cough. The main molecular mechanisms of peripheral sensitisation are summarised below:

- Many mediators of inflammation such as bradykinin and prostaglandins, acting on G-protein-coupled receptors, increase the excitability of nerve membranes by activating intracellular protein kinases (PKA and PKC). Protein kinases mediate the phosphorylation of the transducing receptors (e.g. TRPV1) and voltage-gated sodium channels on the membranes of sensory nerve terminals. This phosphorylation results in enhanced activity and trafficking of the receptors and thus lowering the threshold for activation and increasing the magnitude of response. This form of sensitisation is rapid and reversible when there are no further modulatory substances [129, 130].
- Long-lasting chronic changes within the afferent sensory neurons involve upregulation of genes encoding receptors and neuropeptides, and phenotypic switch of non-nociceptive afferent fibres, leading to activation of cough pathways by subthreshold stimuli (discussed below) [129, 130].

The section entitled “Transduction of Cough Stimuli”, discusses evidence supporting the sensitisation of sensory nerve receptors and voltage-gated sodium channels by bradykinin, prostaglandin E2, and protons, which are seen in inflammation.

Neurotrophins and Peripheral Sensitisation

Neurotrophins (e.g. nerve growth factor [NGF] and brain-derived neurotrophic factor [BDNF]) are protein molecules crucial for the growth and function of neurons in both the peripheral and central nervous systems [131]. Upon binding of neurotrophins to a receptor (tropomyosin-related kinase [TrK]) on peripheral nerve terminals, a neurotrophins-receptor complex is transported intracellularly to the nerve body where it influences expression of genes [131]. In the airways, neurotrophins are released by epithelial cells and inflammatory cells including mast cells, macrophages and T lymphocytes [132, 133].

Neurotrophins are thought to play an important role in the sensitisation of neural pathways contributing to respiratory diseases such as asthma [134]. Levels of neurotrophins in the airways have been shown to be increased after allergen challenge in guinea pig experimental models and in atopic asthmatics [132, 135]. Afferent pathways mediating cough may also be modulated by neurotrophins. For example, pre-clinical studies have indicated that NGF up-regulate the expression of TRPV1 [136] and P2X3 [137] receptors. TTX-resistant sodium current in cultured visceral nociceptive sensory neurons were augmented after exposure to NGF [129].

In addition to the increased expression of receptors/channels, neurotrophins contribute to the novel expression of transducing receptors and neuropeptides in low-threshold afferent fibres that usually do not participate in sensing noxious irritant stimuli, a process called “phenotypic switch” [130]. Lieu et al have examined the effect of neurotrophins on TRPV1 expression in nodose-derived low-threshold mechanosensor Aδ fibres in guinea-pig airways [64] (The role for C- and Aδ fibres in cough is summarized in a previous section). Using retrograde labelling, Lieu et al identified the cell body of tracheal C- and Aδ vagal afferent fibres in jugular and nodose ganglia, respectively. Single cell retrograde PCR method was applied to evaluate the expression of TRPV1 and neurotrophin receptors. Jugular ganglion neurons were positive for TRPV1 mRNA, but nodose ganglion neurons were not. The majority of jugular neurons expressed TrKA receptors while nodose neurons expressed TrKB receptors. Two weeks after topical application of BDNF (selective for TrKB) to the trachea, TRPV1 mRNA was detected in nodose derived neurons [64]. This suggests that an additional group of Aδ fibres arising from the nodose ganglion, which were previously insensitive to TRPV1 activation, could be recruited by BDNF. This is an

important concept from pre-clinical animal work which could help explain a novel mechanism into the pathological cough associated with airway inflammation.

Similar to the change in receptors/ion channels gene expression, phenotypic switch also affects neurotransmitters. Exposure to airway allergens and viral infections in animal models induces expression of peptide neurotransmitters including substance P, CGRP and neurokinins in myelinated non-nociceptive A-fibres (see previous section, vagal A-fibres do not typically contain neuropeptides) [46, 138, 139]. This is likely to be mediated via actions of neurotrophins on nerve endings as intact vagal nerve has been shown to be mandatory for this effect to happen [138]. Substance P and neurokinins are involved in strengthening the synaptic transmission (see next section).

Another mechanism by which neurotrophins could enhance the peripheral processing of cough is through airway nerve density [130]. In bronchial epithelium from endobronchial biopsies, although not statistically significant, increased density for PGP-immunoreactivity (general neuronal marker) has been observed in chronic cough patients compared with healthy volunteers [140]. However, chronic cough patients had significantly higher nerve density for CGRP-containing nerves compared with controls.

Taken together, neurotrophins are likely to be involved in mediating peripheral sensitisation of the cough reflex. However, neither the level of serum nor airway neurotrophins in chronic cough has been demonstrated to differ significantly from healthy controls [141, 142]. It remains uncertain whether this means that neurotrophins are important in the pathophysiology of chronic cough or indicate that peripheral neuronal alterations persist in the absence of neurotrophins that possibly mediated permanent modifications.

1.4.2 Central Sensitisation

The concept of central sensitisation was first introduced by Woolf in 1983. He created an animal model in which he applied thermal injury to the lateral hind paw of decerebrate rats to mimic changes in humans following tissue injury [143]. Following the injury, electrophysiological recordings from the femoral biceps efferent fibres (as a measure of the withdrawal reflex) in the ipsilateral site showed spontaneous activity. Additionally, in both the site ipsilateral and contralateral to the foot with thermal injury, the threshold for activation by mechanical stimulation was reduced, with greater and prolonged action potentials evoked by noxious stimuli. Furthermore, the receptive fields for those neurons widened. However, after injection of local anaesthetics in the injury

area, there was no reduction in the receptive fields, suggesting that hyper excitability of the dorsal horn neurons, rather than peripheral nerve endings, is the main mechanism contributing to post-injury increased sensitivity and responsiveness [143].

Subsequently, those results have helped in providing the basis of plausible pathophysiological explanations for a variety of chronic somatosensory and visceral disorders such as chronic pain, irritable bowel syndrome, and fibromyalgia [144].

Mechanisms of Central Sensitisation

A key mechanism of the initiation and maintenance of central nervous system plasticity involves N-Methyl-D-Aspartate receptors (NMDARs) [145].

NMDARs

NMDARs are named after the N-methyl-D-aspartate molecule (an analogue of glutamate) which was the first molecule used in research to activate these receptors [146]. NMDARs are ligand-gated ion channels to which the major central nervous system excitatory neurotransmitter (glutamate) binds, allowing the flow of calcium and sodium into the cell cytoplasm. They are distributed throughout the CNS neuronal membranes [146].

The receptor is composed of a combination of 4 or 5 of seven subunits (NR1, NR2A, NR2B, NR2C, NR2D, NR3A and NR3B). NR1 subunit is essential for a functioning NMDAR while the other subunits serve a modulatory function. Each subunit has an extracellular terminal, 3 trans-membrane domains (M1, M3, and M4), a pore forming domain (M2) and an intracellular terminal. The ligand-binding site is formed by the extracellular terminal joined by the extracellular loop generated by the M3 and M4 domains [146]. Magnesium molecules block the channels at rest, however, when the membrane is depolarised enough, the magnesium molecule is removed permitting the opening of the channel [147].

Molecular Mechanisms

The proposed molecular mechanism, as understood in the somatosensory system, leading to CNS plasticity and the contribution of NMDARs are summarised here.

Low frequency stimulation of nociceptive C-fibres (such as in physiological pain) leads to the release of glutamate from central nerve terminals (Figure 3). Glutamate then binds to and activates the ligand-gated ion channels AMPA and kainite on postsynaptic membranes, initiating fast excitatory postsynaptic potentials (EPSP). These EPSPs last only a fraction of a second (milliseconds) [128, 146]. NMDARs would not open in this situation because they would be still blocked by the voltage-gated magnesium molecule.

In contrast, high frequency and/or intense stimulation of the C-fibres results in the release of glutamate as well as neuromodulator molecules such as substance P into the central synapse [128] (Figure 4). So in addition to the binding of glutamate to AMPA/kainite, the neuromodulators bind to their postsynaptic receptors (neurokinins receptors) to further augment the membrane depolarisation. As a result, the voltage-gated magnesium blockage is removed from the NMDARs, allowing the influx of calcium and sodium ions. This leads to slow EPSP, which contributes to amplification of the membrane depolarisation and action potential discharges with subsequent stimuli; a process known as “wind up”. The “wind up” lasts tens of seconds and is thought to contribute to central sensitisation [128, 148].

However, in the presence of inflammatory mediators or nerve injury, in addition to the “wind up” phenomenon, further changes occur that result in a much more lasting upregulation of central neuronal responses (Figure 5). Given that NMDARs are highly permeable to calcium, activation of NMDARs increases the intracellular calcium. Elevated calcium plays a role in trafficking and phosphorylation of membrane receptors, including NMDARS, (via complex intracellular signalling pathways involving kinases) [128, 148]. The phosphorylated receptors demonstrate heightened sensitivity and function. Altogether, these processes would enhance the synaptic strength [148]. Furthermore, upregulation of receptor gene expression and induction of enzymes (e.g. COX-2) give rise to more long-lasting plasticity changes of the central neurons [128].

Figure 3: Fast Excitatory Post Synaptic Potential

Activated somatosensory C-fibres release glutamate into the central synapse in the spinal cord. Glutamate binds to AMPA/Kainate receptors, allowing the influx of cations. NMDARs are blocked by magnesium molecules.

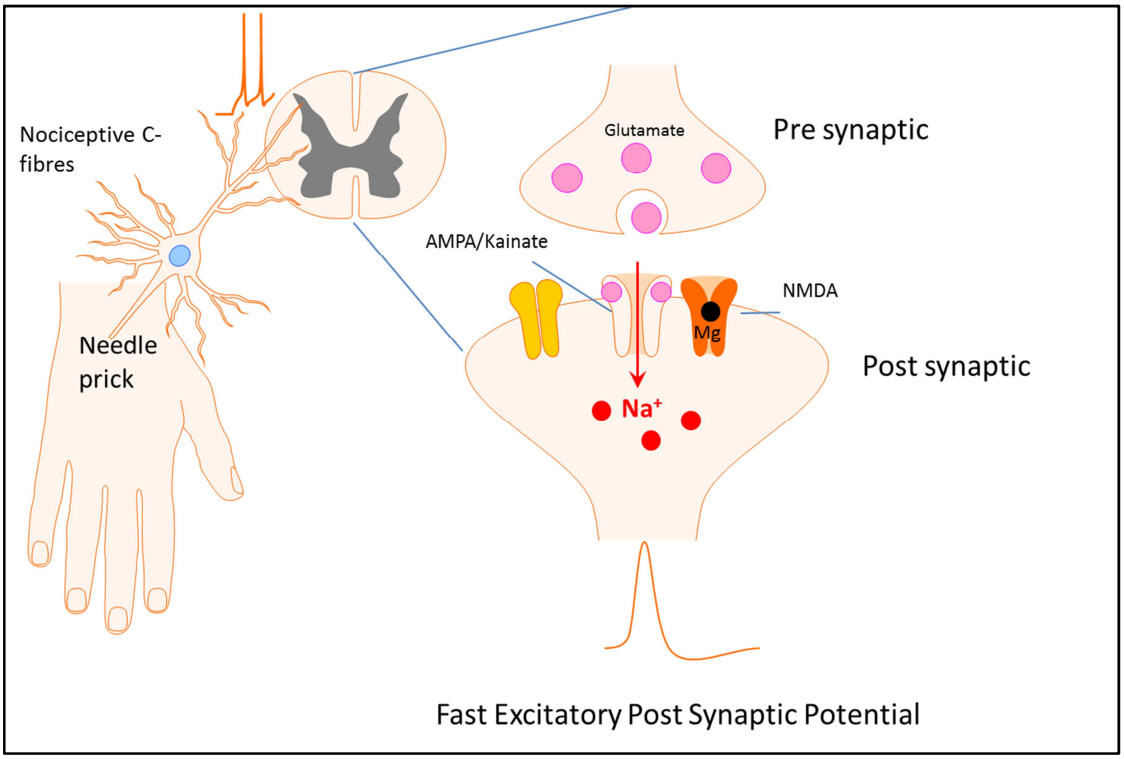


Figure 4: Slow Excitatory Post Synaptic Potential

Intense somatosensory stimulation leads to the release of both glutamate and neuromodulators such as substance P (SP). Consequently, membrane depolarisation reaches a threshold for magnesium molecules to move out of NMDARs. Calcium ions then flow through NMDARs into postsynaptic cells. NK=neurokinins.

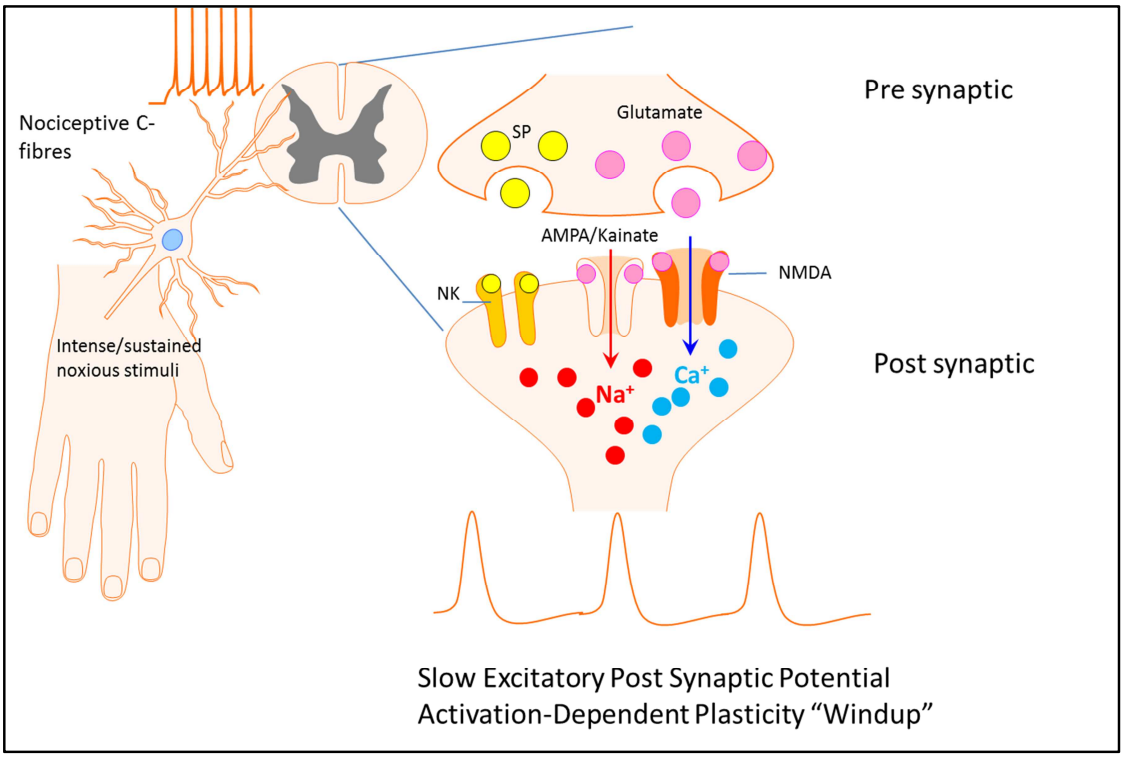
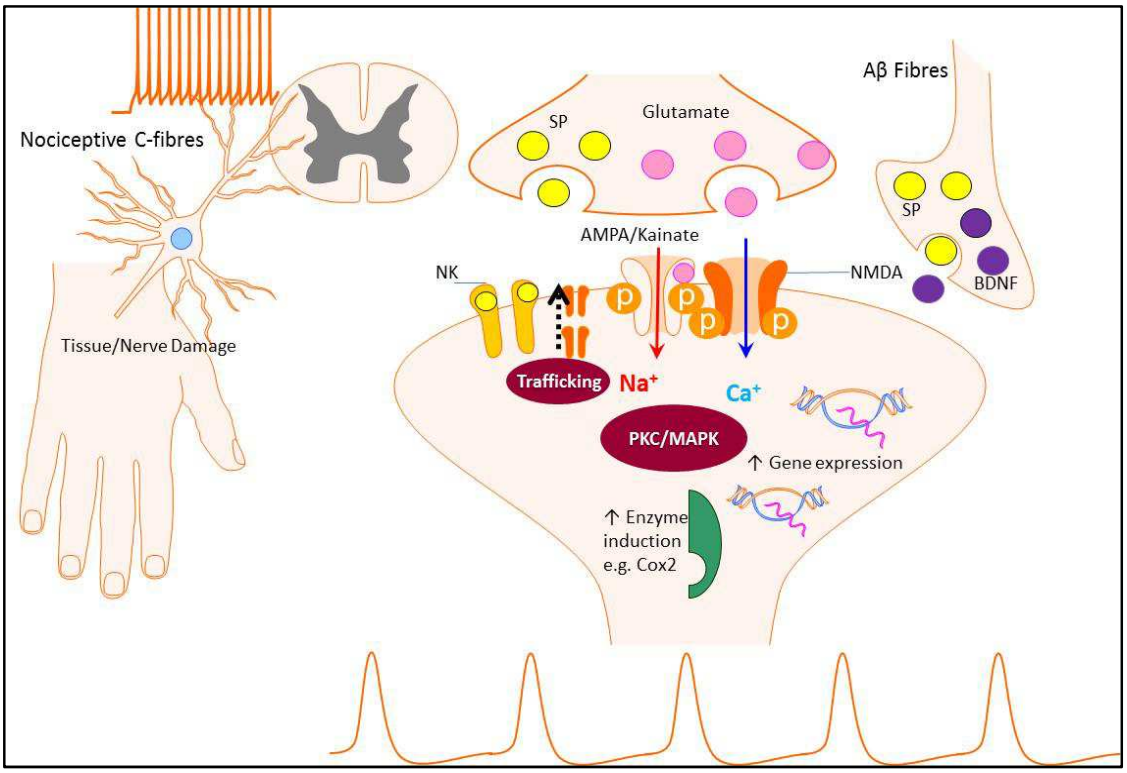


Figure 5: Central Sensitisation

Long-term structural and functional synaptic changes occur in situations of tissue damage. These include phosphorylation and trafficking of channels, increased gene expression and enzymes induction. In addition, phenotypic switching of afferents is seen. BDNF= brain-derived neurotrophic factor.



The Clinical Implications of Central Sensitisation

Sensitisation of the primary activated synapses (homosynaptic sensitization) is responsible for the hyperalgesia (increased pain in response to noxious stimuli) and allodynia (pain with innocuous stimuli). Sensitisation may also involve the adjacent synapses (heterosynaptic sensitization), which gives rise clinically to the secondary (in areas surrounding the injury site) hyperalgesia and allodynia [148].

Cough and Central Sensitisation

Experimental animal studies have demonstrated that upregulation of NTS neurons occur [149-151]. Plasticity within this site is likely to alter the cough response to a variety of tussive stimuli [152]. In a model of allergic airway inflammation, monkeys were challenged repetitively with house dust mite (HDM) allergen [151] and the excitability of NTS neurons was studied using electrophysiological recordings. Compared to controls, NTS neurons from HDM-challenged monkeys were hyper excitable with significantly higher resting membrane potentials. In addition, increasing amplitudes of electrical stimuli evoked increasing numbers of action potentials and increasing peak frequencies.

Previous work by Mazzone and Canning in anaesthetised guinea pigs has shown that although the stimulation of tracheal and laryngeal C-fibres by capsaicin and bradykinin did not elicit coughs, it lowered the voltage threshold for coughs evoked by electrical stimuli. Similarly, injection of capsaicin into the NTS sensitised the cough reflex. The mechanism of this sensitisation is thought to be mediated by central action of SP since the central (but not peripheral) application of NK antagonists blocked this effect [153].

In conscious guinea pigs exposed to environmental tobacco smoke (ETS) from the age of 1 to 6 weeks, compared with filtered air, the number of inhaled citric acid-evoked coughs was higher [149]. This effect was attenuated by pre-treatment of NTS injection of a NK1 antagonist in sensitised (ETS-exposed) animals but not in controls. This finding supports a role for SP and NK1 receptors in enhancing the excitability of central neurons processing cough information. The lack of effect of NK1 antagonists in control animals highlights the importance of studying novel anti-tussives in disease rather than in health.

In humans, central sensitisation provides a possible explanation for the association between chronic cough and events happening outside the lung. For example,

convergence of oesophageal and airway vagal afferents at the level of NTS is considered to be the most likely explanation for gastro-oesophageal reflux-associated cough [154, 155]. In the study by Smith et al in a group of unselected patients with chronic cough, around half of the chronic cough events were preceded by distal oesophageal reflux events more than expected by chance alone [156]. Patients with such an association had a more sensitive cough reflex than the group who did not have this association. The authors suggested that central sensitisation was the likely mechanism in patients with the reflux-cough temporal association.

1.5 Studies in Human Cough

1.5.1 The Urge to Cough Sensation

Patients with chronic cough often are aware of a sensation (typically in the throat or in the chest) which precedes cough and is called an 'urge to cough' [157]. The urge to cough sensation may positively or negatively regulate the cough motor response [158]. For example, a person may either decide to inhibit the cough response, unless the sensation is irresistible, if he/she is in a social meeting, or produce coughs to satisfy this sensation ("sensory derived cough") [159]. However, when there is a need to instantly protect the airways, for example when aspirating a foreign body, there is very little, if any, conscious control and the resulting cough represents a true reflex response ("reflex cough"). Conversely, humans can produce coughs voluntarily without any sensory component. In contrast to sensory-driven and voluntary cough, reflex cough is still maintained during general anaesthesia and sleep, providing the body with a vital defence mechanism [30, 160].

Activation of cough afferent pathways by a tussive stimulus results in stimulation of the cough centre in the brain stem and, in conscious humans, can also lead to cortical/subcortical areas of the brain to be activated and thus results in the perception of an urge to cough [25]. It has been proposed that a "gating-out" mechanism exists in the brain in which only supra threshold signals are associated with perceiving this sensation [158]. The urge to cough might be mediated by the activation of C-fibres [30, 161, 162]. However, the exact neural pathways involved are not fully known yet.

Widespread sensory and motor cortical areas were active on functional MRI following capsaicin induced urge to cough in humans [25]. However, the rating of the urge to cough was only positively correlated with the signal change in some, and not all of the activated areas (mainly, the anterior midcingulate cortex, the right primary somatosensory cortex and the supplemental motor area). The exact role and contribution of the different activated cortical areas is uncertain. The authors provided some circumstantial evidence that the cingulate cortex and the supplementary motor area play an inhibitory function (i.e. subjects trying to control the sensation). Of note, capsaicin-induced activation of the different areas of the brain could also be explained by secondary responses like eye watering and burning sensation rather than a primary effect of capsaicin-induced urge to cough [159].

Experimental studies have used a modified Borg scale or a visual analogue scale to measure this sensation in response to inhaled irritants [157, 158, 163, 164]. Urge to cough is perceived before coughs and the threshold for experimentally eliciting the urge to cough is lower than the threshold for evoking cough [163-165]. Although healthy females and those with chronic cough have been shown to have more sensitive cough reflex sensitivity [10, 11, 166], measured by the concentration of chemical irritants producing 5 or more coughs (C5), gender differences are less clear for the urge to cough. Mazzone et al demonstrated that, on average, healthy females had a lower threshold for inhaled capsaicin-induced urge to cough [25]. In contrast, two subsequent experimental studies in healthy volunteers have not shown a gender difference in the threshold for the urge to cough [163, 164]. Of interest, compared to men, healthy non-smoking women experienced greater magnitude of irritant-induced urge to cough in a dose-dependent manner [164]; this may contribute to the lower cough threshold in females and the higher cough frequency in female chronic cough patients [12]. One similarity between the urge to cough and cough reflex is that factors influencing the cough reflex sensitivity such as respiratory viral infections have been shown also to lower the threshold for the urge to cough or vice versa [167].

Davenport et al examined the relationship between the intensity of a tussive stimulus (different concentrations of inhaled capsaicin), the urge to cough, and the cough motor response [165]. Healthy volunteers rated their urge to cough on a modified Borg scale after each inhalation. Evoked coughs were counted and their intensity was measured using EMG recordings from the abdominal and intercostal muscles, and also by measuring the expiratory airflow during the expulsive phase of cough. The urge to cough increased with increasing concentrations of capsaicin. Both the cough counts and intensity had a positive linear relationship with the urge to cough intensity [165].

1.6 Measuring Cough

Studying the mechanisms of cough and evaluating the effectiveness of cough medicines requires the ability to measure cough using validated and reproducible tools. In clinical trials, the use of both subjective and objective outcome measures is recommended as they provide different information about cough and its impact [168].

1.6.1 Objective Measures

Objective Cough Recording

Although subjective measures of cough give important information on how patients perceive their symptom, it is paramount to have objective evidence of the presence of cough and its extent. This enables the study of the mechanisms of cough and assessment of the therapeutic benefit of any potential anti-tussive medication. The attempt to record cough sounds started almost 50 years ago [169-172] using large reel tapes and microphones to record cough sounds. Understandably, this required subjects to be admitted to an institution and therefore it did not reflect the daily activities and environmental exposures of subjects. Similarly, video recordings of subjects enabled the identification of cough events, but could not be ambulatory so did not represent a true picture of life.

The next stage in the field was the development of ambulatory cough monitors. In 1994, Hsu et al reported the use of a 24 hour ambulatory cough audio monitoring device [173]. Nowadays, the availability of digital audio systems has made 24-hour ambulatory cough recordings and storage much easier. However, manual listening and counting of cough sounds is still required, which is time consuming and tedious. Several groups have tried to develop automated ambulatory systems for example Vivometrics lifeshirt™ [174], Hull automated cough counter [175], Leicester cough monitor [176] and VitaloJAK™ [177]. These systems face a difficulty of reliably distinguishing coughs from other sounds such as background noise, sneezes, throat clears and speeches. Consequently, none of these systems has yet achieved full automation in monitoring cough [178].

A number of methods to quantify coughs have been described: number of explosive cough sounds, time spent coughing, and number of cough epochs [179] . Time spent coughing is usually reported as the time in seconds in which there is at least one cough

sound. A cough epoch is a peel of cough sounds with no pause lasting at least 2 seconds. All three methods correlate well with each other [173, 179], and therefore, no one method can be recommended over the others [178]. A limitation of using acoustic recorders alone is the lack of appreciating the cough intensity. However, capturing cough intensity is challenging.

Inhalational Cough Challenges

The concentration of an inhaled tussive agent (e.g. capsaicin, citric acid) eliciting at least 2 (C2) or 5 (C5) coughs is used as a reproducible tool to measure the sensitivity of the cough reflex in humans [180, 181]. Previously, there were no agreed upon standards for performing these challenges, making it difficult to compare the results of various studies. In 2007, the European Respiratory Society cough task force published guidelines on how to assess cough including recommendations on how to perform the inhalational challenge tests [178]. The main two methods for performing the challenges (coughs occurring in the first 15 seconds after inhalation are regarded as evoked coughs) are:

1. Single dose method: this involves inhaling a single concentration of the tussive agent. The single dose method is not recommended because of the large inter subject variability in the cough response to any single concentration of a tussive agent.

2. Dose-response method: this involves inhaling doubling concentrations of the tussive agent. Subjects might modify their cough response as a result of the incremental concentrations of capsaicin, so placebo (normal saline) is randomly interspersed in between doses for blinding purposes [180]. The dose-response test can be performed in two different ways, either as a single breath inhaled from a dosimeter nebuliser equipped with a valve that controls the inspiratory flow rate or as tidal breathing over a certain period (15 – 60 seconds). More commonly, the single breath method is applied because of the concern over variability of the delivered dose with the tidal breathing method in terms of flow and particle deposition [182].

Although chronic cough patients as a group have heightened cough reflex sensitivity, *i.e.* lower C2/C5, compared to healthy volunteers, the inter subject variability of C2/C5 is large and therefore it overlaps substantially with the values for a healthy person [183, 184]. This implies that subjects need to be compared to themselves, *i.e.* crossover design, when designing interventional studies. Interestingly, a recently published paper suggests that the maximum cough response (Cmax) beyond C5 to the maximum

tolerated tussigen dose (capsaicin in this study) is a more reliable tool in differentiating health from disease [185]. In addition, while C5 only moderately relates to 24-hour ambulatory objective cough recording [186], Cmax correlates strongly with cough frequency [185].

There has been discordance between the reduction in cough reflex sensitivity and the change in objective cough frequency with cough treatments. For example, codeine attenuated the cough reflex sensitivity in healthy humans [187], but has failed to reduce objectively recorded cough sounds compared to placebo in COPD patients [188]. Furthermore, a novel TRPV1 antagonist led to just over two-fold increase in C5 for capsaicin over placebo in chronic cough patients; however, it did not change the objectively recorded cough rate compared to placebo [59]. These examples highlight a limitation of using these challenge tests as primary endpoints in clinical trials of anti-tussives. Nevertheless, inhalational cough challenges are still important tools in investigating mechanisms of cough in both experimental animal models and human studies. Inhalation of chemical irritants also provides evidence of the mechanism of action of drugs; for example, the change in threshold for capsaicin-induced cough by the TRPV1 antagonist indicates that the drug engaged the target receptor (capsaicin is an agonist of TRPV1).

1.6.2 Subjective measures

Subjective measures of cough are important tools to understand patients' experience of cough. However, they have only moderate correlation with objective cough counts [179]. The severity of cough and impairment in quality of life is likely to be influenced not only by the frequency of cough, but also by the cough intensity and its interference with daily activities [189].

Visual Analogue Scale (VAS)

Patients are asked to indicate the severity of their cough on a 10-cm line ("no cough" up to "worst cough"). Cough severity VAS responds to change [190]. No repeatability testing of VAS in chronic cough patients has been done previously, but short-term (2 weeks) reproducibility has been demonstrated in cough secondary to COPD [191].

Quality of Life Questionnaires

Two validated and reproducible self-completed cough-specific quality-of-life questionnaires have been developed to assess the impact of chronic cough on quality of life and to monitor treatment effect.

Cough Quality of Life Questionnaire (CQLQ)

CQLQ is a 28-item questionnaire, with a four-point Likert scale response [192]. Overall scores range from 28 (no impairment in quality of life) to 112 (worst impact on quality of life). The questionnaire covers 6 domains: physical complaints, psychosocial issues, functional abilities, emotional well-being, extreme physical complaints, and personal safety. The minimal important difference (MID) is estimated to be 10.58 using the global rating of change. However, Fletcher et al argues that the global rating of change overestimates a change and therefore it is suggested that, using a prospective assessment tool to assess the change (Punum ladder), a difference of at least 21.89 is necessary to be clinically significant [193].

Leicester Cough Quality of Life (LCQ)

LCQ has 19 items; each item has a seven-point Likert scale response [190]. The 19 items are separated into 3 different domains: physical, social, and psychological. Total scores range from 3 to 21, with higher scores representing better quality of life. The MID for LCQ is 1.3 [194].

In chronic cough patients, scores from CQLQ and LCQ relate moderately to each other [195]. Indeed, extreme physical complaints such as incontinence and vomiting are contained within CQLQ but not LCQ.

1.7 Aims and Hypothesis

The aim of this thesis is to understand the therapeutic value of ion channel antagonists in chronic cough; this additionally gains insights into the mechanistic contribution of those ion channels. I will specifically focus on NaV, P2X3, and NMDA receptors.

I hypothesise that;

1. Blocking voltage gated sodium channels on airway afferents results in reduction of daytime cough

2. P2X3 channels contribute to cough neuronal hyper excitability and their antagonists would be effective as an anti-tussive.

3. NMDAR mediated central sensitisation is an important mechanism in chronic cough.

I planned three experimental clinical studies in patients with chronic cough. In chapters 2 and 3, randomised placebo-controlled crossover studies were conducted to investigate the efficacy of nebulised lidocaine (pan NaV blocker) and AF-219 (potent and selective oral P2X3 antagonist) in improving daytime cough. In chapter 4, I performed a feasibility study of the tolerability and optimal dosing of Memantine (low affinity and uncompetitive NMDAR antagonist) in subjects with chronic cough to inform future randomised controlled trials.

CHAPTER 2

The Effect of Lidocaine and its Delivery in Patients with Chronic Cough

2.1 Background and Rationale

Voltage-gated sodium channels (VGSCs) (see chapter one for details) are crucial for the initiation and conduction of action potentials. Nine subtypes (1.1 – 1.9) of NaV have been identified, based on different αsubunits of the channel [116]. Subtypes 1.7, 1.8 and 1.9 are primarily expressed in primary sensory neurons including airway vagal afferents [87, 115]. However, currently there is no subtype-selective blocker, but such molecules are being developed as therapeutic agents.

Lidocaine is a non-selective voltage-gated sodium channel blocker. Its sites of action include the conduction system of the heart, the central and peripheral nervous system. Its use in the treatment of arrhythmias and as a local anaesthetic is well established. In bronchoscopy, lidocaine is widely used as a local anaesthetic to inhibit cough and the gag reflex [196]. Nebulised anaesthetics are used routinely in the field of palliative care [197] for the suppression of cough, but its use is not widespread and not licensed in the routine clinical care of patients with chronic cough. This could be explained by a number of factors including: a lack of robust, objective evidence of its efficacy, a concern that aspiration could result from the loss of protective reflexes accompanying anaesthesia, and potential cardiac and neurological side effects.

Experimental animal studies indicate that local anaesthetics are effective in inhibiting cough induced by chemical and mechanical stimuli [198-202]. In humans, various studies [203-205] and case-reports [206-209] have suggested that lidocaine is anti-tussive, on the basis of subjective symptoms or sensitivity to irritant stimuli.

Hansson *et al.* randomised 10 healthy volunteers to nebulised lidocaine (20 mg), adrenaline with lidocaine, adrenaline alone and placebo (saline) [204]. This was a double-blinded study. Using a microphone and tape recorder, the authors counted capsaicin induced coughs at 5, 15, 25, 45 and 60 minutes post treatment. Although subjective oropharyngeal anaesthesia lasted less than 15 minutes, nebulised lidocaine significantly decreased capsaicin induced coughs at 5 – 25 minutes (mean inhibition of 35 %, 95% CI 14% - 55 %, $p < 0.05$). The anti-tussive effect of nebulised lidocaine lasted longer than the anaesthetic effect. This shows that lidocaine might exert its anti-tussive effect by a mechanism separate from its anaesthetic action.

So far, there have been no studies of nebulised lidocaine in patients with chronic idiopathic cough that used 24-hour objective cough frequency as an outcome measure. There are a number of small case studies of patients with chronic cough and mixed

respiratory disorders (COPD, asthma, sarcoidosis) treated with nebulised lidocaine [206-209]. Their primary and only outcome measurement was subjective assessment of cough by the patients. Howard *et al.* [206] claimed that patients reported improvement in their cough for 1 – 6 weeks after nebulisation of 400 mg of lidocaine, although, oropharyngeal anaesthesia was short living (< 30 minutes) as would be expected

This study aimed to answer the question of whether blocking airway VGSCs by using nebulised lidocaine would improve objectively recorded cough in patients with chronic cough. Nebulised lidocaine is associated with oropharyngeal anaesthesia; thus, in an attempt to enhance blinding of the study, I planned to deliver lidocaine as a throat spray as well as via nebuliser. Often patients with chronic cough describe an irritation in their throat, which gives them an urge to cough [210]; other patients have a sense of chest irritation. Therefore, including lidocaine throat spray would also be useful in examining the effect of treating throat irritation on cough.

2.2 Hypothesis

I hypothesise that blocking VGSCs using lidocaine will prevent generation of action potentials in afferent airway nerves responsible for evoking cough. Therefore, I would predict treating the airways with nebulised lidocaine would be an effective anti-tussive in patients with chronic cough. Furthermore, throat irritation/urge to cough sensation in patients with chronic cough is thought to be a referred sensation; therefore, I predict that lidocaine throat spray would be ineffective.

2.3 Study Aims

Primary Aim:

- To investigate the effect of nebulised lidocaine on cough.

Secondary Aim:

- To investigate the effect of lidocaine throat spray on cough.

2.4 Study Objectives

Primary Objective:

- To quantify the effect of nebulised lidocaine over placebo on objective cough frequency.

Secondary Objectives:

- To quantify the effect of nebulised lidocaine over placebo on cough severity VAS.
- To quantify the effect of nebulised lidocaine over placebo on the urge to cough VAS.
- To quantify the effect of lidocaine throat spray over placebo on objective cough frequency.
- To quantify the effect of lidocaine throat spray over placebo on cough severity VAS.
- To quantify the effect of lidocaine throat spray over placebo on the urge to cough VAS.

2.5 Study Endpoints

Primary Endpoint:

- Objective cough frequency over 10 hours post treatment (i.e. from the end of both nebulisation and throat spray) after nebulised lidocaine compared to placebo.

Secondary Endpoints:

- Cough visual analogue scale scores over 10 hours post treatment after nebulised lidocaine compared to placebo.
- Urge to cough visual analogue scale scores over 10 hours post treatment after nebulised lidocaine compared to placebo.

- Objective cough frequency over 10 hours post treatment after lidocaine throat spray compared to placebo.
- Cough visual analogue scale scores over 10 hours post treatment after nebulised lidocaine compared to placebo.
- Urge to cough visual analogue scale scores over 10 hours post treatment after lidocaine throat spray compared to placebo.

2.6 Methodology

2.6.1 Study Subjects

Patients with idiopathic chronic cough or chronic cough resistant to treatment of specific triggers were recruited from our tertiary cough clinic (University Hospitals of South Manchester, UK) over a 4-month period from February 2011 to May 2011. Patients were investigated according to a diagnostic algorithm [211, 212]. The diagnostic algorithm enabled patients to be investigated thoroughly for specific triggers of chronic cough. All patients had full lung function testing, methacholine/histamine challenge testing, nasoendoscopy, high resolution computerised tomography of the chest, and bronchoscopy (with lavage for differential cell count, and endobronchial biopsies). All Patients were treated for any detected triggers of chronic cough (inhaled corticosteroid for asthma/eosinophilic bronchitis, corticosteroid nasal spray and anti-histamines for post-nasal drip syndrome, and proton pump inhibitor twice a day and nocturnal ranitidine for gastro-oesophageal reflux). Patients with cough refractory to treatment of underlying triggers were considered eligible for the study.

Approvals from Central Manchester Research and Ethics Committee (reference: 10/H1008/95) and the Medicines and Healthcare Products Regulatory Agency (reference number 21463/0217/001) were obtained prior to the start of the study. The trial was registered at clinicaltrials.gov (NCT01252225). All patients signed a written informed consent form and the study was conducted according to the Declaration of Helsinki.

2.6.2 Inclusion and Exclusion Criteria

Inclusion Criteria

- Male and female subjects, age 18 years and over
- History of cough for more than 8 weeks
- Normal CXR
- Chronic idiopathic cough or chronic cough resistant to treatment of specific triggers

Exclusion Criteria

- History of chest or upper airway infection within the past 6 weeks
- Current smokers; Ex-smokers with history of smoking > 20 pack years or those who have given up < 6 months ago
- Prohibited medications: medications likely to suppress/affect cough including: codeine, morphine, pregabalin, gabapentin, amitriptyline, angiotensin converting enzyme inhibitors and baclofen, any anti-arrhythmic medication, cimetidine, beta blockers
- Cardiovascular conditions: sinoatrial disease, bradycardia or all types of heart blocks, history of ischaemic heart disease or heart failure, clinically significant abnormal electrocardiogram (ECG) at screening or baseline, history of cardiac surgery
- Asthma
- History of Epilepsy or myasthenia gravis
- Pregnancy or breast feeding
- Participation in another trial within the preceding 6 weeks
- History of hepatic or renal dysfunction, porphyria
- History of hypersensitivity to lidocaine or related drugs
- Trauma or ulceration to oral mucosa
- Conditions that may affect cough response such as stroke, diabetes, Parkinson's Disease

2.6.3 Study Design

This was a single dose, randomised, double-blind, double dummy, placebo-controlled, three-way crossover study, with a minimum 2-day washout period between treatments.

On each treatment day patients were randomised to receive:

- **Nebulised lidocaine** 600 mg: nebulised lidocaine followed by placebo throat spray OR,
- **Placebo** (Normal Saline): nebulised placebo followed by placebo throat spray, OR,
- **Lidocaine throat spray** 100 mg: nebulised placebo followed by lidocaine throat spray

2.6.4 Randomisation and Allocation

The randomisation was provided by our hospital statistical department using a computer generated permuted block design with mixed block sizes and random seed. The randomisation list was kept in the hospital pharmacy and the allocation sequence was concealed from the research team.

Blinding of the Study

Although the patients and I were blinded to the treatment allocation, patients may have been able to distinguish lidocaine from placebo because of the accompanying anaesthetic effect of the lidocaine. In an attempt to minimise this, nebulisation was followed by throat spray. This way, patients may have found it difficult to know whether the nebulisation or the throat spray caused the anaesthesia. Patients were instructed not to report oropharyngeal anaesthesia to me, but could report it to the research assistants.

2.6.5 Study Visits & Procedures

In the first visit, written informed consent was obtained and eligibility criteria were checked. Patients who met the eligibility criteria were then randomised to the three treatment visits.

In each treatment visit the following procedures were performed:

- Baseline blood pressure, pulse rate, and respiratory rate

- Baseline spirometry and ECG
- Baseline cough visual analogue scale & urge to cough visual analogue scale
- Urge to cough questionnaire to describe its location (Appendix 1)
- Start of the 24 hour cough recording before commencing the treatment
- Nebulisation of lidocaine or placebo followed by throat spray of lidocaine or placebo according to the randomisation schedule
- Repeat ECG, blood pressure, pulse rate, and respiratory rate 15 minutes after the completion of treatment
- Repeat cough visual analogue scale and urge to cough visual analogue scale at 15 minutes, 30 minutes, 45 minutes, 60 minutes, 75 minutes, 90 minutes, 105 minutes and 120 minutes post treatment and then hourly for 8 hours
- Repeat spirometry 2 hours after the completion of treatment to rule out significant bronchoconstriction (drop in $FEV_1 > 20\%$).
- Following the repeat spirometry at 2 hours post treatment, patients were discharged from the research ward but continued to wear the ambulatory cough monitor.

Patients were asked not to have drinks for 2 hours and not eat for 4 hours after the finish of treatment to minimise the risk of aspiration.

Approximately 24 hours after the end of treatment, patients were asked to repeat the cough VAS and urge to cough VAS.

At the end of study (24 hours after last treatment visit), patients were asked to report what treatment they thought they had in each visit.

2.6.6 Detailed Description and Justification of Methodology

Porta-Neb Ventstream Nebuliser (Philips Respironics, Surrey, UK)

The nebuliser was compressor driven. It provides flow rate of 6 l/min and 80 % of its output is less than 5 microns. Compared with conventional nebulisers, Ventstream

nebuliser is breath-enhanced, delivering more during inhalation with less wastage during exhalation. After filling the drug container with 6 ml of the lidocaine/placebo, I asked patients to hold the Ventstream upright and seal their lips around the mouthpiece. Patients then had tidal breathing through the mouthpiece until no further medication could be aerosolised.

Figure 6: Porta-Neb Ventstream Nebuliser

Dose Rationale

I designed the study so that the dose deposited in the throat from the nebuliser would be as similar as possible to the dose delivered by the throat spray. Therefore, any additional benefit of the nebulised lidocaine could be attributed to the dose deposited in the lower airways.

I-Neb AAD (Adaptive Aerosol Delivery) nebuliser, delivering medication only on inhalation, achieves around 65% (of the delivered dose) deposition in the lung [213]. The lung deposition with ventstream nebuliser is twice that of conventional Hudson nebuliser [214]. Using radiolabelled saline, nebulisers which produce aerosols with Mass Median Diameter (MMD) of around 1.8 – 4.6 micron result in 25 % of the dose wasted during exhalation, 45 - 60 % lung deposition and 10 - 30 % deposition in the oropharynx [215]. I estimated that the Porta Neb-Ventstream nebuliser would deliver 50% of its output into the lung; 25 % would be deposited in the throat and 25 % would be wasted during exhalation.

Since the nebuliser has a dead space of 2 ml, of the 6 ml placed into the nebuliser, 4 ml will be emitted. Of the delivered dose (4 ml), I estimate that 2 ml (200 mg lidocaine) will be deposited into the lung, 1 ml (100 mg lidocaine) deposited into the throat, and 1 ml (100 mg) wasted during exhalation. The total dose available for absorption would be 3 ml (300 mg lidocaine).

In an average 70 kg adult, up to 560 mg (8 mg/kg) of lidocaine will be sprayed into the bronchi during bronchoscopy with safe plasma level [196]. In 2 previous studies using ultrasonic nebuliser, 600 mg of lidocaine [216] and 530 mg of lidocaine [217] resulted in plasma level of 0.5 and 1.3 microgram/ml respectively. Toxic plasma level of lidocaine is thought to be around 6 microgram/ml [218]. Thus, the dose I administered was well below toxic level. Following treatment with nebulised lidocaine, peak plasma level is reported to be approximately 15 minutes after treatment [219]. Therefore, repeat ECG was done at 15 minutes post treatment to assess for any cardiac toxicity.

Rationale for 48-hour Wash Out

The terminal half-life of lidocaine following systemic administration is 1.5- 2 hours [220]. By allowing 48-hours washout period, at least 20 half-lives would have elapsed. This is to ensure that no effect is left to alter the results of subsequent visits.

Study Medication

To allow the use of a dose of 600 mg lidocaine with a volume not exceeding the capacity of the nebuliser pot, 10 % lidocaine was chosen. 10 % w/v lidocaine hydrochloride in water (pH 4 - 5.5) and matching placebo (0.9% saline) was supplied by Calderdale and Huddersfield Pharmacy Manufacturing Unit. 10% lidocaine/placebo was delivered in a pair of matching glass bottles; one labelled for use in nebulisation and the other one for use as a throat spray. Each bottle had 7 ml of 10 % lidocaine or placebo (normal saline). Three pairs of the glass bottles represented the different treatment visits patients were randomly assigned.

A syringe and a needle were used to draw 6 ml of 10% lidocaine or matched placebo, which is then placed in a Porta-Neb Ventstream nebuliser. For the throat spray, metered pumps and nozzles were provided by GlaxoSmithKline. A metered pump and nozzle were attached to the glass bottle labelled for use as a throat spray. The metered pump delivered 100 microlitres per actuation. Ten sprays (1 ml of 10% lidocaine) were sprayed into the throat following nebulisation, delivering 100 mg (dose rationale

explained before). No estimation could have been made on the size of particles delivered by the nozzle as it was more of a jet than a spray. This indicates that they were of large particle sizes that could not terminate in the lower airways.

Ambulatory Objective Cough Recording

For the purpose of obtaining objective cough frequency, the VitaloJAK™ cough monitor (Vitalograph Ltd, UK), a custom-built digital recording device, was used (Figure 7). VitaloJAK™ features an air microphone attached to the subject's lapel and an adhesive sensor attached to the chest wall over the sternum. The device records all sounds (8 kHz, 16 bit wave format) continuously over 24 hours. Data was written to a 4 Gigabyte compact flash data card, which was then downloaded onto a personal computer and archived on a digital versatile disc. Validated custom-written software was then used to compress the recording from 24 hours to a shorter file by detecting all potential cough sounds and cutting out non-cough sounds such as silence, background noise and speech [221]. Trained staff and myself (I counted 10% of the total cough recordings) then manually listened to the compressed file and counted the number of explosive cough sounds using an audio editing software package (Adobe® Audition® 3.0). The results were expressed as explosive cough sounds per hour. This is a validated and repeatable objective measure of cough frequency [186], with excellent intra-and inter-observer agreement [179].

Although lidocaine has a short plasma half-life, there is no prior data from an objective cough recording of the length of its potential anti-tussive effect. The 10 hour period following treatment was chosen as an endpoint to cover the change of cough count over daytime.

Figure 7 VitaloJAK™ Cough Monitor

2.6.7 Sample Size and Statistical Analysis

Sample Size Determination

A previous study has suggested that around a 30% reduction in cough frequency (SD of change 37%) is detected by patients [222]. A sample size of 20 would have 90% power to detect a 30% reduction in daytime objective cough frequency for nebulised Lidocaine over placebo (using a simple paired t-test and assuming a standard deviation of change of 37%). A conventional two-sided 5% significance level is used.

Statistical Analysis

In view of the within-subject design of the study which results in repeated measurements for each subject, and to take account of the correlation of data within subjects appropriately, I used generalised estimating equations (GEE) [223](SPSS version 20, Chicago, IL, USA) to investigate the effect of nebulised lidocaine and lidocaine throat spray on study outcome measures, compared to placebo. This technique also enables all subjects to be included in the analysis, even if there are some missing data amongst the repeated measurements. No adjustment for the multiple pairwise comparisons was made for this proof-of-concept study. Intention-to-treat observed case analysis was applied. Graphs were generated by Prism (version 6, GraphPad Software Inc., CA, USA). Statistical advice about using the GEE models has been sought from Julie Morris, Honorary Reader in Medical Statistics, University Hospital of South Manchester.

The following models were used:

1. To investigate the effect of the different interventions on the average 10-hour cough rate, I used a regression model with intervention and treatment period as within-subject main factors and included the interaction between intervention and period. I used an exchangeable working correlation matrix, which assumes a uniform correlation for all pairs of the within-subject variables. A natural $\log_e$ transformation was applied to the 10-hour cough rate to obtain a reasonable approximation to a normal distribution and a linear model was used. The $\log_e$ means were de-transformed to produce geometric mean summary statistics.

The influence of gender or urge to cough location was investigated in a sub analysis by fitting the main effect term for the factor and the interaction term between the factor and intervention.

2. To explore the effect of time post treatment on the hourly cough rate, I used an autoregressive GEE model with time, period and intervention as factors and the interaction terms intervention*time and intervention*period. An Autoregressive working correlation matrix was chosen because of the temporal nature of these repeated measures and that correlation for cough counts further separated in time is not as strong as the correlation for cough counts taken close in time within a subject. The cough count in each hour was not normally distributed and were assumed to follow a Poisson distribution in the model (a conventional distribution for counts).

3. To investigate the effect of intervention on the overall cough and urge to cough VAS, I used a GEE model with intervention, period and time as factors and the interaction terms intervention*period and intervention*time. Two baseline covariates were used – the first is the average of the loge baseline VAS scores for the subject, and the second is the period specific baseline (the difference of the period's Log_e baseline from the subject's average baseline). The average baseline covariate adjusts for the between-subjects' variation and the period specific baseline adjusts for the within-subject's variation between periods.

   Similar to the hourly cough count model, an autoregressive working correlation matrix was chosen. The VAS scores were not normally distributed and contained zeros. Therefore, to apply a linear model, 1 was added first to the VAS scores before they were log_e transformed.

4. Additionally, and as an exploratory analysis, I described the cough count during treatment; non-parametric tests were used to examine the statistical significance for the cough count during nebulisation among the different treatments.

Summary of the various GEE models used to analyse the data is presented in the table below.

Table 1*:* Summary of the GEE models

Dependent Variable	Model Type	Working Correlation Matrix	Model Effects	Covariates
Log_e transformed 10 hour cough rate	Linear	Exchangeable	Intervention Period Intervention*Period	None
Log_e transformed 10 hour cough rate	Linear	Exchangeable	Intervention Period Gender Intervention*Period Intervention*Gender	None
Log_e transformed 10 hour cough rate	Linear	Exchangeable	Intervention Period UTC location Intervention*Period Intervention*UTC location	None
Hourly cough rate	Poisson Log-linear	Autoregressive	Intervention Period, Time Intervention*Period Intervention*Time	None
Log_e transformed cough VAS	Linear	Autoregressive	Intervention Period Time Intervention*Period Intervention*Time	Average baseline. Period baseline.
Log_e transformed UTC VAS	Linear	Autoregressive	Intervention Period Time Intervention*Period Intervention*Time	Average baseline. Period baseline.

2.7 Results

2.7.1 Demographics and Baseline Characteristics of Subjects

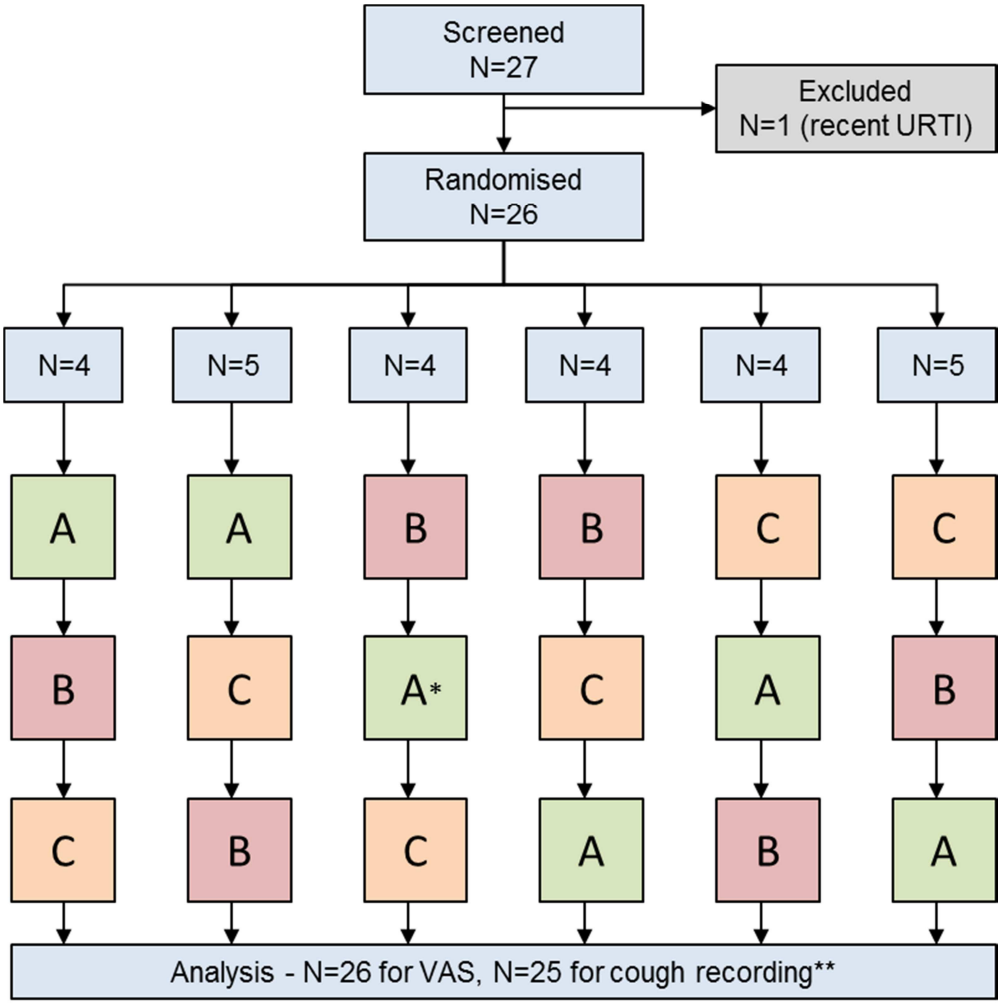
A summary of the demographics and baseline characteristics is presented in Table 2. Study recruitment, treatment allocation and data analysis is summarised in Figure 8.

Table 2: Demographics and Baseline Characteristics

Sample Size	26
Age (years)	53.5 (12.1) *
Gender (M:F)	4:22
Smoking History (never smoked:ex-smokers)	18:8
Ex-smokers Pack Years	5.5 (2.5 – 8) **
FEV_1 (L)	2.7 (0.7) *
FEV_1 (%predicted)	105.2 (16.8) *
FVC (L)	3.5 (0.9) *
FVC (%predicted)	112.4 (18) *
Cough Duration (years)	10 (7-16) **

*Mean (SD), **Median (IQR)

Figure 8: Flow Diagram of Study Recruitment, Treatment Allocation and Data Analysis.



Treatment Block A= Nebulised Lidocaine followed by placebo throat spray. Treatment Block B= Nebulised placebo followed by placebo throat spray. Treatment Block C= Nebulised placebo followed by Lidocaine throat spray.

*One patient did not receive the allocated treatment because of fault with the nebuliser.
**One patient was not included in the analysis of cough frequency because of difficulty in distinguishing coughs from other sounds.

2.7.2 Missing Data

Missing data is summarised in Table 3. Given the repeated measures design of the study, some subjects failed to complete the VAS at some time points. One subject had no nebuliser treatment because of fault with the nebuliser machine during the visit day.

Table 3: Missing Data

Outcome variable	Intervention	Total no. of repeated measures	Missing data no.
Urge to cough VAS	Placebo	468	4
	Lidocaine throat spray	468	8
	Nebulised lidocaine	468	23
Cough VAS	Placebo	468	4
	Lidocaine throat spray	468	8
	Nebulised lidocaine	468	22
10-hour cough rate	Placebo	25	0
	Lidocaine throat spray	25	0
	Nebulised lidocaine	25	1

2.7.3 Characteristics of the Urge to Cough

Characteristics of the urge to cough (n= 26) are summarised in Table 4, Table 5, and Table 6. Most subjects felt their urge to cough sensation in the throat and/or neck area. They could locate the area with one finger. This area was mainly in the midline.

Table 4: Location of the urge to cough

UTC location	Frequency*
Nose	1
Throat	6
Neck	18
Supraclavicular notch	1
Sternum	4
Chest	1

*more than one location was chosen by some subjects

Table 5: Lateralisation of the urge to cough location

Site	Frequency (percentage)
Left	1 (3.8%)
Right	1 (3.8%)
Central	20 (76.9%)
Bilateral	4 (15.4 %)

Table 6: Area of the urge to cough

Area	Frequency (percentage)
Localised	18 (69.2%)
Diffuse	8 (30.8%)

2.7.4 Cough Frequency

Intervention effect on the 10-hour cough rate post treatment (after the delivery of both nebuliser and throat spray)

Figure 9 shows the 10 hour cough rate after nebulised lidocaine vs. placebo and lidocaine throat spray vs. placebo.

There was no significant main effect of intervention (*P*= 0.06) *i.e.* the cough rate did not differ significantly between the three interventions however there was a trend towards significance. The paired comparisons of treatment arms suggest a significant difference in the cough rate between lidocaine throat spray and placebo (*P*=0.026). There was no significant difference in the cough rate between nebulised lidocaine and placebo (*P*=0.915) or nebulised lidocaine and lidocaine throat spray (*P*=0.106).

Table 7 shows the estimated marginal geometric means (95% CI) for the cough rate. Table 8 shows the contrast estimates for the difference between lidocaine spray and placebo and the difference between placebo and nebulised lidocaine. Since we are looking at differences in the Log_e-transformed cough rate, these are expressed as ratios of cough rate.

Table 7: Estimated marginal geometric mean 10 hour cough rate (cough/hr) and 95 % confidence interval for the interventions.

Intervention	Geometric Mean	95 % CI*
Placebo	27.8c/hr	18.7 - 41.2
Lidocaine throat spray	22.6c/hr	14.7 - 34.6
Nebulised lidocaine	27.4c/hr	18.8 - 39.9

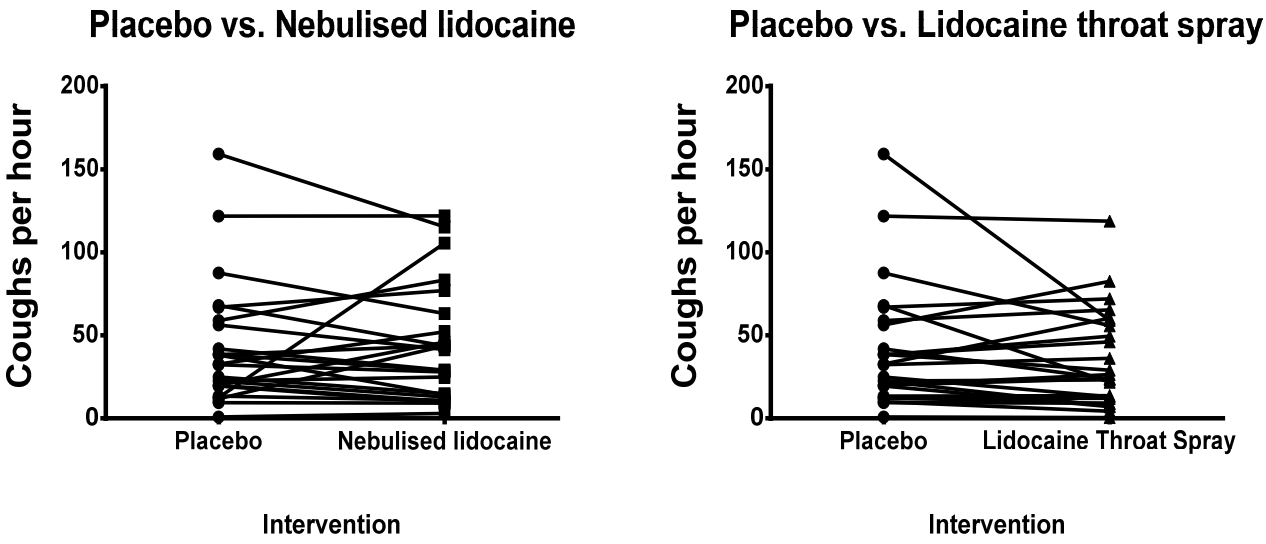
*95 % CI is wide because it represents the between subject variance and does not take into account the within subject study design. Hence comparisons of these confidence intervals between interventions do not reflect the variability of differences within subjects.

Table 8: Interventions 10 hour cough rate pairwise comparisons

Intervention	Mean difference (95%CI)*(Δ)	Ratio	Estimated % change	*P* value for Δ
Lidocaine throat spray vs. placebo	-0.21 (-0.39 to -0.02)	0.81 (0.68 -0.98)	-19% (-32% to -2%)	0.026
Nebulised lidocaine vs. placebo	-0.01 (-0.25 to 0.22)	0.99 (0.78 -1.25)	-1% (-22% to+25%)	0.915
Lidocaine throat spray vs. nebulised lidocaine	-0.1937 (-0.43 to 0.04)	0.82 (0.65 -1.04)	-18% (-35% to +4)	0.106

*Mean difference is the model predicted mean difference in the Log_e transformed 10 hour cough rate.

Figure 9: The cough rate after placebo compared with nebulised lidocaine and lidocaine throat spray



Period Effect on the 10 Hour Cough Rate

There was a significant main effect of period (*P*= 0.001). Estimated marginal geometric means (95% CI) for the different periods are summarised in Table 9. The pairwise comparisons of periods showed that the cough rate averaged across the three interventions was significantly lower in the second period compared with the first and third periods (Table 10).

Table 9: Estimated marginal geometric mean cough rate (cough/hr) and 95 % confidence interval for the periods

Period	Geometric Mean	95 % CI*
First	28.5 c/hr	19.9 – 41.0
Second	20.9 c/hr	13.8 – 31.7
Third	28.8 c/hr	18.9 – 43.8

*95 % CI is wide because it represents the between subject variance and does not take into account the within subject study design. Hence comparisons of these confidence intervals between interventions do not reflect the variability of differences within subjects.

Table 10: Periods cough rate pairwise comparison

Period	Mean difference (95%CI)*(Δ)	Ratio	Estimated % change	*P* value for Δ
Second vs. First	-0.31 (-0.51 to -0.12)	0.73 (0.6 – 0.89)	-27% (-40% to -11%)	0.002
Second vs. Third	-0.32 (-0.54 to -0.10)	0.73 (0.58 - 0.91)	-27% (-42% to -9%)	0.005
First vs. Third	-0.01 (-0.25 to 0.24)	0.99 (0.78 - 1.27)	-1% (-22% to +27%)	0.9490

*Mean difference is the model predicted mean difference in the $\log_e$ transformed 10 hour cough rate.

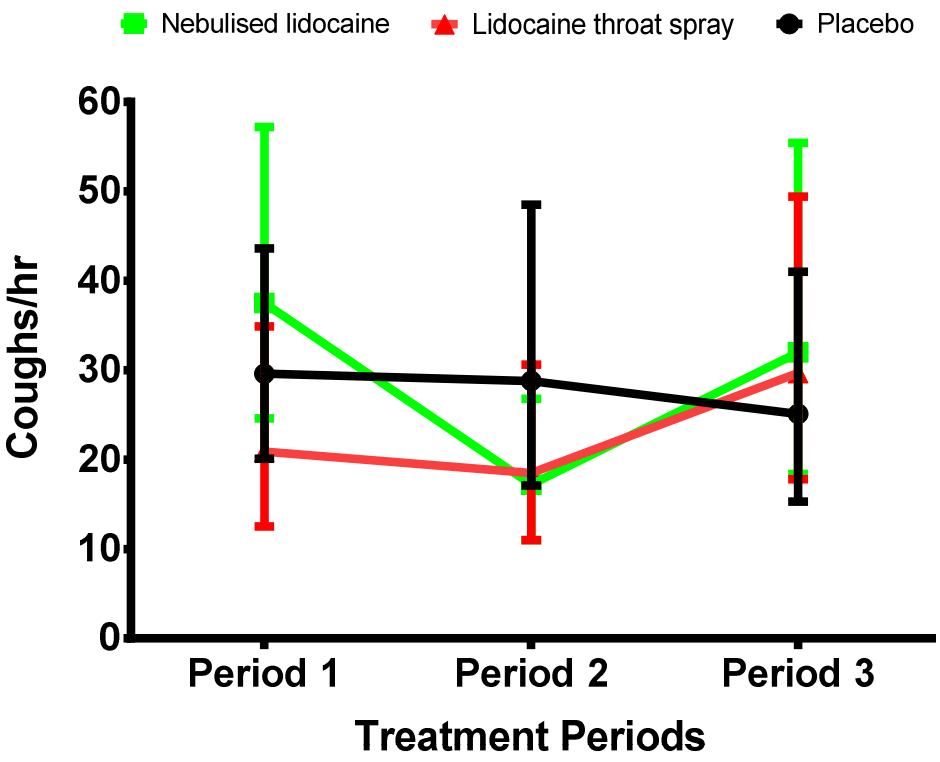
Interaction between Intervention and Treatment Period

There was no significant interaction between intervention and period (*P*= 0.132) i.e. the relationship between the cough rate and periods was not significantly different for the different interventions (Table 11, Figure 10). It appears however that placebo cough rate remained stable for the three treatment periods, whereas it dropped in period 2 with nebulised lidocaine and increased in period 3 with lidocaine throat spray.

Table 11: Estimated marginal geometric mean cough rate (cough/hr) and 95 % confidence interval for the intervention*period interaction

Intervention	Period	Geometric Mean	95% CI
Placebo	First	29.6 c/hr	20.1 - 43.6
	Second	28.8 c/hr	17.1 - 48.5
	Third	25.1 c/hr	15.3 - 41.0
Lidocaine throat Spray	First	20.9 c/hr	12.5 - 34.9
	Second	18.5 c/hr	11.2 - 30.6
	Third	29.7 c/hr	17.8 - 49.4
Nebulised lidocaine	First	37.5 c/hr	24.6 - 57.2
	Second	17.2 c/hr	11.0 - 26.8
	Third	32.0 c/hr	18.4 - 55.4

Figure 10: The interaction between intervention and treatment periods



The cough rate is the estimated marginal geometric mean cough rate. Error bars are for the 95% confidence intervals between subjects.

Effect of Gender

There were only 4 males in the study hence any formal statistical comparison of gender is exploratory and the results need to be interpreted cautiously. Only gross gender differences are able to be detected statistically.

There was no significant main effect of gender (*P*=0.137) (Table 12). There was also no significant intervention*gender interaction (*P*=0.493) i.e. the relationship between the cough rate and the different interventions is not different for males and females (Table 13). From the graph (Figure 11) for the intervention*gender interaction, it appears that the cough rate was lower with lidocaine throat spray compared to placebo and nebulised lidocaine in females, but males did not show such a change. However, the lack of statistical significance is not surprising given the very small number of males.

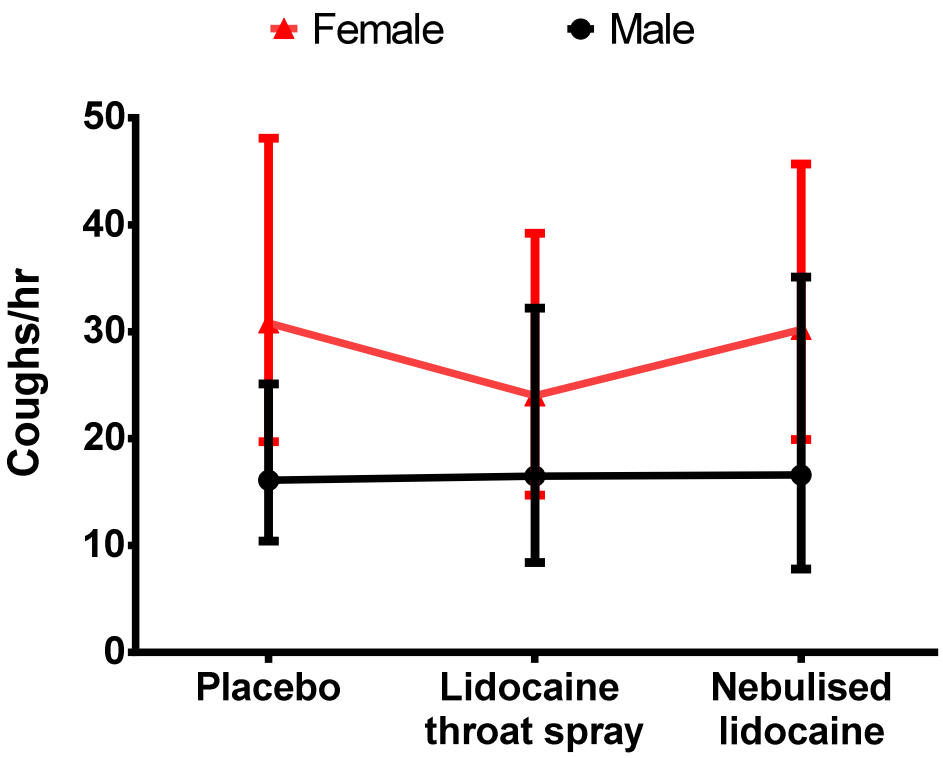
Table 12: Estimated marginal geometric mean cough rate (coughs/hr) and 95 % confidence interval for males and females

Gender	Geometric Mean	95 % CI
Male (n=4)	16.4 c/hr	9.4 – 28.7
Female (n=21)	28.2 c/hr	18.3 – 43.2

Table 13: Back transformed model derived mean cough rate (coughs/hr) and 95 % confidence interval for intervention*gender interaction

Intervention	Gender	Mean	95 % CI
Placebo	Male	16.1 c/hr	10.4 – 25.1
	Female	30.8 c/hr	19.7 – 48.1
Lidocaine throat spray	Male	16.5 c/hr	8.4 – 32.2
	Female	24.0 c/hr	14.7 – 39.2
Nebulised lidocaine	Male	16.6 c/hr	7.8 – 35.1
	Female	30.2 c/hr	19.9 – 45.7

Figure 11: The interaction between intervention and gender



The cough rate is the derived estimated marginal geometric mean cough rate. Error bars are 95 % CI between subjects.

Effect of Urge to Cough Location

I categorised the urge to cough location as mainly throat and/or neck area (throat/neck) or any other area as summarised in table 3. Most patients had urge to cough in their throat/neck area (n=21) compared to only 4 who reported UTC in other areas.

There were only 4 subjects with cough located other than in the throat/neck Hence, again, any formal statistical comparison is exploratory and the results need to be interpreted cautiously. Only gross differences are able to be detected statistically.

There was a significant main effect of the urge to cough location (P=0.018) and significant interaction between the urge to cough location and the interventions (P<0.001) i.e. the relationship between the cough rate and interventions is different for the two urge to cough locations (Table 14, Table 15, Table 16). From the graph (Figure 12), it appears that subjects who reported an urge to cough in the throat/neck had a lower cough rate with lidocaine throat spray compared to placebo and nebulised lidocaine, but subjects who reported an urge to cough in other areas had a higher cough rate with lidocaine throat spray.

Table 14: Estimated marginal geometric mean cough rate (coughs/hr) and 95 % confidence interval for urge to cough location

Urge to cough location	Geometric mean	95 % CI
Throat & Neck (n=21)	23.0 c/hr	14.9 – 35.3
Other (n=4)	47.5 c/hr	31.5 – 71.7

Table 15: Estimated marginal geometric mean cough rate (coughs/hr) and 95 % confidence interval for intervention*urge to cough location interaction

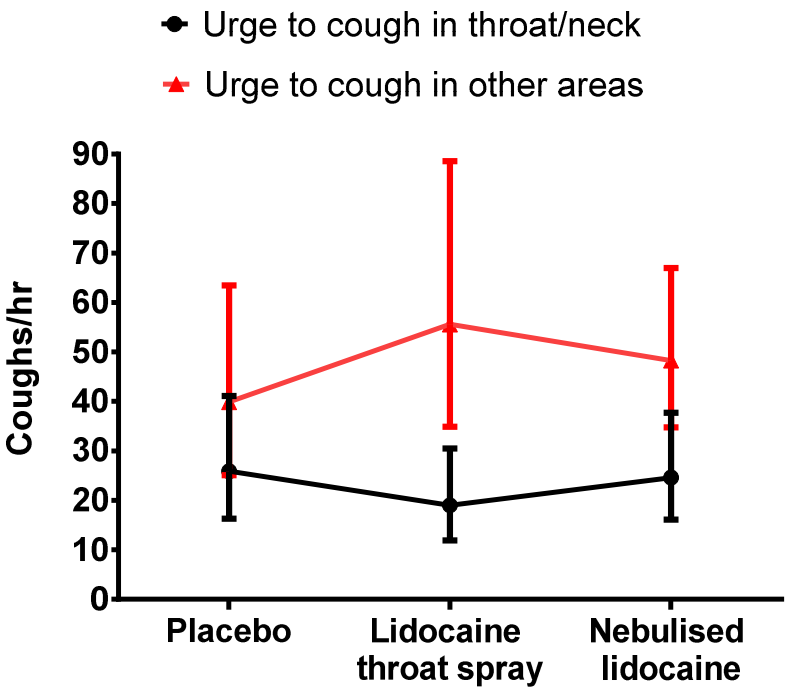
Intervention	UTC Location	Geometric mean	95 % CI	P value for mean difference
Placebo	Throat/neck	25.9 c/hr	16.3 – 41.1	0.220
	Other	39.9 c/hr	25.1 – 63.5	
Lidocaine Throat Spray	Throat/neck	19.0 c/hr	11.9 – 30.5	0.002
	Other	55.6 c/hr	34.9 – 88.6	
Nebulised Lidocaine	Throat/neck	24.6 c/hr	16.1 – 37.7	0.013
	Other	48.3 c/hr	34.8 – 67.0	

Table 16: Estimated marginal geometric mean, mean percentage difference with different interventions based on the urge to cough location

UTC Location	Intervention	Geometric mean (95% CI)*	Mean % difference **(95% CI)	*P* Value**
Throat & Neck	Placebo	25.9 (16.3-41.1)		
	Lidocaine throat spray	19.0 (11.9-30.5)	-26% (-39% to -11%)	0.001
	Nebulised lidocaine	24.6 (16.1-37.7)	-5% (-29% to +28%)	0.743
Other areas	Placebo	39.9 (25.1-63.5)		
	Lidocaine throat spray	55.6 (34.9-88.6)	+39% (+21% to +60%)	<0.001
	Nebulised lidocaine	48.3 (34.8-67.0)	+21% (-1% to +47%)	0.059

*95% CI is wide because it refers to the between-subjects' variability and does not take into account the within subject design of the study.
** The mean % difference and *P* value is compared to placebo.

Figure 12: Intervention and urge to cough location interaction

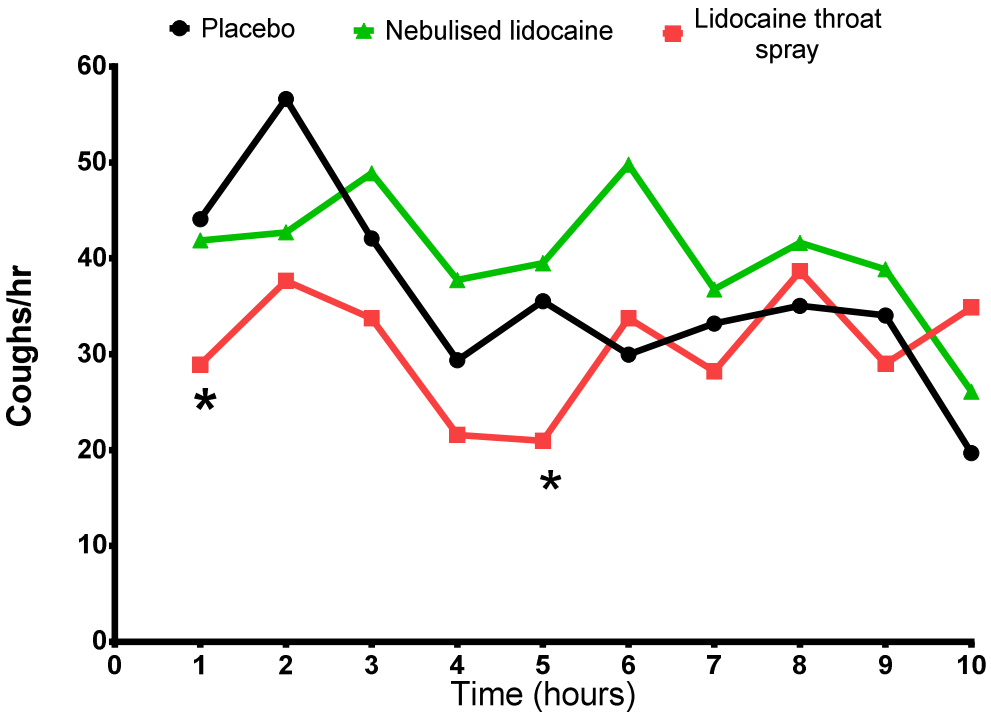


The cough rate is the derived estimated marginal geometric mean cough rate. Error bars are 95 % CI between subjects.

Effect of Interventions on the Cough Rate over the 10-hour Time Period

There is a significant main effect of time ($P<0.001$). The interaction between intervention and time is also significant ($P<0.001$) i.e. the relationship between coughs and time is different for the different interventions. Estimated marginal means for the cough count in each hour is summarised in Table 17. The graph (Figure 13) shows the change of the cough count over time for the three different treatments. The lines cross each other at some points and hence the significant interaction between intervention and time i.e. the three treatments had different trends over time. It appears that mainly in the first hour, the lidocaine throat spray had a significantly lower cough count compared to placebo.

Figure 13: Changes in hourly cough counts over time



*Compared to placebo, there is a significant ($P<0.05$) decrease in the cough count at 1 and 5 hours after lidocaine throat spray.

Table 17: Model predicted mean (95%CI) cough rate and changes over time following each intervention

Intervention	Time (Hours)									
	1	2	3	4	5	6	7	8	9	10
Placebo	44.1 (31.6-61.5)	56.6 (35.7-89.9)	42.1 (30.9-57.2)	29.4 (21.8-39.6)	35.5 (25.2-50.2)	30.0 (21.3-42.1)	33.2 (25.4-43.4)	35.0 (25.5-48.2)	34.0 (21.7-53.5)	19.7 (13.4-29.0)
Lidocaine throat spray	28.9 (19.6-72.8)	37.7 (23.6-60.0)	33.7 (24.9-45.7)	21.6 (14.7-31.7)	21.0 (12.8-23.3)	33.8 (23.1-49.4)	28.2 (19.0-41.8)	38.7 (26.7-56.0)	29.0 (20.3-41.4)	34.9 (20.4-59.5)
Nebulised lidocaine	41.9 (23.2-75.6)	42.7 (26.6-68.6)	48.9 (34.3-69.7)	37.7 (24.4-58.5)	39.5 (24.4-63.9)	49.8 (33.0-75.0)	36.8 (27.2-49.7)	41.6 (26.6-65.1)	38.9 (24.1-62.6)	26.1 (18.0-37.9)

2.7.5 Cough During Treatment

The length of treatment varied from one individual to another, but on average the nebulisation lasted around 23 minutes. In both the placebo arm and lidocaine throat spray arm, patients had nebulised placebo (normal saline). The median (IQR) number of coughs recorded during nebulisation is: 5.5 (0 – 20.5) for placebo group, 2 (0 – 16.8) for lidocaine throat spray group and 15.5 (0.75 – 31.3) for the nebulised lidocaine group.

From the graph (Figure 14), it appears that there are more coughs occurring during lidocaine nebulisation compared to nebulised placebo (in both the placebo and lidocaine throat spray treatments). Using a non-parametric test (because the data followed a highly skewed, non-Normal distribution), this was significantly different among the three different treatments (Friedman's ANOVA, P=0.018).

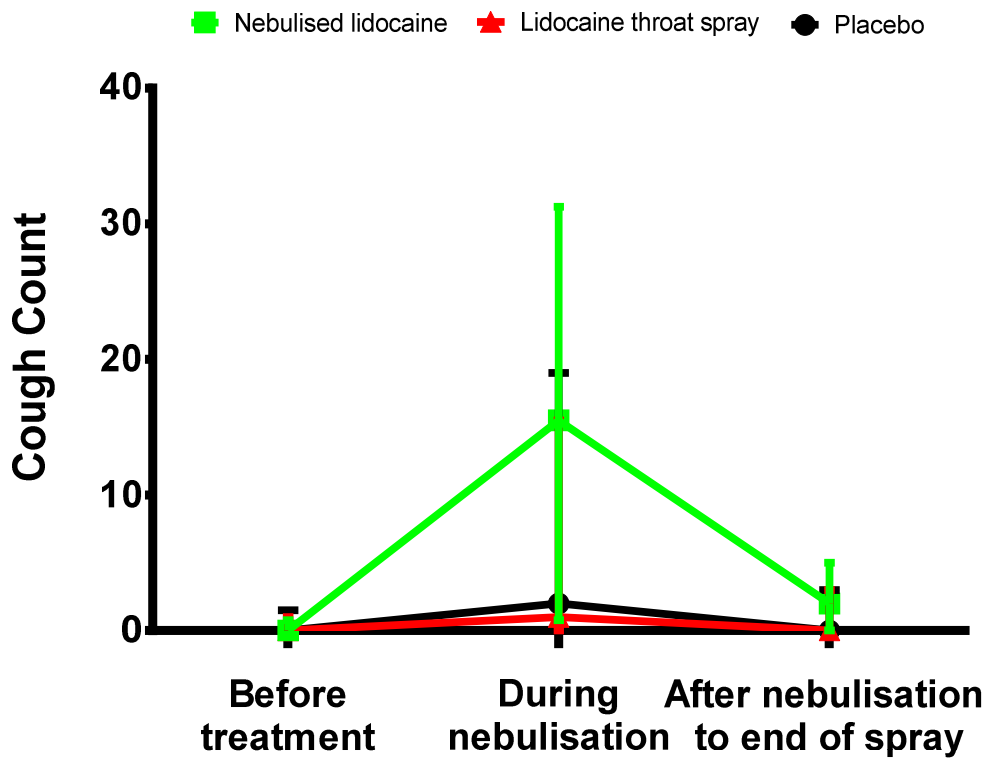
I then carried out non-parametric post hoc tests using Wilcoxon signed-rank tests. I did not use any correction for these post hoc tests, as this is mainly an exploratory analysis for the coughs happening during treatment. Post hoc results:

Placebo (nebulised placebo) vs. nebulised lidocaine: P=0.126

Lidocaine throat spray (nebulised placebo) vs. nebulised lidocaine: P=0.03

Lidocaine throat spray (nebulised placebo) vs. placebo: P=0.338

Figure 14: Cough count during treatment



The graph represents median (IQR) cough counts. Before treatment is the short period (few minutes) before the start of nebulisation. Nebulisation time on average is 23 minutes. Following nebulisation, throat spray was applied. Both lidocaine throat spray and placebo treatments had placebo (normal saline) nebulisation. Subjects had significantly more coughs during nebulised lidocaine compared with normal saline nebulisation. In contrast administration of the throat spray evoked little coughing, regardless of whether it was saline or lidocaine therapy.

2.7.6 Visual Analogue Scales

Cough Severity VAS

There was a trend towards significance for the main effect of intervention (*P*=0.08) for cough severity VAS scores. The nebulised lidocaine and lidocaine throat spray were very similar to one another and showed a trend towards a reduction compared with placebo. The average cough VAS over 10 hours and mean differences compared to placebo are shown in Table 18 and Table 19. The change over time is shown in Figure 15.

There was a significant interaction with time (*P*<0.001). In the first few hours the decrease in VAS scores appeared to be smaller for the placebo group compared to the other two intervention groups, as seen in Figure 15. However, compared to baseline, post treatment cough VAS scores declined substantially with all the interventions including placebo (*P*<0.05).

Similarly, extending the model to include the cough VAS done at 24 hours, there was a trend towards significance for the main effect of intervention (*P*=0.09).

Table 18: Estimated marginal geometric mean cough VAS over 10 hours (mms) and 95 % confidence interval for the interventions

Intervention	Geometric mean VAS	95 % CI*
Placebo	12.5 c/hr	5.7 – 26.1
Lidocaine throat spray	8.2 c/hr	3.6 – 17.5
Nebulised lidocaine	8.3 c/hr	3.8 – 16.8

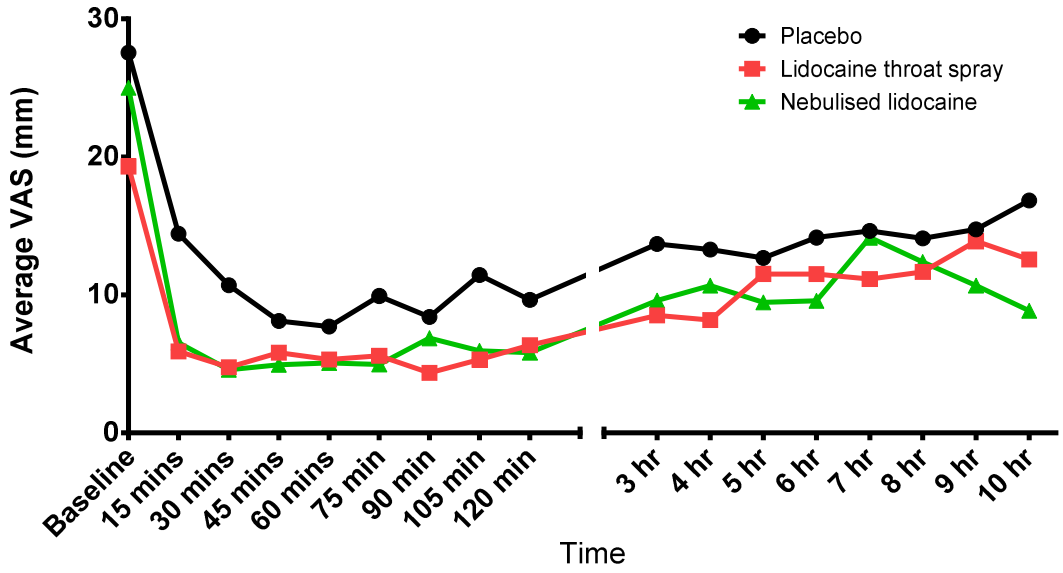
*95 % CI is wide because it represents the between subject variance and does not take into account the within subject study design. Hence comparisons of these confidence intervals between interventions do not reflect the variability of differences within subjects.

Table 19: Pairwise Comparison for the cough VAS over 10 hours

Intervention	Mean difference (95%CI)*(Δ)	Ratio	Estimated % change	*P* value for Δ
Lidocaine throat spray vs. placebo	-0.39 (-0.73 to -0.03)	0.68 (0.48-0.97)	- 32% (-52 to -3)	0.031
Nebulised lidocaine vs. placebo	-0.38 (-0.73 to -0.01)	0.69 (0.48 – 0.99)	- 31% (-52 to -1)	0.042

*Mean difference is the model predicted mean change in the Log_e transformed VAS

Figure 15: Cough VAS



Urge to Cough VAS

There was a trend towards significance for the main effect of intervention (P=0.094). The average urge to cough VAS over 10 hours and mean differences compared to placebo are shown in Table 20 and Table 21.

The change over time is shown in Figure 16. There was a significant interaction with time (*P*<0.001). In the first few hours the decrease in VAS scores appeared to be smaller for the placebo group compared to the other two intervention groups as seen in Figure 16. However, compared to baseline, all post treatment UTC VAS scores were significantly lower for all the interventions (*P*<0.001).

Extending the model to include the urge to cough VAS done at 24 hours, there was no significant main effect of intervention (*P*=0.102).

Table 20: Estimated marginal geometric mean urge to cough VAS over 10 hours (mms) and 95 % confidence interval for the interventions

Intervention	Geometric mean VAS	95 % CI*
Placebo	15.5 c/hr	6.2 – 36.8
Lidocaine throat Spray	12.0 c/hr	4.8 – 28.3
Nebulised lidocaine	10.9 c/hr	4.7 – 24.0

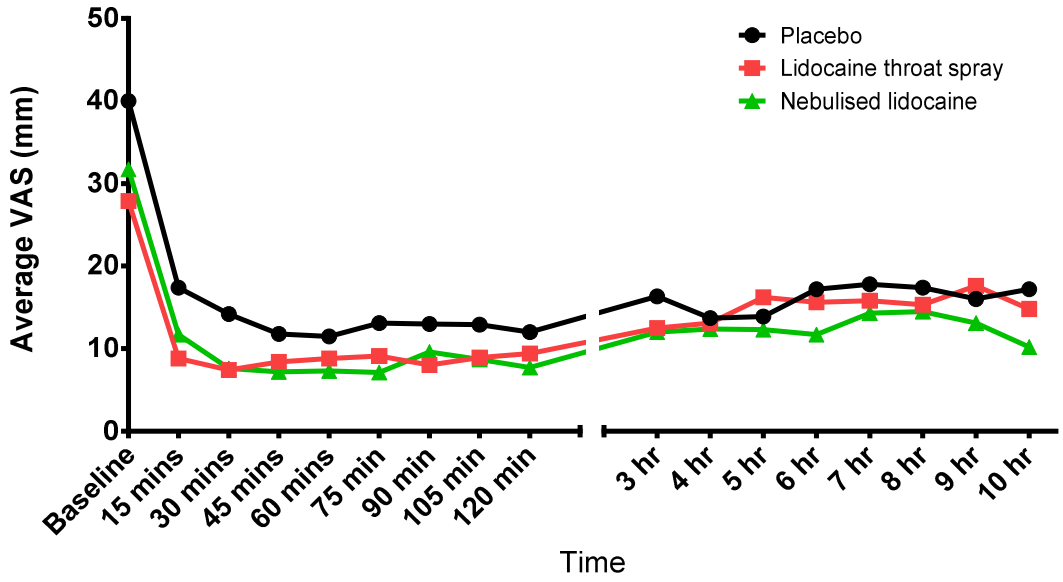
*95 % CI is wide because it represents between subject variance and does not take into account the within subject study design.

Table 21: Pairwise comparison for the urge to cough VAS over 10 hours

Intervention	Mean difference (95%CI)*(Δ)	Ratio	Estimated % change	*P* value for Δ
Lidocaine throat spray vs. placebo	-0.23 (-0.47 to 0.00)	0.79 (0.63-1)	-21% (-37 to 0)	0.054
Nebulised lidocaine vs. placebo	-0.32 (-0.64 to -0.01)	0.73 (0.53-0.99)	-27% (-47 to -1)	0.044

*Mean difference is the model predicted mean change in the Log_e transformed VAS.

Figure 16: Urge to Cough VAS



2.7.7 Debriefing

Table 22: Debriefing

Debriefing	Actual Treatment			
	Nebulised lidocaine	**Lidocaine throat spray**	**Placebo**	**Total**
Nebulised lidocaine	19	3	4	26
Lidocaine throat spray	2	22	2	26
Placebo	5	1	20	26
Total	26	26	26	

As can be seen in Table 22, most patients (19/26 for nebulised lidocaine, 22/26 for lidocaine throat spray and 20/26 for placebo) predicted what treatment they had on the different visits despite the attempt to enhance blinding with including throat spray application. This was retrospect information and therefore subject to recall bias.

2.7.8 Adverse events

None of the adverse events (AEs) were serious; they were mild or moderate in severity. Table 23 summarises the AEs for each intervention. There were no significant changes in ECG or heart rate and no bronchoconstriction was observed in any patient.

Table 23: Adverse events with lidocaine

Adverse event	Nebulised lidocaine (N)	Lidocaine throat spray (N)	Placebo (N)
Swallowing difficulty	1	0	0
Sore throat	1	0	2
Heartburn	1	0	0
Breathlessness	1	0	0
Headache	1	1	1
Panic attack	0	1	0
Itching	0	1	1
Palpitation	0	1	0
Skin bruise	0	1	0
Painful hand	0	1	0

2.7.9 Overall Summary of Results

- The 10 hour cough rate following treatment was not statistically significant among the three different interventions. However, there was a trend towards a significant reduction with lidocaine throat spray compared to placebo.
- The effect of lidocaine throat spray on cough rate might be restricted to patients who reported their urge to cough in the throat.
- During nebulisation, there were significantly more coughs with lidocaine than normal saline.
- The VAS scores for both cough and urge to cough declined substantially after all treatments, including placebo.
- The cough severity VAS for nebulised lidocaine and lidocaine throat spray over 10 hours were similar to one another and had a trend towards reduction compared with placebo.
- The urge to cough VAS over 10 hours for lidocaine throat spray and nebulised lidocaine had a trend towards reduction compared with placebo.
- Most patients predicted correctly which treatment they had on the different visits.

2.8 Discussion

2.8.1 Summary of Main Findings

This study showed that overall a single dose of nebulised lidocaine or lidocaine throat spray did not significantly reduce the 10-hour objective or subjective measures of cough compared to placebo. Even though nebulised lidocaine was previously demonstrated to attenuate the evoked cough reflex sensitivity in healthy volunteers [204] , it did not reduce the spontaneous cough in patients here. This is contrary to the earlier case reports [207, 224], which suggested an anti-tussive effect (even for weeks) with nebulised lidocaine.

2.8.2 *Nebulised Lidocaine*

Given the fundamental role of VGSCs in the initiation and conduction of action potentials in sensory neurons, I did predict a reduction in cough with inhaled lidocaine. There are several possible explanations for nebulised lidocaine having no effect on the cough rate in this study:

1. The administered dose of nebulised lidocaine could have been insufficient. Safety considerations have limited the use of higher doses of nebulised lidocaine. Previous studies [207, 224] that suggested the improvement in cough with nebulised lidocaine had used lower doses (300 – 400 mg) than the one in this study. Noteworthy, observed increased coughs during lidocaine nebulisation may have reduced the delivery of aerosolised particles to the airways, with significantly less dose delivered than had been planned.
2. Lidocaine has a short half-life of approximately 90 minutes [220]. Longer duration of action or repeated doses may have been needed to translate into any anti-tussive effect. This is unlikely as nebulised lidocaine was not associated with significantly lower cough count at any time point, including immediately after treatment (hour 1), compared with placebo.
3. Inhaled lidocaine inhibits the activity of vagal Aδ-fibres and both bronchial and pulmonary C-fibres in guinea pigs [201]. While bronchial C-fibres and Aδ-fibres are believed to activate the cough reflex, animal data suggests that C-fibres in the distal airways and lung parenchyma (pulmonary C-fibres) originate from the nodose ganglion and have an inhibitory function on evoked cough [225, 226].

Therefore, delivery into the smaller airways and pulmonary C-fibres could remove this inhibitory function and explain why nebulised lidocaine did not significantly reduce cough. Indeed a novel VGSC blocker, RSD931, which showed a significant anti-tussive effect in guinea pigs, stimulated (rather than inhibited) the action of pulmonary C-fibres [201].

4. The sensitivity of VGSCs in cough vagal afferents to lidocaine may be different to the somatosensory afferents. Therefore, more potent and novel VGSCs blockers may be needed to explore this. Certain subtypes of VGSCs (NaV 1.7 – 1.9) are distributed specifically on primary sensory neurons [114]. Targeting those channels may be more appropriate as an anti-tussive medication. This would also allow the use of more potent drugs and avoid potential cardiac and CNS toxicity. For example, in guinea pigs silencing RNA directed against NaV 1.7 gene blocked the conduction of action potentials in airway C-fibres, and inhibited citric acid induced cough [125]. In addition, PGE induced upregulation of NaV 1.8 sodium currents has been shown previously [126]. Targeting TTX-resistant (NaV 1.8 and 1.9) NaV channels could result in reducing the cough hyper-responsiveness associated with airway inflammation [227].
5. The negative study findings could support central sensitisation as the most likely mechanism, at least in some patients, with chronic cough. Sensitisation of central neurons in the NTS would likely enhance the cough response to a wide variety of stimuli. Chronic cough patients demonstrate heightened sensitivity to various environmental and chemical exposures. Therefore, central sensitisation could be a better explanation for chronic cough rather than wide spread upregulation of peripheral transducing receptors. Also, as summarised in section 1.4.2, the association between distal oesophageal reflux events and cough is probably explained by the convergence of airway and oesophageal vagal afferents in the NTS.

The increased cough during lidocaine nebulisation was unanticipated, but there are a number of possible explanations for this observation:

1. Lidocaine may directly activate TRPV1 channels on the nociceptive sensory nerves. [228]. This action of lidocaine might explain the painful burning sensation that immediately accompanies the local injection of lidocaine into the skin or into mucous membranes; the effect subsides once lidocaine blocks the voltage-gated sodium channels, inhibiting signal transmission.

2. Acid solutions are thought to trigger cough via TRPV1/ASIC mechanisms [62]; the pH of such solutions is usually less than 4 [229]. The pH of 10% lidocaine is 4 – 5.5, which is an acceptable pH range for nebulisation. Furthermore, normal saline has a similar pH of 5.4 and had lower cough counts than lidocaine. Therefore, the increased cough count during lidocaine nebulisation is unlikely to be related to the pH of the solution.
3. The osmolarity of 10% lidocaine is 693 mosmol/L and normal saline is 308 mosmol/L. Thus, the hypertonicity of the 10% lidocaine solution is a possible explanation.

2.8.3 *Lidocaine Throat Spray*

Somatic sensations from the pharynx are projected in the pharyngeal branches of the glossopharyngeal and vagus nerves; both terminating in the spinal nucleus of the trigeminal nerve (Figure 17 and Figure 18). This is adjacent to second-order neurons of cough afferents in the nucleus of the solitary tract in the medulla. It is possible that there is a cross talk between afferents from the pharynx and airways either in the vagus nerve or in the central nervous system because of the close proximity of the nucleus of the solitary tract to the spinal nucleus of the trigeminal nerve (Figure 19). This is analogous to the referred left arm pain from myocardial ischemia because of the convergence of both cardiac afferents and arm somatic sensory afferents in the dorsal horn cells of the spinal cord. The sensation of irritation and urge to cough arising from the throat has been assumed to be a referred sensation from the airways, as a result of cross-organ talks between the airways and the pharynx. Thus, lidocaine throat spray was not predicted to be anti-tussive. An alternative explanation would be that the pharynx becomes hypersensitive in chronic cough patients and plays a role in the initiation of cough. This could occur via a specific effect in the pharynx or represent a general vagal sensitivity.

Although, the cough frequency after lidocaine throat spray was lower than placebo, the overall result of the study did not reach statistical significance. The estimated change (-19%) is less than what the study was powered to detect and it is also questionable whether this is a clinically important effect. Nevertheless, it remains an interesting secondary finding. In the sub-analysis, the effect of lidocaine throat spray was restricted to those patients with an urge to cough located in their throat/neck, but this needs to be interpreted with caution because most subjects (n=21) reported

an urge to cough in their throat/neck compared with only 4 subjects who reported an urge to cough arising in the chest. This suggests the upper airway (pharynx and larynx) may play a role in initiation of cough; however the size of the effect was not large. To explore this further, a RCT of an anti-tussive delivered as a throat spray vs. placebo in chronic cough patients with throat irritation vs. those without throat irritation would be needed. Blinding would be a limitation if lidocaine was used, given this and the observed period effect, a parallel design would be optimal. Novel molecules that block VGSCs without anaesthetising the oropharynx would be ideal.

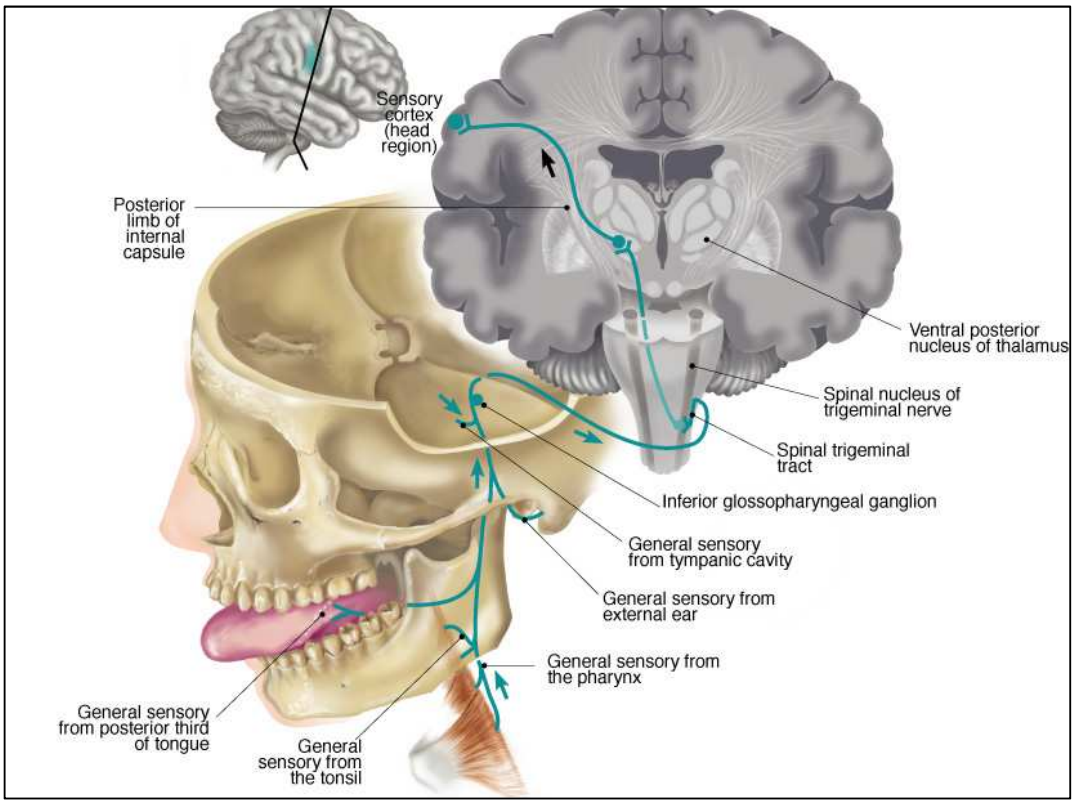
Given that I estimated that the fraction of nebulised lidocaine deposited in the throat is comparable to the lidocaine throat spray dose, the observed reduction in cough count with lidocaine throat spray should have been seen as well with nebulised lidocaine. However, the estimation is difficult and may have not been accurate. Increased coughing during the lidocaine nebulisation as mentioned above may have reduced the delivery to the throat. Other reasons for this discrepancy are:

1) Saline throat spray, which immediately followed nebulised lidocaine treatment, may have washed off lidocaine particles deposited in the throat,

2) The inhibitory effect of lidocaine on pulmonary C-fibres, as described above, may have counteracted any anti-tussive effect on the throat,

3) Lidocaine may have reduced the cough rate by acting on distal oesophageal vagal afferents after it was swallowed and the dose swallowed may have been greater with lidocaine throat spray than with nebulisation. To investigate this is challenging; this would require a further study on the effect of lidocaine infused by a catheter into the oesophagus on the cough rate. Infusing lidocaine into the oesophagus via a catheter is invasive, and the presence of the catheter influences cough significantly [222]; therefore interpretation would be difficult. Although lidocaine solution could potentially be swallowed, this would inevitably also expose the throat.

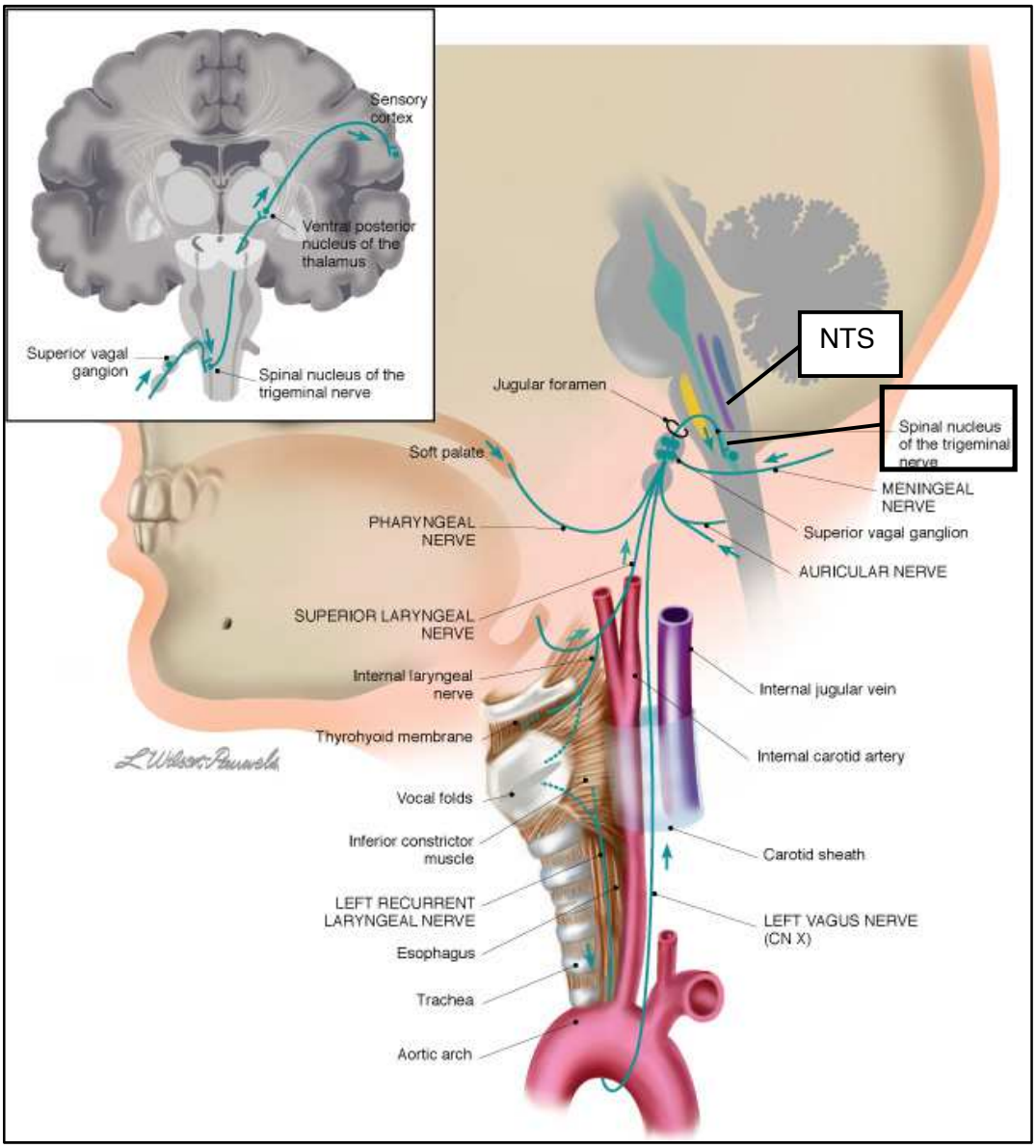
Figure 17: Glossopharyngeal nerve innervating the pharynx



Afferents from the glossopharyngeal nerve convey sensory signals from the pharynx to second-order neurons in the spinal nucleus of the trigeminal nerve.

Reproduced with permission from "Cranial nerves in Health and Disease" 2002, Wilson-Pauwels, Akesson, Stewart, Spacey, B C Decker Inc.

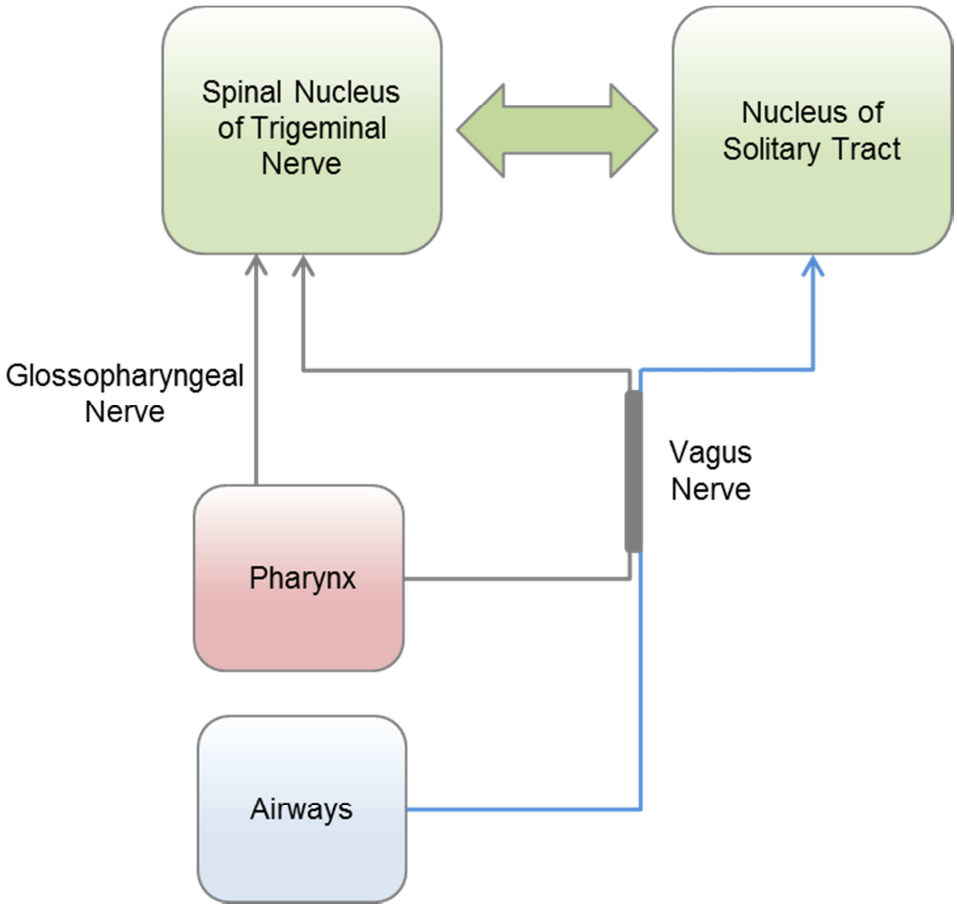
Figure 18: Vagal innervation of the pharynx



Afferents from the vagal nerve also convey sensory signals from the pharynx to second-order neurons in the spinal nucleus of the trigeminal nerve, which lies adjacent to the nucleus of the solitary tract (NTS) allowing cross talks with airway vagal afferents terminating in the NTS.

Reproduced with permission from "Cranial nerves in Health and Disease" 2002, Wilson-Pauwels, Akesson, Stewart, Spacey, B C Decker Inc.

Figure 19: Cross talks between the airways and pharynx



2.8.4 Study Limitations

- The main limitation of this study was the inadequate blinding because of the accompanying oropharyngeal anaesthesia with lidocaine; this was partially offset by having two active lidocaine arms. Future subtype-specific voltage-gated sodium channel blockers may be able to provide an anti-tussive effect without the oropharyngeal anaesthesia.

- The period effect, with cough rates at visit 2 being lower than at visits 1 and 3, was surprising and hard to explain. However, this was accounted for in the modelling.

2.9 Conclusions

The following conclusions can be drawn from the study:

- Nebulised lidocaine was not effective in reducing the cough rate in patients with chronic cough.

- This study suggests that in patients with chronic cough, and urge to cough arising from the throat, lidocaine throat spray could be an effective anti-tussive agent. To investigate this further, placebo controlled parallel group study in patients with and without throat irritation would be needed.

- Future subtype (1.7 – 1.9) specific voltage gated sodium channel blockers have potential as anti-tussive agents in chronic cough especially if applied to the pharynx.

CHAPTER 3

P2X3 Antagonism in Chronic Cough

3.1 Background and Rationale

Patients with chronic cough often report coughing in response to minimal stimuli such as talking, laughing and exposure to perfumes [230], and have been shown to have heightened sensitivity to inhaled irritants such as capsaicin compared to healthy humans [100, 183]. Hyper excitability of the vagal sensory pathways mediating the cough reflex is considered to be the most likely mechanism leading to chronic cough.

P2X3 receptors are ATP-gated ion channels and are thought to play an important role in neuronal hyper excitability [68]. This is supported by data from both pre-clinical and clinical studies of healthy volunteers investigating the effect of ATP and P2X3 receptors in mediating both physiological and exaggerated somatosensory and visceral pain [231] . In guinea pigs, P2X3 receptors have been shown to be expressed in jugular and nodose C-fibres innervating the airways [87]. The role of P2X3 in mediating cough and cough hypersensitivity is not very clear and warrants further investigations. Aerosolised αβ-methylene blue ATP, a non-degradable form of ATP and selective P2X agonist, did not induce cough in conscious guinea pigs [30]. In humans, the only two studies of ATP inhalation have shown contradictory results. The aim of both studies was to evaluate ATP-induced bronchoconstriction. One study made a comment about cough provoked by ATP inhalation [90], whereas the other one did not [91]. Nevertheless, it has been demonstrated that aerosolised ATP augments the cough response to citric acid in guinea pigs in a concentration dependent manner, suggesting that ATP plays a role in sensitising the cough reflex [88]. This effect was abolished by pre-treatment with an inhaled P2X1-4 antagonist, TNP-ATP [88]. It is not known yet whether an upregulation of the P2X3 receptors happens in subjects with chronic cough.

The development of compounds that could antagonize P2X3 channels and be approved for use in clinical trials has been quite challenging [231]. Thus far, the lack of selective and potent P2X3 antagonists suitable for use in humans has hindered our ability to explore the therapeutic potential of this class of medications. More importantly, our understanding of the clinical relevance of the mechanistic role of P2X3 in sensitisation of neuronal pathways implicated in various diseases has also been limited. AF-219 is a first-in-class potent and selective oral antagonist of P2X3-containing receptors (P2X3-P2X2/3) that has progressed to the stage of clinical trials. This could potentially shed light on the role played by P2X3 receptors in

cough neuronal hyper excitability and suggest a novel class of anti-tussive therapies. In addition, if a significant and marked anti-tussive effect is observed, it will aid exploration of the correlation between the change in objective cough frequency and patient related outcomes.

3.2 Hypothesis

I hypothesised that P2X3 receptors play a role in the hyper excitability of vagal pathways mediating the cough reflex leading to chronic cough.

3.3 Aim

- To investigate the role played by P2X3-containing receptors in chronic cough

3.4 Study Objectives

Primary Objective:

- To evaluate the effectiveness of AF-219 in reducing daytime objective cough frequency.

Secondary Objectives:

To evaluate the effectiveness of AF-219 in:

- reducing night time objective cough frequency;
- reducing subjective scores of cough severity;
- showing global rating of change scale; and
- improving cough-specific quality of life.

To evaluate the safety of AF-219 in a subject population with chronic cough.

3.5 Study Endpoints

Primary Endpoint:

- Change from baseline in daytime objective cough frequency after 2 weeks of AF-219 therapy compared with placebo treatment.

Secondary Endpoints:

Change from baseline after 2 weeks of AF-219 therapy compared with placebo treatment in:

- night time objective cough frequency
- cough VAS day/night
- urge to cough
- global rating of change
- cough quality of life questionnaire (CQLQ)

3.6 Methods

3.6.1 Study Subjects

Patients with idiopathic chronic cough or chronic cough resistant to treatment of specific triggers were recruited from our tertiary cough clinic (University Hospitals of South Manchester, UK) over a 19-month period from August 2011 to March 2013. Patients were investigated according to a diagnostic algorithm [211, 212]. The diagnostic algorithm allowed patients to be investigated thoroughly for specific triggers of chronic cough. All patients had full lung function testing, methacholine/histamine challenge testing, nasoendoscopy, high resolution computerised tomography of the chest, and bronchoscopy (with lavage for differential cell count, and endobronchial biopsies). All Patients were treated for any detected triggers of chronic cough (inhaled corticosteroid for asthma/eosinophilic bronchitis, corticosteroid nasal spray and anti-histamines for post-nasal drip syndrome, and proton pump inhibitor twice a day and nocturnal ranitidine for gastro-oesophageal reflux). Patients with cough refractory to treatment of underlying triggers were considered eligible for the study.

Approvals from Greater Manchester South Research Ethics Committee (REC: NW/11/0231) and the Medicines and Healthcare Products Regulatory Agency (39495/0001/001) were obtained prior to the start of the study. The trial was registered at clinicaltrials.gov (NCT01432730). All patients signed an informed consent form and the study was conducted according to the Declaration of Helsinki.

3.6.2 Inclusion and Exclusion Criteria

Inclusion Criteria

- Men and women ≥18 and ≤80 years of age
- Normal chest radiograph
- Idiopathic or treatment resistant cough
- Women of child-bearing potential must have had a negative pregnancy test and agreed to use one of the following acceptable birth control methods from screening visit to follow-up contact: true complete abstinence, surgical sterilisation of either the female subject in study or of her male partner,

established hormonal contraception, an intrauterine device (IUD) or intrauterine system (IUS), double barrier method.

Exclusion Criteria

- Current smoker, individuals who had given up smoking within the past 6 months, or those with >20 pack-year smoking history
- Abnormal spirometry
- History of upper respiratory tract infection within 4 weeks of the baseline visit
- Body mass index (BMI) <18 or >35
- History of urinary tract infection within 6 months prior to screening
- History or symptoms of renal disease, including nephro/urolithiasis, pyelonephritis
- History of conditions or disorders which predispose to nephrolithiasis
- History of stroke or transient ischemic attack within 2 years prior to screening
- History of life-threatening neoplasms within 5 years prior to study entry
- History of drug or alcohol dependency or abuse within approximately the last 5 years
- Clinically significant depression or a history of suicide behaviour or suicidal ideation
- Uncontrolled or unstable clinically significant disease
- Any condition possibly affecting drug absorption (e.g., gastrectomy, vagotomy, or bowel resection)
- Estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2
- Screening systolic blood pressure greater than 160 mm Hg or a diastolic blood pressure greater than 90 mm Hg
- Clinically significant abnormal electrocardiogram (ECG) at screening
- Significantly abnormal laboratory tests
- Screening haemoglobin A1C ≥7.0

- Screening microscopic haematuria, defined as ≥ 5 red blood count per high-power field on microscopic urinalysis
- Post-void residual >200 mL at screening
- Clinically significant abnormalities on renal/bladder ultrasound at screening
- History of a cutaneous adverse drug reaction to sulphonamides
- Treatment with an investigational drug within 60 days preceding the first dose of study medication
- Flu vaccination within 30 days of Day 0
- Subjects who are known to be Human Immunodeficiency Virus (HIV) positive or known to have viral hepatitis (A, B, or C)

Prohibited Medications

Medications that may affect the cough reflex: Opioids were not allowed from 1 week prior to the baseline visit through to the follow-up visit. Pregabalin/Gabapentin was not allowed 4 weeks prior to the baseline visit through to the follow-up visit. Treatment with an ACE-inhibitor during the study or within 4 weeks prior to Day 0 was not allowed.

Medications that might interact with AF-219, including histamine H2 antagonists, proton-pump inhibitors, sucralfate, and antacids (all of which may reduce AF-219 absorption) were not allowed from Day 0 through to the follow-up visit. If previously treated with these agents, last use must have been at least 2 weeks prior to Day 0.

Medications or supplements which may increase the risk of renal stones, including acyclovir, sulfadiazine, sulfonamide, triamterene, vitamin C in excess of 2000 mg/day, vitamin D in excess of 4000 IU/day, and calcium supplements in excess of 2500 mg/day, were not allowed from Day 0 through to the follow-up visit. If previously treated with these agents, last use must have been at least 4 weeks prior to Day 0.

3.6.3 Study Design

This was a randomised, double-blind, placebo-controlled, crossover, single centre, phase 2, proof-of-concept study with two 2-week treatment periods separated by a 2-week washout period.

3.6.4 Interventions

- Oral AF-291 600 mg (two 300 mg tablets) twice daily or matched placebo given for two weeks. Study medication is taken on a full stomach.

3.6.5 Randomisation: Sequence Generation

Subjects were randomly allocated one of the treatment sequences (AF-219/Placebo or Placebo/AF-219) according to a computer-generated randomisation schedule provided by the hospital statistical department. In each treatment period, the hospital pharmacy dispensed AF-219 or the matched placebo according to the randomisation schedule.

3.6.6 Study Visits and Procedures

After obtaining informed consent, the following screening procedures were performed: medical history, physical examination, vital signs, weight, height, CXR, collecting blood for FBC, U&Es, LFTs, calcium, fasting glucose, HbA1C and serum pregnancy test, 12-lead ECG, spirometry, renal ultrasound including post void bladder volume, and urinalysis.

Following the screening period of up to 3 weeks, eligible patients were randomised into two 2-week treatment periods (AF-219 or placebo) with a 2-week washout period.

In each treatment period, patients were scheduled to have the following visits and procedures (Figure 20):

Day 0:

- Baseline ambulatory 24 hour cough recording.
- Baseline visual analogue scales for cough severity and urge to cough
- Baseline CQLQ (Appendix 2)

Day 1: Randomisation to one of the study treatments: AF-219 or placebo

Day14: End of treatment period ambulatory 24 hour cough recording.

Day 15: End of treatment period outcome measures:

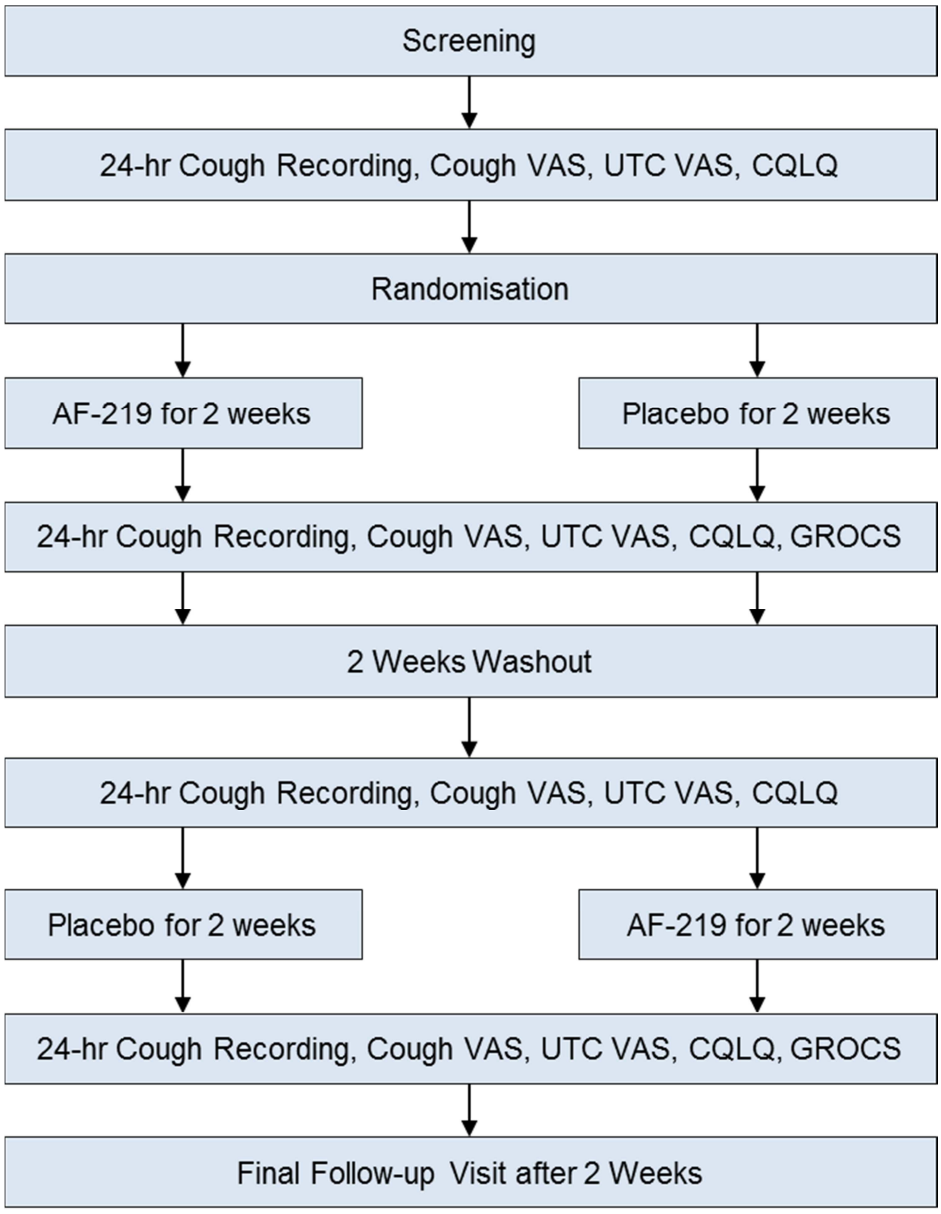
- Cough severity VAS
- Urge to cough VAS
- CQLQ
- Global rating of change scale (GROCS) (Appendix 3)

After a washout period of 2 weeks, patients then crossed over to the other study treatment with repeat of visits Day 0, 1, 14 and 15.

Safety measures/monitoring:

At the beginning and end of each treatment period, routine blood tests, ECG and renal ultrasound were performed.

Figure 20: Study procedures



3.6.7 Detailed Description and Justification of Methodology

Study Medication and Dose Rationale

The following information is summarised from the investigator's brochure for AF-219.

- AF-219 is a potent (IC_{50} 30-200 nM) and selective antagonist of the homomeric P2X3 and the heteromeric P2X2/3 receptors (over 100-fold selectivity over other P2X channels and no activity at other receptors or enzymes in *in vitro* experiments).

- AF-219 reduced pain sensitivity in animal models of inflammatory and neuropathic pain. It also reduced bladder reflexes triggered by filling volumes in anaesthetised rats.

- Peak concentration is reached 1 – 2 hours post dose (Tmax).

- Terminal half-life is approximately 8 – 10 hours.

- AF-219 has a low penetration (<10%) to the central nervous system in animal studies.

- 600 mg BD was chosen to obtain an exposure associated with safe maximum efficacy. This was based on nonclinical models and clinical phase 1 studies examining the plasma concentrations after exposure to different doses of AF-219.

Ambulatory Objective Cough Recording

For the purpose of obtaining objective cough frequency, the VitaloJAK™ cough monitor (Vitalograph Ltd, UK), a custom-built digital recording device, was used. VitaloJAK™ features an air microphone attached to the subject's lapel and an adhesive sensor attached to the ches t wall over the sternum. The device records all sounds (8 kHz, 16 bit wave format) continuously over 24 hours. Data was written to a 4 Gigabyte compact flash data card, which was then downloaded onto a personal computer and archived on a digital versatile disc. Validated custom-written software was then used to compress the recording from 24 hours to a shorter file by detecting all potential cough sounds and cutting out non-cough sounds such as silence, background noise and speech [232]. Trained staff then manually listened to the compressed file and counted the number of explosive cough sounds using an audio editing software package (Adobe® Audition® 3.0). The results were expressed as explosive cough sounds per hour. This is a validated and repeatable objective measure of cough frequency [186], with excellent intra-and inter-observer agreement [179].

Validation and Quality Control checks of the cough recordings by the VitaloJAK™

Processing of the 24hr recordings with the cut down software has been shown to retain a median of 100 % (100-99.8) of the coughs manually counted in the full 24 hour recordings [232]. Ten per cent (equating to 2.5 hours) of every 24 hour recording was double counted by experienced research assistants to quality control check the manual cough counting. The Median difference between the original cough count and the repeat (QC) cough count was 1.2 cough/hr. There was excellent inter-observer agreement (intra-class correlation co-efficient =0.999, $p<0.001$). Nine (10.4%) of the recordings fell outside the predefined 95% limits of agreement (+4.5 to -4.8 cough/hr). Eight of the nine recordings were from three individual subjects. All visits were counted by the same person so may not be an issue for detecting within-subject changes. Also, some patients had extremely high cough count so errors are proportionately small.

3.6.8 Sample Size and Statistical Analysis

Sample Size Determination

With 20 subjects completed, this study had approximately 90% power to achieve statistical significance, with a 5% significance level (2-sided test), if there was a 50% change in daytime objective cough frequency with AF-219. This assumed a mean daytime cough frequency of 25coughs/hour at baseline and a fall in the placebo treated group of 5 coughs/hour (based on data from [186]).

Statistical Analysis

To determine the significance of change in primary and secondary endpoints from baseline to treatment (week 2) for AF-219 compared with placebo, linear mixed-effects models were used (SPSS, version 20.0). The models accounted for treatment period, sequence, and subjects nested within sequence by fitting intervention, period, and sequence as fixed effects and subjects within a sequence as a random effect. Two baseline covariates were included: the average of the baseline measures for the subject and the period specific baseline (difference of the period baseline from the average baseline for the subject). The average baseline covariate accounts for the between subject's variability and the period specific baseline accounts for within subject's variability between periods. Any carryover effect was examined by the sequence term in the model. Cough frequency was $\log_{10}$ transformed to obtain normal distribution before being applied in the model; in the case of night time cough frequency, 0.1 was added to all data before transformation because it contained zero numbers. If data were log transformed, change from baseline is shown as a ratio of treatment/baseline. Model estimated mean change for AF-219 and placebo, and difference (AF-219 vs. Placebo), 95% confidence interval, and 2-sided p value are presented. $P < 0.05$ is considered to be significant.

Rationale for Use of Mixed-Effect Models Rather than Fixed-Effect Models

In fixed-effects models, only subjects with data from 2 periods will contribute to the analysis. However, with fitting random effects in the model, such as in mixed-effect models, subjects with only one period data will still contribute (likely small contribution) to the model estimates. This is because subjects with one period worth

of data will generate a between subject comparison, and this is then combined with the within subject comparison. Since the between subject variance is high in chronic cough patients, and the contribution of the two estimates are based upon weights driven by the variance of the estimates, the contribution will still be low.

Pre-Defined Subject Populations for Analysis

Intention to Treat (ITT): Subjects who were randomised and received at least one dose of the study medication.

Per Protocol (PP): Subjects who have completed 2 weeks of both treatment periods.

Handling of Missing Values

To assess the influence of missing data on outcome measures, three different analyses were conducted - ITT observed case, Per Protocol, and ITT worst case.

- ITT observed case analysis: this included ITT subject population with at least one period of data (baseline and week 2). Subjects who withdrew early from the study were asked to complete patient related outcome measures based on the period that they were taking the study medication; these data were included in the observed case analyses. Similarly, if week 2 cough recording was done when the subject had stopped taking the medication before that, data were still included in this analysis.

- Per protocol complete-case analysis: Subjects who have complete data (baseline and week 2) from both treatment periods and had not withdrawn early from the study.

- ITT worst-case analyses: This was performed only on the daytime cough frequency i.e. the primary endpoint. It involved imputing the worst change, i.e. the largest increase from baseline as opposed to a decrease, in the relevant treatment group, provided baseline measures were not missing. If the week 2 cough monitor was performed when the subject had stopped taking the medication before that, the data was considered missing and replaced by the worst change. In the case of missing data because of a failed recording, this was considered to be uninformative missing data; hence, no worst-case imputation was done.

I have also explored the relationship between changes in daytime cough rate and changes in subjective measures (cough severity VAS, urge to cough VAS, and CQLQ) using Pearson's correlation coefficient.

3.7 Results

3.7.1 Demographics and Baseline Characteristics

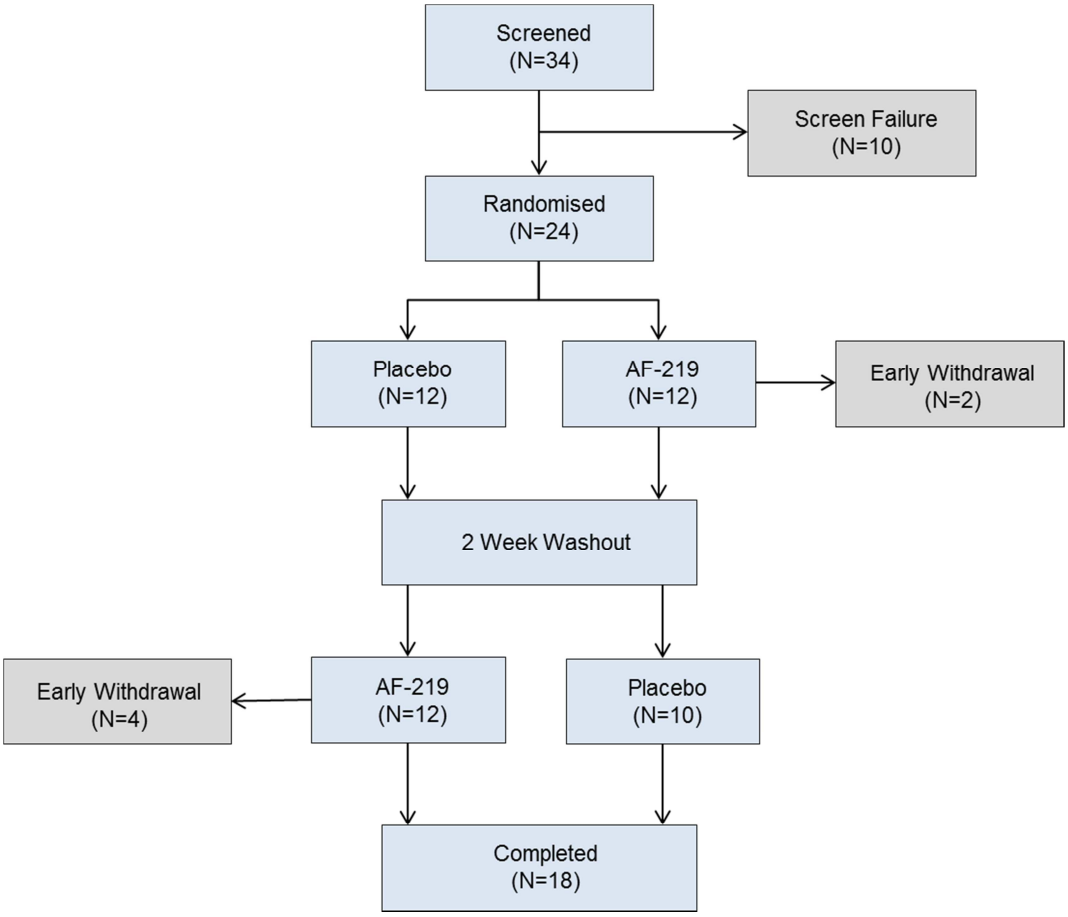
A total of 24 subjects were enrolled into the study (see the flow diagram in Figure 21). Study subjects were predominately middle-aged females, which is representative of the population attending chronic cough clinics. Table 24 summarises the subject characteristics.

Table 24: Demographics and baseline characteristics of all randomised subjects

Total participants, n	**24**
Women, n (%)	18 (75)
Age, yr	54.5 (±11.1)
Race, n (%)	
White, n	23
Mixed, n	1
Body mass index	25.9 (±3.9)
Smoking history	
Never smoker, n (%)	16 (66.7)
Ex-smoker, n (%)	8 (33.3)
Pack years for ex-smokers	5.7 (4.3)
Cough duration, yr	11 (±6.8)
Nature of Cough	
Dry, n (%)	18 (75)
Productive, n (%)	6 (25)
FEV1, % predicted	103.4 (±15.2)
FVC, % predicted	108.2 (±10.7)
FEV1/FVC ratio	79.5 (5.3)
Total cough frequency[¥], c/hr	20.8 (12.8 – 34.0)
Daytime cough frequency[¥], c/hr	30.7 (18.7 – 50.2)
Night-time cough frequency[¥], c/hr	1.8 (0.8 – 4.0)
Daytime cough VAS, mm	58.2 (21.1)
Night-time cough VAS, mm	26.3 (24.6)
Urge to cough VAS, mm	68.7 (21)
CQLQ (n=23)	58.3 (10.5)

Results shown are mean (SD) unless otherwise stated. ¥ geometric mean (95% CI). Cough frequency, VAS and CQLQ are from baseline period 1. CQLQ missing n=1.

Figure 21: CONSORT Flow diagram



Screen Failures = High BMI (1), High BP (2), High HbA1C (2), Hypercalcaemia (1), Residual bladder volume > 200 ml (2), Previous history of septrin induced rash (1), episodic cough (1).

Early withdrawal = Taste disturbance (6) +/- increase salivation (1), throat tightness and soreness (1), worsening of cough, acid reflux and sensation of globus (1).

3.7.2 Analysis Populations

ITT: 24 patients were randomised and received at least one dose of study medication. 2 subjects withdrew from the study after taking AF-219 in treatment period 1; therefore 24 subjects have received AF-219, but only 22 subjects had placebo. For each study endpoint, the number of patients with missing data and the reasons for this are explained.

PP: 18 patients completed both treatment periods (Figure 21). However, prior to unblinding the study, 2 subjects who had developed symptoms of cold during one of the treatment periods were excluded from the PP analysis; therefore, only 16 subjects were included in the PP population. For each study endpoint, the number of subjects included in the PP analysis and explanation of missing data is provided.

3.7.3 Effect of AF-219 on Daytime Cough Rate

ITT Observed Case Analysis (n=24)

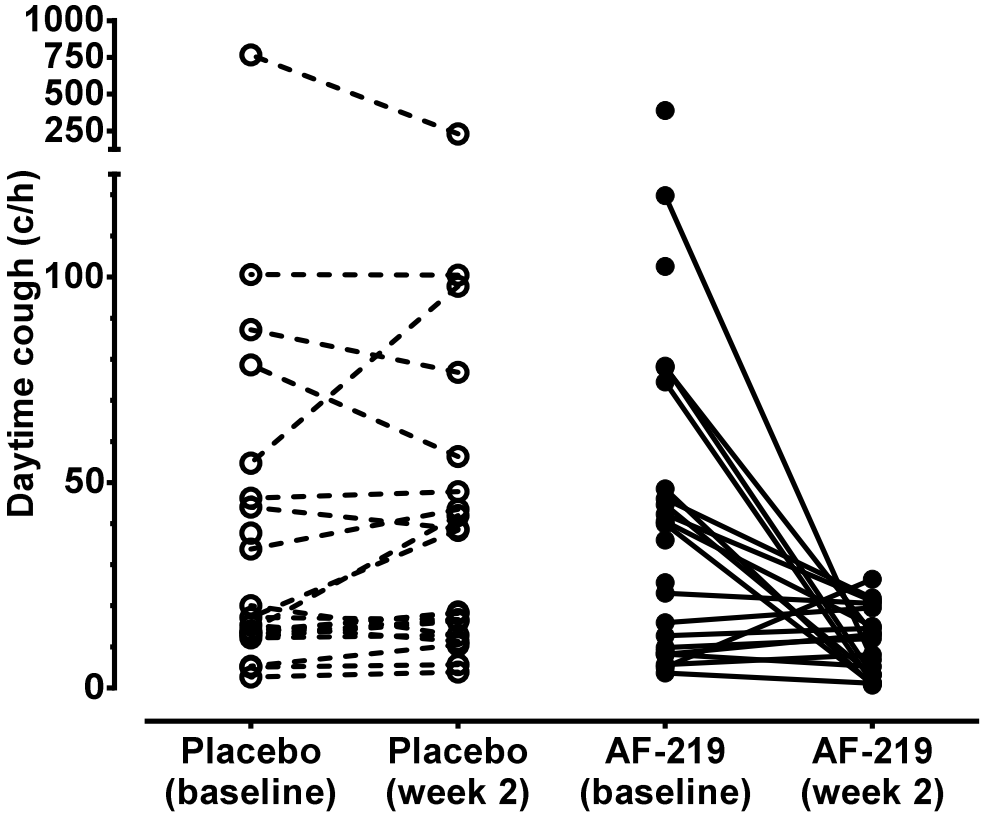
Summary of metric data for daytime cough rate included in the ITT observed-case analysis is summarised in Table 25. In the table below and subsequent tables, 'All Baselines' column refers to any available baseline data. 'Baseline' and 'Week 2' columns refer only to complete data from both baseline and week 2, from which the within subject change is calculated. Individual data are plotted in Figure 22.

Table 25: Daytime cough rate included in the ITT observed-case analysis

	All Baselines	Baseline	Week 2
Placebo			
Number	22	21	21
Geometric mean	24.1	23.6	26.6
Median (range)	17.2 (2.7-767.6)	17.2 (2.7-767.6)	18.5 (3.9-230.3)
AF-219			
Number	24	19	19
Geometric mean	27.6	23.6	6.9
Median (range)	38 (3.7 -389.1)	39.9 (3.7-119.8)	12.0 (0.7 – 26.5)

During placebo treatment, one week 2 cough recording failed. Even though 6 out of the 24 subjects discontinued taking AF-219 before the efficacy visit, 3 subjects had cough recordings at that visit. Further, one subject had cold in week 2, and one cough recording failed; therefore, no week 2 data was available for those subjects. Therefore, 19 cough recordings were included in the ITT analysis for AF-219 treatment.

Figure 22: Daytime cough (ITT OC population) for both placebo and AF-219 at baseline and week 2.



The unconnected points are for subjects who either discontinued taking the medication before the efficacy visit or represent failed cough recordings in week 2.

12/24 subjects had > 30 % reduction in daytime cough rate and 10/24 had more than 50 % reduction with AF-219, whereas only 2 subject had > 30 % reduction with placebo (Table 26 and Table 27).

Table 26: Change in day time cough with AF-219

		Frequency	%	Valid %	Cumulative %
Valid	No change or increase	6	25.0	31.6	31.6
	≤30% to >0% reduction	1	4.2	5.3	36.9
	≤50% to >30% reduction	2	8.3	10.5	47.4
	>50 % to <90% reduction	4	16.7	21.0	68.4
	≥90 % reduction	6	25.0	31.6	100.0
	Total	19	79.2	100.0	
Missing		5	20.8		
Total		24	100.0		

Missing data are explained in Table 25.

Table 27: Change in day time cough with placebo

		Frequency	%	Valid %	Cumulative %
Valid	No change or increase	14	63.6	66.7	66.7
	≤30% to >0% reduction	5	22.7	23.8	90.5
	≤50% to >30% reduction	1	4.5	4.8	95.2
	>50 % to <90% reduction	1	4.5	4.8	100.0
	Total	21	95.5	100.0	
Missing		1	4.5		
Total		22	100.0		

Results from the mixed-effects model are shown in Table 28, Table 29, and Table 30. The effect of intervention on daytime cough rate was highly significant with no sequence (i.e. carryover) or period effect (Table 28). Interestingly, the average baseline cough rate significantly affected the cough rate (Table 28). As can be seen in Table 29, daytime cough slightly increased with placebo but substantially fell with AF-219 treatment. In comparison to placebo, AF-219 significantly reduced daytime cough rate by 75% (Table 30).

Table 28: Mixed-Effects Model for daytime cough rate in the ITT observed case analysis

Source	Numerator (df)	Denominator (df)	F	P value
Intervention	1	34	16.431	<0.001
Sequence	1	34	.149	0.702
Period	1	34	.033	0.857
Average baseline	1	34	19.109	<0.001
Period specific baseline	1	34	.096	0.759

Table 29: Model estimate for the change in daytime cough rate with placebo and AF-219 (ITT)

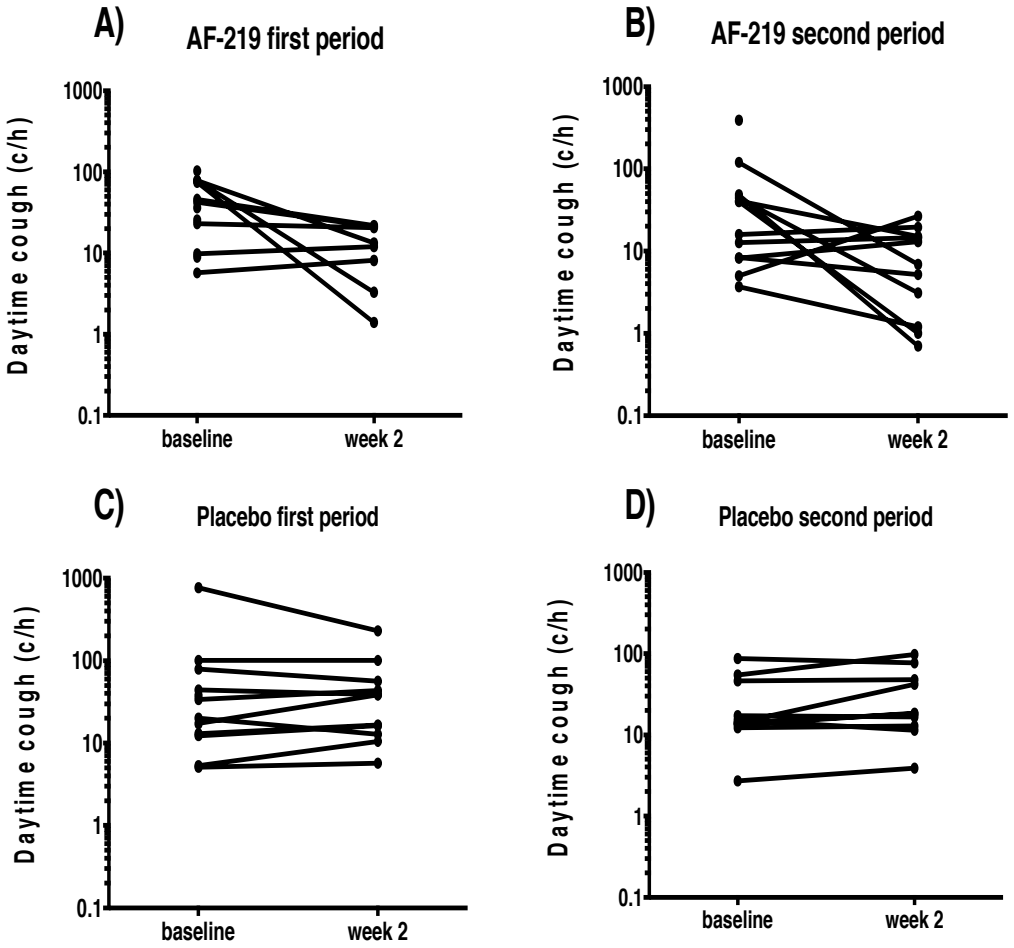
Intervention	Mean change	df	95% Confidence Interval	
			Lower Bound	Upper Bound
Placebo	+16%	34	-28%	+84%
AF-219	-71%	34	-83%	-52%

Table 30: Model estimated difference (AF-219 vs. Placebo) in daytime cough rate (ITT)

(I) intervention	(J) intervention	Mean Difference (I-J)	df	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
AF-219	Placebo	-75%	34	<0.001	-88%	-50%

Figure 23 shows separate graphs for daytime cough based on whether patients had AF-219 or placebo first. As can be seen in the figure, the change in cough rate with AF-219 or placebo does not seem to be affected by the treatment order.

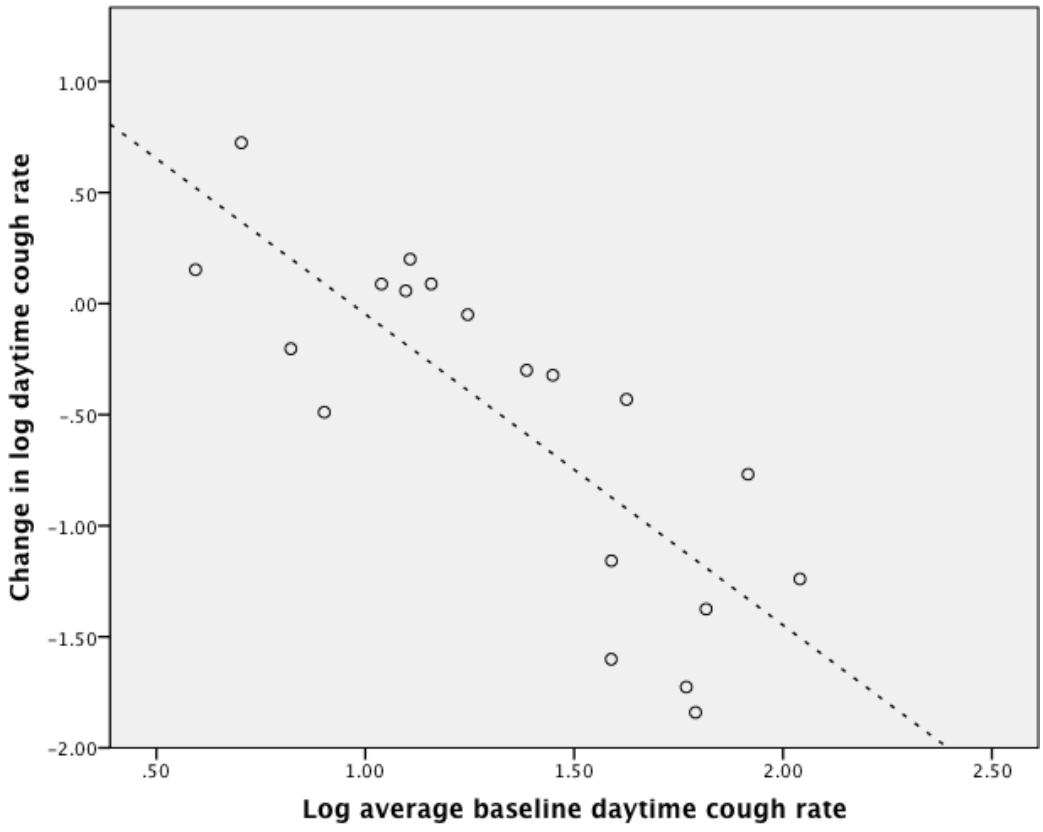
Figure 23: Daytime cough with placebo and AF-219 based on treatment order



The change in cough rate was not different if AF-219 was given first (panel A) or second (panel B). Also, very little change is seen with placebo and it did not matter whether it was given first (panel C) or second (panel D).

Average baseline daytime cough rate (i.e. average of the two periods baselines) had a significant effect on the change in daytime cough rate ($p < 0.001$). I explored this further by examining the relationship between baseline cough rate and the change with AF-219 (Figure 24). As there were only 2 subjects who had > 30 % reduction in daytime cough, I did not examine the relationship for placebo. There was a significant and negative strong correlation (Pearson correlation coefficient $r = -0.803$, $p < 0.001$), which means subjects with the highest baseline cough rate had the highest reduction in cough.

Figure 24: The relationship between average baseline daytime cough rate and change with AF-219



Average baseline cough rate represents the average rate from the baseline in both treatment periods.

As can be seen in Table 26, 12 subjects had at least more than 30 % reduction in daytime cough rate with AF-219. Around a 30% fall in cough rate is thought to be

clinically meaningful [222]. Now, I want to examine if there were any differences in demographics or baseline daytime cough rate between these 2 groups (responders vs. non-responders). In here, I include all available cough recordings even if they were performed after early discontinuation of treatment.

Table 31: Comparison of patients with and without > 30% reduction in daytime cough rate

	> 30 % reduction N = 12	**≤ 30% reduction N = 7**	***P* value**
Female, n (%)	10 (83)	4 (57)	0.305§
Age, yr	54.2	53.7	0.938≠
BMI	24.5	26.8	0.202≠
Smoking history, N, ex-smokers	4	3	1.00§
Cough duration, yr	11.6	9.8	0.583≠
Average daytime cough (c/hr)¥	36.1	9.8	0.004≠

¥Geometric mean, § Fisher's exact test, ≠ independent sample t-test

In summary, daytime cough rate fell significantly with AF-219 compared with placebo. Patients who responded to treatment had significantly higher cough rate.

Daytime Cough Rate – Per Protocol Analysis

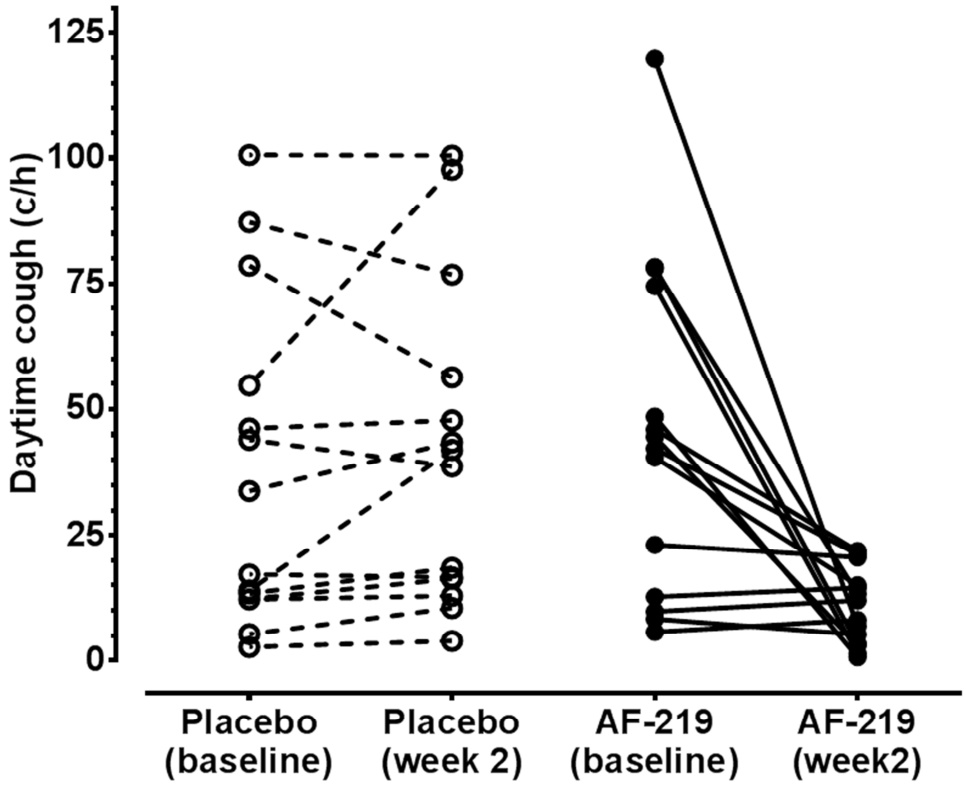
Table 32: Daytime cough rate (PP)

	All baselines	Baseline	Week 2
Placebo			
Number	16	14	14
Geometric mean	23.6	23.7	29.5
Median (range)	25.5 (2.7 – 100.6)	25.5 (2.7 –100.6)	40.4 (3.9 – 110.5)
AF-219			
Number	16	14	14
Geometric mean	32.8	32.2	7.2
Median (range)	41.4 (5.7 – 119.8)	43.5 (5.7 – 119.8)	10.1 (0.7 – 21.9)

16 subjects were in the PP population. However, 2 subjects were excluded from the PP analysis for the daytime cough because one cough monitor failed on placebo week 2 and one on AF-219 week 2.

Individual data are plotted in Figure 25.

Figure 25: Daytime cough (PP population) for both placebo and AF-219 at baseline and week 2



In the per protocol analysis, the reduction in daytime cough with AF-219 compared with placebo is even larger at 84% (Table 33).

Table 33: Model estimated difference (AF-219 vs. Placebo) in daytime cough rate (PP)

(I) intervention	(J) intervention	Mean Difference (I-J)	df	P value	95% Confidence Interval for Difference	
					Lower Bound	Upper Bound
AF-219	Placebo	-84%	22.000	< 0.001	-94%	-60%

Daytime Cough Rate – ITT WC Analysis

Even in the worst-case analysis, daytime cough rate fell by a large percentage and was highly statistically significantly with AF-219 compared with placebo (Table 34).

Table 34: Model estimated difference (AF-219 vs. Placebo) in daytime cough rate (worst case analysis)

(I) intervention	(J) intervention	Mean Difference (I-J)	df	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
AF-219	Placebo	-65%	38	0.005	-82%	-29%

3.7.4 Effect of AF-219 on Night Time Cough Rate

Table 35: Night time cough rate included in the ITT observed-case analysis

	All baselines	Baseline	Week 2
Placebo			
Number	21	20	20
Geometric mean	1.7	1.6	1.9
Median (range)	1.7 (0-108.3)	1.6 (0-108.3)	2.3 (0-19.1)
AF-219			
Number	24	18	18
Geometric mean	1.4	1.4	0.6
Median (range)	1.3 (0-50.2)	1.4 (0-32)	0.4 (0-11.3)

Night cough added 0.1 then subtracted after back transformation.

Figure 26: Night time cough (ITT OC population) for both placebo and AF-219 at baseline and week 2

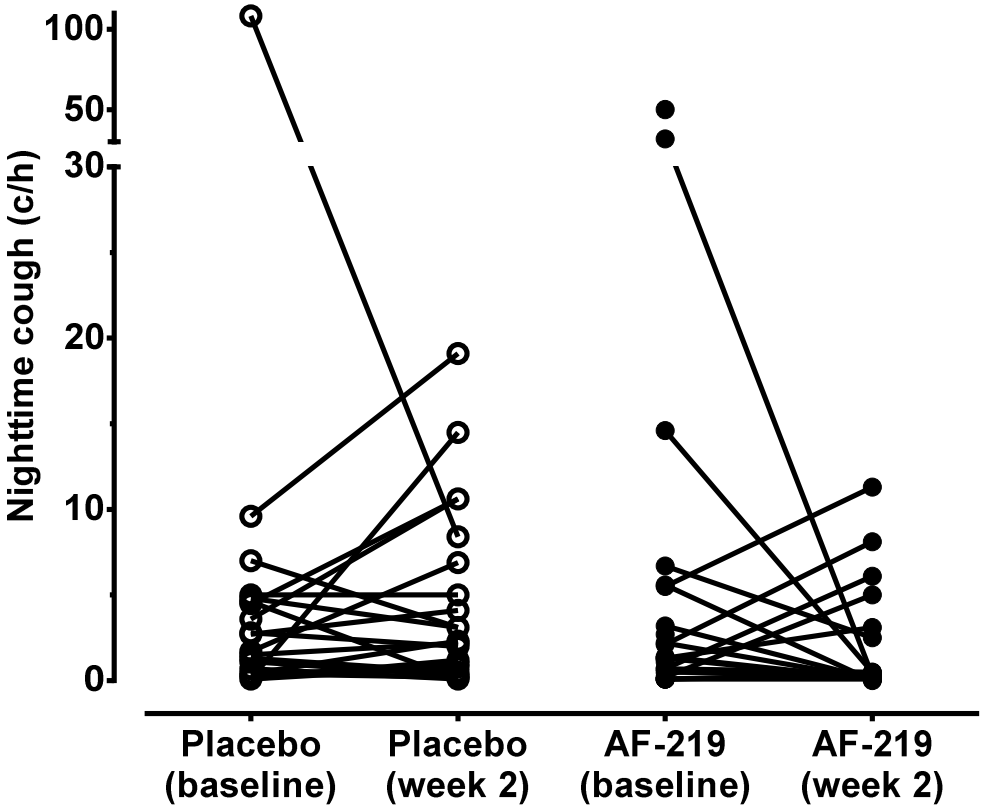


Table 35 and Figure 26 show that night time cough frequency is generally low at baseline. Placebo resulted in a small rise in night time cough, whereas AF-219 led to reduction in cough.

The modelled data showed borderline significant effect of intervention (Table 36). Night time cough fell with AF-219 (Table 37) and compared to placebo it reduced cough by 62% (Table 38). In summary, patients coughed infrequently at night, so there was low cough frequency to start with. Nevertheless, night time cough trended in the same direction as daytime cough.

Table 36: Mixed-Effects Model for night time cough rate (ITT OC)

Source	Numerator df	Denominator df	F	P value
Intervention	1	19.119	4.093	0.057
Sequence	1	19.698	3.609	0.072
Period	1	20.112	.186	0.671
Average baseline	1	22.183	11.184	0.003
Period specific baseline	1	17.585	14.749	0.001

Table 37: Model estimate for the change in night time cough rate with placebo and AF-219 (ITT OC)

Intervention	Mean	Df	95% Confidence Interval	
			Lower Bound	Upper Bound
Placebo	+28%	31.820	-37%	+158%
AF-219	-52%	31.912	-77%	+3%

Table 38: Model estimated difference (AF-219 vs. Placebo) in night time cough rate (ITT OC)

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Df	P Value	95% Confidence Interval	
					Lower Bound	Upper Bound
AF-219	Placebo	-62%	19.119	0.057	-86%	+3%

Night Time Cough Rate – PP

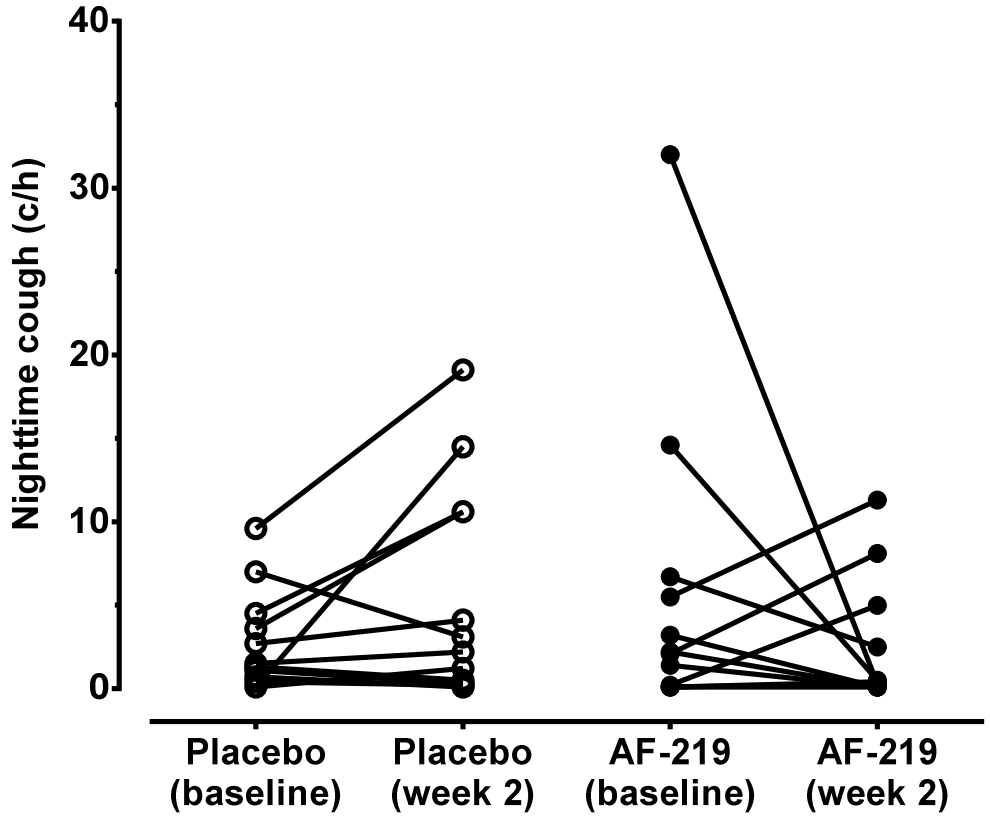
Descriptive summary of night time cough frequency in the per protocol group is shown in Table 39. Figure 27 shows the per protocol individual night time cough frequency at baseline and week 2 for both placebo and AF-219.

Table 39: Night time cough (PP)

	All baselines	Baseline	Week 2
Placebo			
Number	15	12	12
Geometric mean	1.4	1.4	2.0
Median (range)	1.5 (0.1 - 9.6)	1.4 (0.1 - 9.6)	2.7 (0 - 19.1)
AF-219			
Number	16	12	12
Geometric mean	1.5	1.5	0.6
Median (range)	1.8 (0 - 32)	2.2 (0 - 32)	0.4 (0 - 11.3)

Geometric mean: 0.1 added before transformation then subtracted. One recording had less than 24 hours in placebo baseline. Only 12/16 had complete cough frequency data from both treatment periods because of failed cough recordings.

Figure 27: Night time cough (PP population) for both placebo and AF-219 at baseline and week 2



As in the ITT analysis, there was a borderline significant effect of intervention in the per protocol group. In comparison to placebo, night time cough rate decreased by 69% (Table 40).

Table 40: Model estimated difference (AF-219 vs. Placebo) in night time cough rate (PP)

(I) Intervention	(J) Intervention	Mean Difference (I-J)	df	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
AF-219	Placebo	-69%	9	0.061	-93%	+7%

3.7.5 Effect of AF-219 on Total 24 Hour Cough Rate

Descriptive summary of the 24 hour cough frequency for both placebo and AF-219 at baseline and week 2 is shown in Table 41.

Table 41: 24hr cough frequency (ITT)

	All baselines	**Baseline**	**Week 2**
Placebo			
Number	21	20	20
Geometric mean	17.3	16.9	18.1
Median (range)	12.6 (1.9- 483.8)	12.1 (1.9-483.8)	14 (2.7-131.5)
AF-219			
Number	24	18	18
Geometric mean	18.9	17.2	4.8
Median (range)	23.4 (2.8-241.3)	25.5 (2.8-81.9)	7.8 (0.6-18.4)

Missing data:

Placebo: One recording from placebo baseline had failed after 7 hours and 20 minutes (daytime), so there is no cough rate for 24 hours. One recording failed completely in week 2.

AF-219: 24 subjects had baseline cough monitor. In week 2, one recording completely failed, one subject had only 10 hours and 34 minutes of recording before the monitor failed so was not included in the 24 hour rate, one subject developed cold and therefore had not had a cough recording, and 3 subjects discontinued the medication and had no cough recording at end of treatment. Note: three more subjects discontinued the AF-219 medication but still had a cough recording several days after; their data is included in the ITT observed case analysis.

The linear mixed-model reveals a highly significant effect on 24 hour cough frequency (Table 42). Cough rate had a minor increase with placebo but markedly decreased with AF-219 (Table 43). The mean difference vs. placebo is -74% (Table 44).

Table 42: Mixed-Effects Model for 24 hour cough rate (ITT OC)

Source	Numerator df	Denominator df	F	P value
Intervention	1	32	14.498	0.001
Sequence	1	32	.057	0.812
Period	1	32	.031	0.861
Average Baseline	1	32	18.592	<0.001
Period Specific Baseline	1	32	.041	0.841

Table 43: Model estimated marginal means for the change in 24 hour cough rate with placebo and AF-219 (ITT OC)

Intervention	Mean	df	95% Confidence Interval	
			Lower Bound	Upper Bound
Placebo	+8%	32	-34%	+75%
AF-219	-72%	32	-83%	-52%

Table 44: Model estimated difference (AF-219 vs. Placebo) in 24 hour cough rate (ITT OC)

(I) intervention	(J) intervention	Mean Difference (I-J)	df	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
AF-219	Placebo	-74%	32	0.001	-87%	-46%

24 Hour Cough Rate – PP

Table 45 summarises the per protocol 24 hour cough frequency for placebo and AF-219 for both placebo and AF-219.

Table 45: 24 hour cough frequency (PP)

	All baselines	Baseline	Week 2
Placebo			
Number	15	12	12
Geometric mean	17.2	18.3	21.9
Median (range)	25.6 (1.9-68.3)	25.7 (1.9-68.3)	27.2 (2.7-74.5)
AF-219			
Number	16	12	12
Geometric mean	22.2	23.6	4.6
Median (range)	28.5 (3.8-81.9)	30.5 (3.8-81.9)	5.8 (0.6-18.4)

One recording had less than 24 hours in placebo baseline. Only 12/16 had complete cough frequency data from both treatment periods because of failed cough recordings.

As seen in the ITT analysis, the 24 hour cough frequency fell significantly with AF-219 compared with placebo and the change is larger here at -89% (Table 46).

Table 46: Model estimated difference (AF-219 vs. Placebo) in 24 hour cough rate (PP)

(I) intervention	(J) intervention	Mean Difference (I-J)	df	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
AF-219	Placebo	-89%	18	0.001	-97%	-67%

3.7.6 Patient-Reported Outcomes

All patient reported outcomes presented here are for the intention to treat population. Results of the per protocol analysis for those outcomes are summarised in Table 78.

Daytime Cough Severity VAS

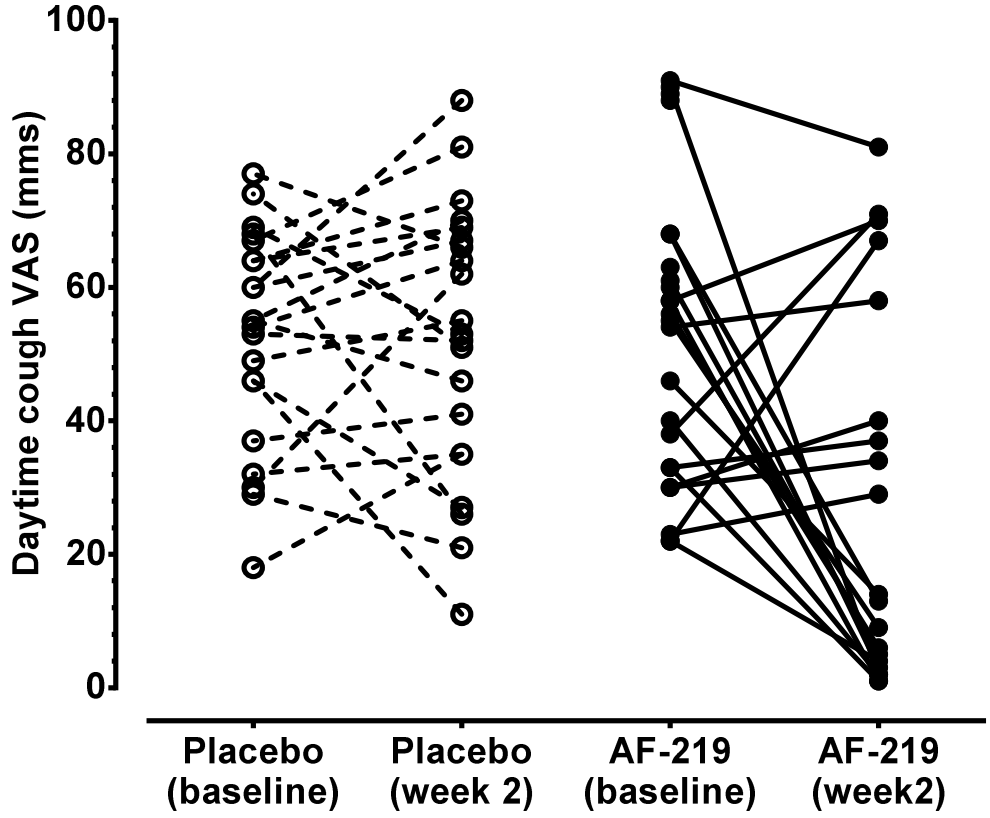
Summary of the daytime cough severity VAS scores is shown in Table 47 and Figure 28. It appears that placebo did not largely change the VAS scores after 2 weeks of treatment, whereas AF-219 reduced the scores substantially.

Table 47: Daytime cough severity VAS (mm)

	All baselines	**Baseline**	**Week 2**
Placebo			
Number	22	21	21
Mean (SD)	53.4 (16)	52.7 (16.1)	52 (20.7)
AF-219			
Number	24	20	20
Mean (SD)	53.2 (22.1)	48.8 (20.7)	27.4 (28)¥

¥ Not normally distributed; the geometric mean is 12.2 and median (range) 13.5 (1 - 81). Only 22 subjects had placebo because 2 withdrew in treatment period 1 after having AF-219. 21/22 in the placebo group had both baseline and week 2 VAS because one subject was not given the VAS in error. 4 subjects in AF-219 had no week 2 VAS because they withdrew from study. 2 more subjects who discontinued the medication several days before week 2 still had their VAS filled in on the assessment day but referring to the period they were on the study medication.

Figure 28: Daytime cough severity VAS for both placebo and AF-219 at baseline and week 2



The mixed-effect model shows that the change in daytime cough severity VAS was significantly affected by the intervention (Table 48). Similar to the cough frequency, the baseline VAS score had a significant effect on the change. Table 49 shows that VAS scores decreased with AF-219 but slightly increased with placebo. There was a statically significant difference in the change between placebo and AF-219 (Table 50).

Table 48: Mixed-Effects Model for daytime cough severity VAS

Source	Numerator df	Denominator df	F	P value
Intervention	1	35	10.586	0.003
Sequence	1	35	1.151	0.291
Period	1	35	0.062	0.805
Average baseline	1	35	6.203	0.018
Period specific baseline	1	35	6.688	0.014

Table 49: Model estimated marginal means for the change in daytime cough severity VAS (mm) with placebo and AF-219

Intervention	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
Placebo	+2.1	5.475	35	-9.1	13.2
AF-219	-23.5	5.582	35	-34.9	-12.2

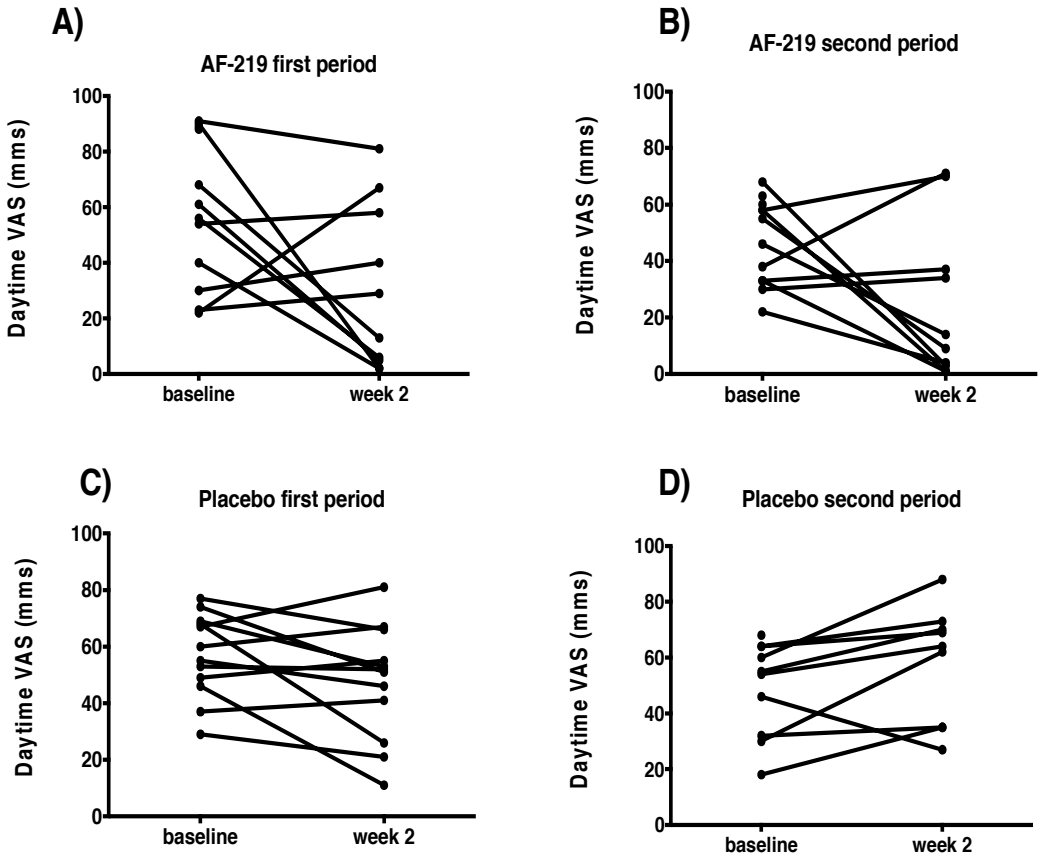
Table 50: Model estimated difference (AF-219 vs. Placebo) in daytime cough severity VAS (mm)

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	df	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
AF-219	Placebo	-25.6	7.860	35	0.003	-41.5	-9.6

In summary, cough severity VAS fell significantly with AF-219 compared with placebo.

As can be seen in Figure 29, the change in cough severity VAS with AF-219 or placebo does not seem to be affected by the order of treatment.

Figure 29: Daytime cough VAS with placebo and AF-219 based on treatment order



The change in cough severity VAS was not different if AF-219 was given first (panel A) or second (panel B). Panels C and D represent the change in VAS with placebo when given in first period (C) or second period (D).

Night Time Cough Severity VAS

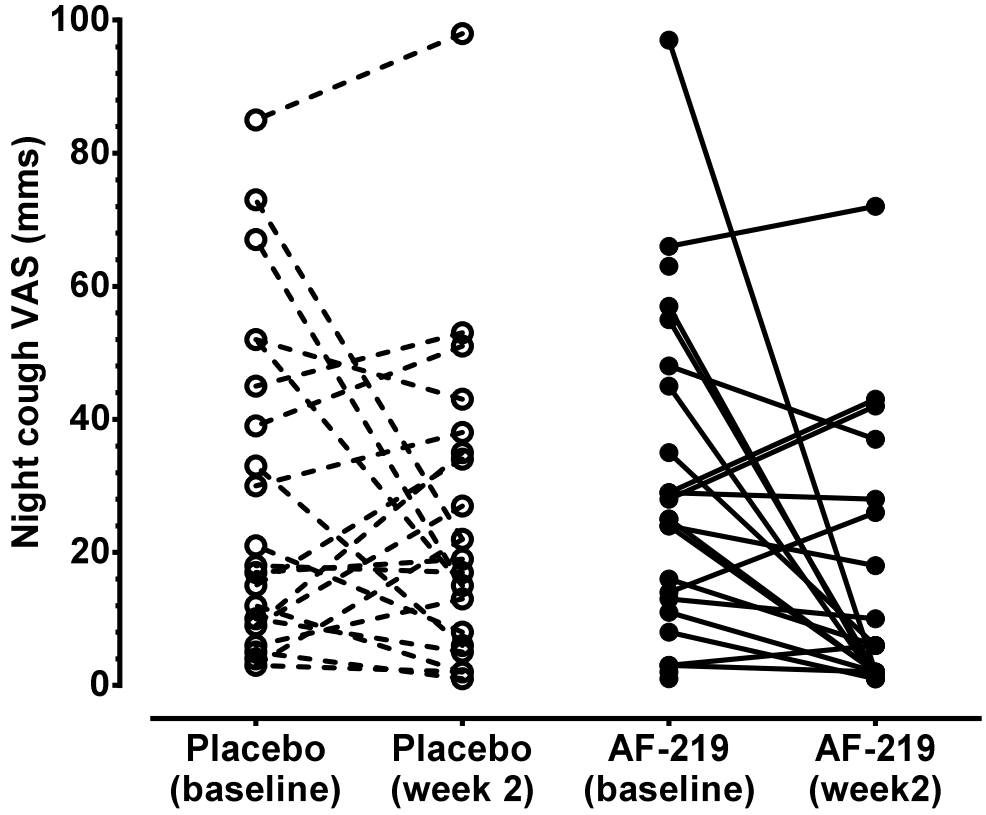
Mean (SD) night time cough severity VAS for both placebo and AF-219 at baseline and week 2 is shown in Table 51. Figure 30 shows the night time cough severity VAS scores for the participants.

Table 51: Night time cough severity VAS (mm)

	All baselines	**Baseline**	**Week 2**
Placebo			
Number	22	21	21
Mean (SD)	27.6 (24.9)	28.8 (24.9)	25 (23)
AF-219			
Number	24	20	20
Mean (SD)	30 (24.3)	31.5 (23.9)	15.5 (19.8)¥

¥ Not normally distributed; the geometric mean is 6.3 and median (range) 6.0 (1 - 72). Only 22 subjects had placebo because 2 withdrew in treatment period 1 after having AF-219. 21/22 in placebo group had both baseline and week 2 VAS because one subject was not given the VAS in error. 4 subjects in AF-219 had no week 2 VAS because they withdrew from study. 2 more subjects who discontinued the medication several days before week 2 still had their VAS filled in on the assessment day but referring to the period they were on the study medication.

Figure 30: Night cough VAS for both placebo and AF-219 at baseline and week 2



From Table 51 and Figure 30, it appears that nighttime cough severity VAS decreased to a greater degree with AF-219 than with placebo. The model estimated means also reveals the same (Table 53). However, the difference in change between treatments did not reach a statistical significance (Table 52 and Table 54).

Table 52: Mixed-Effects Model for night cough VAS (ITT)

Source	Numerator df	Denominator df	F	P value
Intervention	1	35	1.949	0.172
Sequence	1	35	.620	0.436
Period	1	35	.404	0.529
Average baseline	1	35	14.497	0.001
Period specific baseline	1	35	17.587	<0.001

Table 53: Model estimated marginal means for the change in night cough VAS (mm) with placebo and AF-219 (ITT)

Intervention	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
Placebo	-5.3	4.246	35	-13.9	3.4
AF-219	-13.8	4.316	35	-22.6	-5.0

Table 54: Model estimated difference (AF-219 vs. Placebo) in night cough VAS (mm) (ITT)

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	df	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
AF-219	Placebo	-8.5	6.110	35	0.172	-20.9	+3.9

Urge to Cough VAS

VAS for urge to cough fell more with AF-219 compared to placebo (Table 55 and Figure 31).

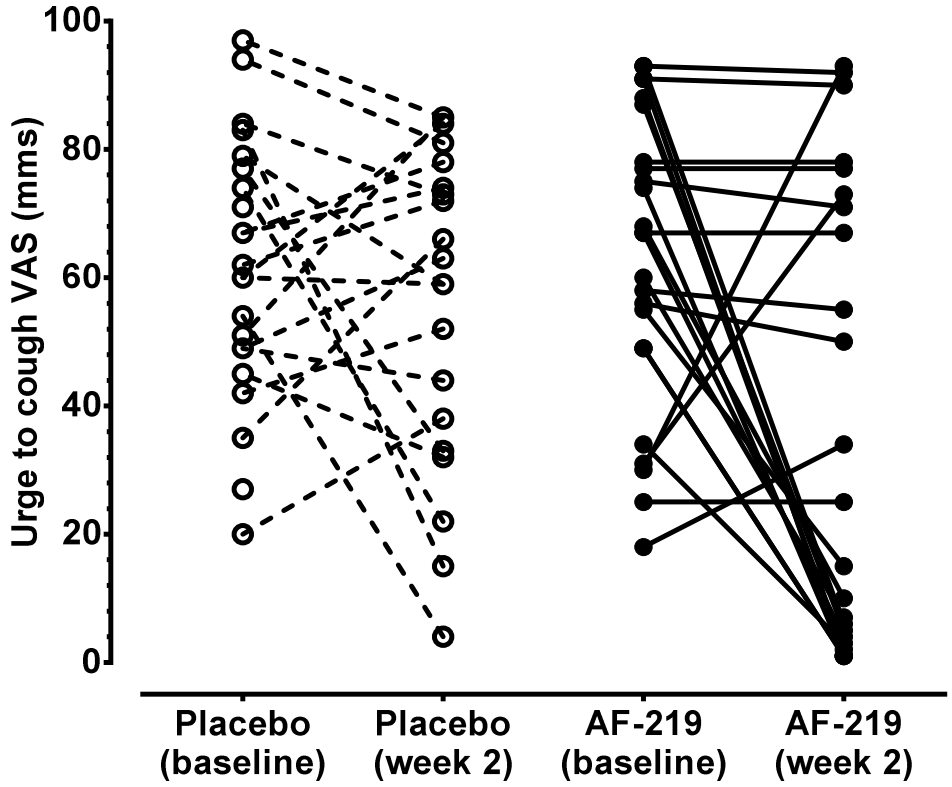
Figure 31 represents the scores before and after treatments for individual patients.

Table 55: Urge to Cough VAS

	All baselines	**Baseline**	**Week 2**
Placebo			
Number	22	20	20
Mean (SD)	61.2 (20.6)	62.5 (20)	56 (24.9)
AF-219			
Number	24	24	24
Mean (SD)	63.1 (23.1)	63.1 (23.1)	35.9 (35.5)¥

2 subjects withdrew after treatment period 1 (AF-219) so had not had placebo. 2 UTC VAS are missing with placebo in week 2 (subjects were not given the VAS in error). ¥ Geometric mean 14 and median (range) 20 (1-93).

Figure 31: Urge to cough VAS for both placebo and AF-219 at baseline and week 2



Results of the mixed-effect model are shown in Table 56, Table 57, and Table 58. The model shows that the change in the urge to cough VAS score was -21.3 mm with AF-219 compared to placebo (*P* = 0.035*).*

Table 56: Mixed-Effects Model for urge to cough VAS

Source	Numerator df	Denominator df	F	P value
Intervention	1	38	4.763	0.035
Sequence	1	38	0.137	0.713
Period	1	38	0.040	0.842
Average baseline	1	38	4.796	0.035
Period specific baseline	1	38	9.634	0.004

Table 57: Model estimated marginal means for the change in urge to cough VAS (mm) with placebo and AF-219

Intervention	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
Placebo	-6.0	7.262	38	-20.6	+8.8
AF-219	-27.2	6.489	38	-40.3	-14.1

Table 58: Model estimated difference (AF-219 vs. Placebo) in urge to cough VAS

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	df	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
AF-219	Placebo	-21.3	9.736	38	0.03	-41.0	-1.5

In summary, the urge to cough VAS scores were reduced significantly with AF-219 compared to placebo.

CQLQ

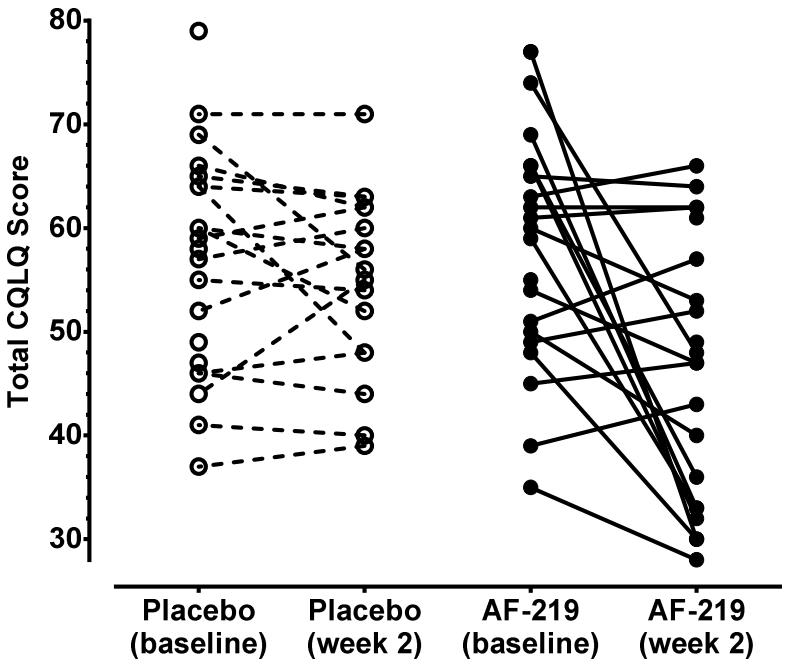
Table 59 shows the mean (SD) of the CQLQ scores for placebo and AF-219 at baseline and week 2. It appears that the reduction in CQLQ scores with AF-219 is greater than with placebo. The before-after graph in Figure 32 illustrates this as well.

Table 59: CQLQ

	All baselines	Baseline	Week 2
Placebo			
Number	22	17	17
Mean (SD)	56.7 (10.6)	56.2 (10.3)	54.9 (8.8)
AF-219			
Number	21	19	19
Mean (SD)	57.2 (10.9)	56.3 (10.4)	45.4 (12.8)

24 subjects completed CQLQ at baseline for the AF-219 treatment period; 3 subjects had missed one or more questions and therefore no total scores were calculable. 5 subjects in the placebo period and 3 in the AF-219 period had incomplete questionnaire answers.

Figure 32: Total CQLQ scores for both placebo and AF-219 at baseline and week 2



Results from the mixed-effects models are shown in Table 60, Table 61, and Table 62.

Table 60: Mixed-effects model for the change in CQLQ total score

Source	Numerator df	Denominator df	F	P value
Intervention	1	30	6.291	0.018
Sequence	1	30	0.200	0.658
Period	1	30	0.191	0.665
Average baseline	1	30	8.066	0.008
Period specific baseline	1	30	0.866	0.360

Table 61: Model estimate for the change in CQLQ with placebo and AF-219

Intervention	Mean	Std. Error	df	95% Confidence Interval	
				Lower Bound	Upper Bound
Placebo	-1.5	2.715	30	-7	+4.1
AF-219	-10.7	2.426	30	-15.6	-5.7

Table 62: Model estimated difference (AF-219 vs. Placebo) in CQLQ change

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	df	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
AF-219	Placebo	-9.2	3.683	30	0.018	-16.8	-1.7

These results demonstrate that CQLQ significantly improved with AF-219 (mean difference vs. placebo -9.2 [95%CI -1.7 to -16.8]; p = 0.018). There was no significant carryover (p = 0.658 for sequence effect) or period (p = 0.665) effect.

CQLQ Domains

Physical complaints:

The physical complaints domain has 9 items; therefore recordable scores could range between 9 and 36.

Table 63: Physical complaints

	All baselines	**Baseline**	**Week 2**
Placebo			
Number	22	18	18
Mean (SD)	17.5 (4.2)	17.8 (4.2)	17.8 (4.1)
AF-219			
Number	22	20	20
Mean (SD)	18.7 (5.4)	18.7 (5.4)	16.5 (4.9)

Missing data:

All baselines: In the AF-219 group, 2 baseline scores are incomplete because of missing answers.

Week 2: 18/22 in the placebo group had data from week 2 because of missing answers from 3 subjects and one subject was not given the CQLQ in error. In the AF-219 group, only 20/22 had data from week 2 because of missing answers.

Figure 33: CQLQ physical complaints domain scores for both placebo and AF-219 at baseline and week 2

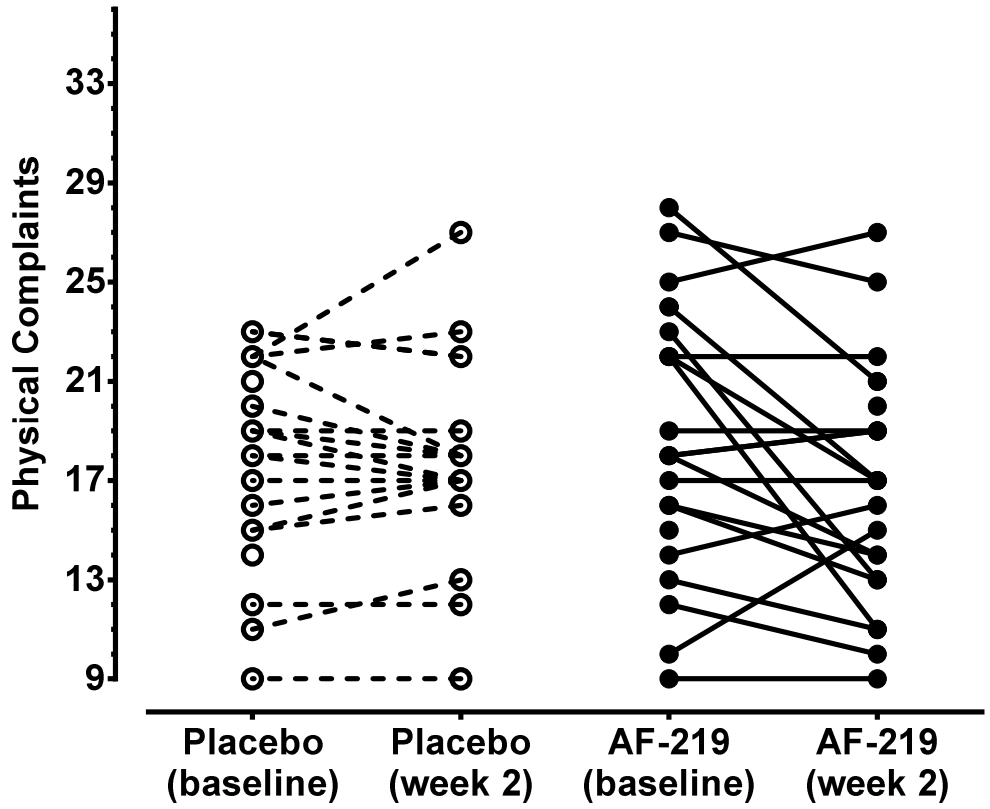


Table 64: Model estimated difference (AF-219 vs. Placebo) in change in physical complaints domain of CQLQ

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	df	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
AF-219	Placebo	-1.2	1.146	32	0.296	-3.6	+1.1

There was no significant change in the physical complaints domain.

Psychosocial Issues:

The psychosocial issues domain has 5 items; therefore, recordable scores could range between 5 and 20.

Table 65: Psychosocial issues

	All baselines	Baseline	Week 2
Placebo			
Number	22	21	21
Mean (SD)	14 (4)	14 (4.1)	13.9 (3.5)
AF-219			
Number	24	23	23
Mean (SD)	13.9 (3.2)	13.7 (3.2)	10 (4.5)

Figure 34: CQLQ psychosocial social domain scores for both placebo and AF-219 at baseline and week 2

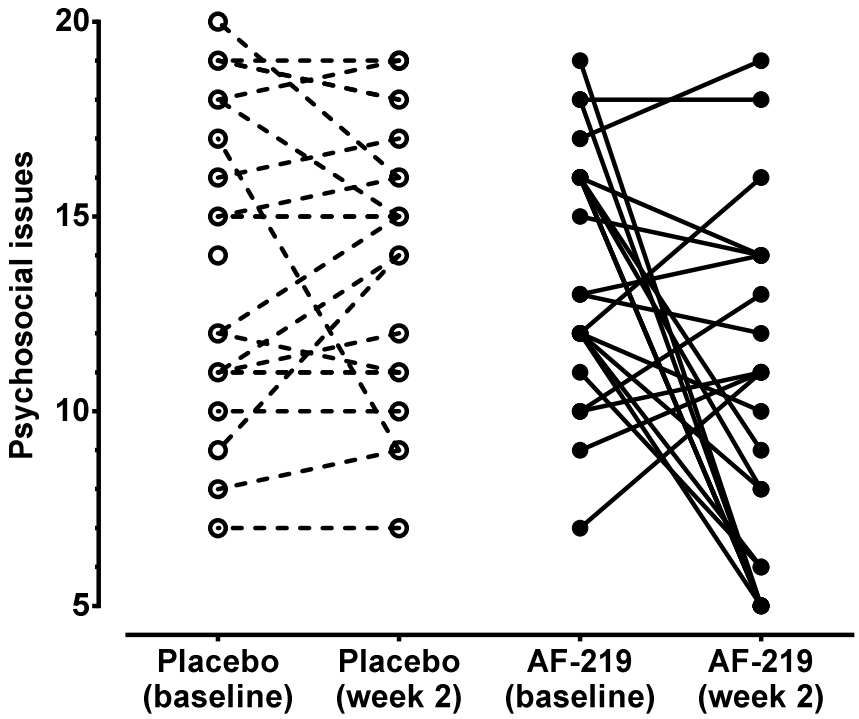


Table 66: Model estimated difference (AF-219 vs. Placebo) in change in psychosocial issues domain of CQLQ

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	df	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
AF-219	Placebo	-3.8	1.218	38	0.003	-6.3	-1.3

The psychosocial issues domain significantly improved with AF-219 compared with placebo.

Functional Abilities Domain:

The functional abilities domain has 5 items; therefore, recordable scores could range between 5 and 20.

Table 67: Functional abilities domain

	All baselines	**Baseline**	**Week 2**
Placebo			
Number	22	20	20
Mean (SD)	9.3 (3.1)	9.1 (3)	8.9 (2.6)
AF-219			
Number	23	21	21
Mean (SD)	9.7 (3.1)	9.5 (3.2)	7.8 (3.1)

Figure 35: CQLQ functional abilities domain scores for both placebo and AF-219 at baseline and week 2

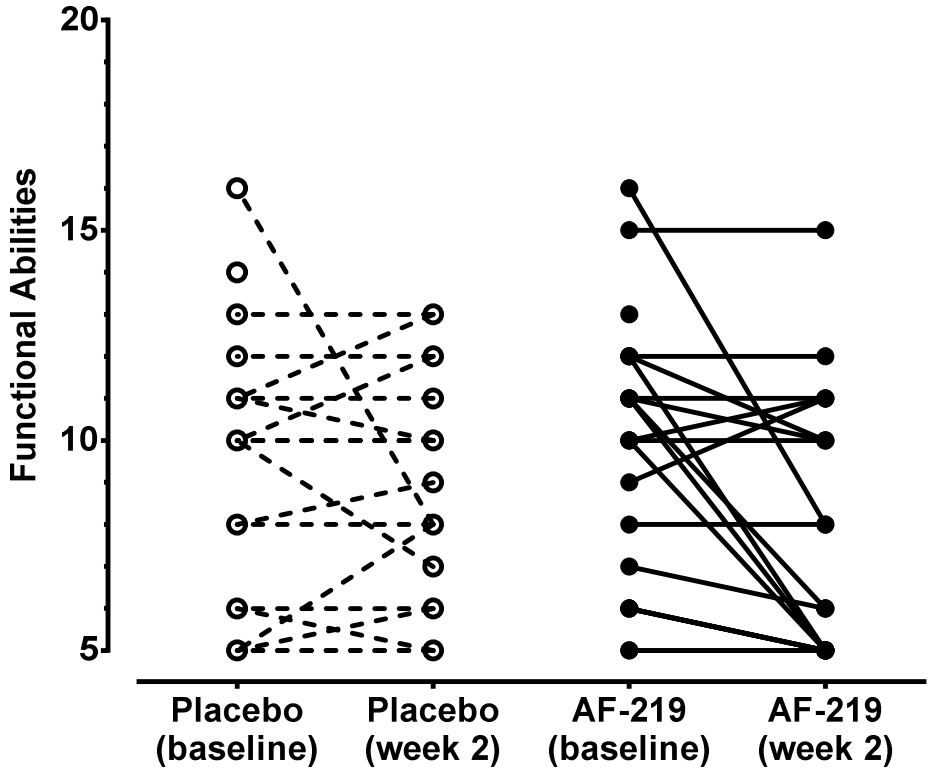


Table 68: Model estimated difference (AF-219 vs. Placebo) in change in functional abilities domain of CQLQ

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	df	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
AF-219	Placebo	-1.1	0.724	35	0.124	-2.6	+0.3

There was no significant change in the functional abilities domain.

Emotional well-being:

The emotional well-being domain has 4 items; scores range between 4 and 16.

Table 69: Emotional well-being

	All baselines	Baseline	Week 2
Placebo			
Number	22	21	21
Mean (SD)	5.7 (2.3)	5.8 (2.3)	5.2 (1.9)
AF-219			
Number	24	22	22
Mean (SD)	5.6 (1.8)	5.5 (1.6)	5.0 (1.4)

Figure 36: CQLQ emotional well-being domain scores for both placebo and AF-219 at baseline and week 2

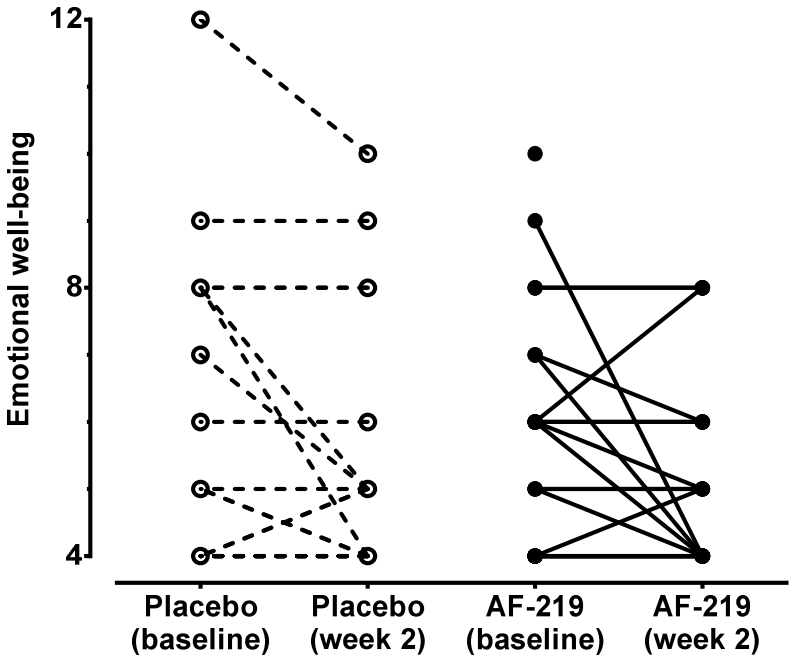


Table 70: Model estimated difference (AF-219 vs. Placebo) in change in emotional well-being domain of CQLQ

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	df	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
AF-219	Placebo	-0.1	0.309	37	0.786	-0.7	+0.5

Emotional well-being domain did not change significantly with AF-219 compared with placebo.

Extreme Physical Complaints:

The extreme physical complaints domain has 4 items; score range between 4 and 16.

Table 71: Extreme physical complaints

	All baselines	**Baseline**	**Week 2**
Placebo			
Number	22	21	21
Mean (SD)	8 (2.4)	8 (2.4)	7.6 (2)
AF-219			
Number	24	22	22
Mean (SD)	8 (2.3)	7.9 (2.3)	6.0 (2.2)

Figure 37: CQLQ extreme physical domain scores for both placebo and AF-219 at baseline and week 2

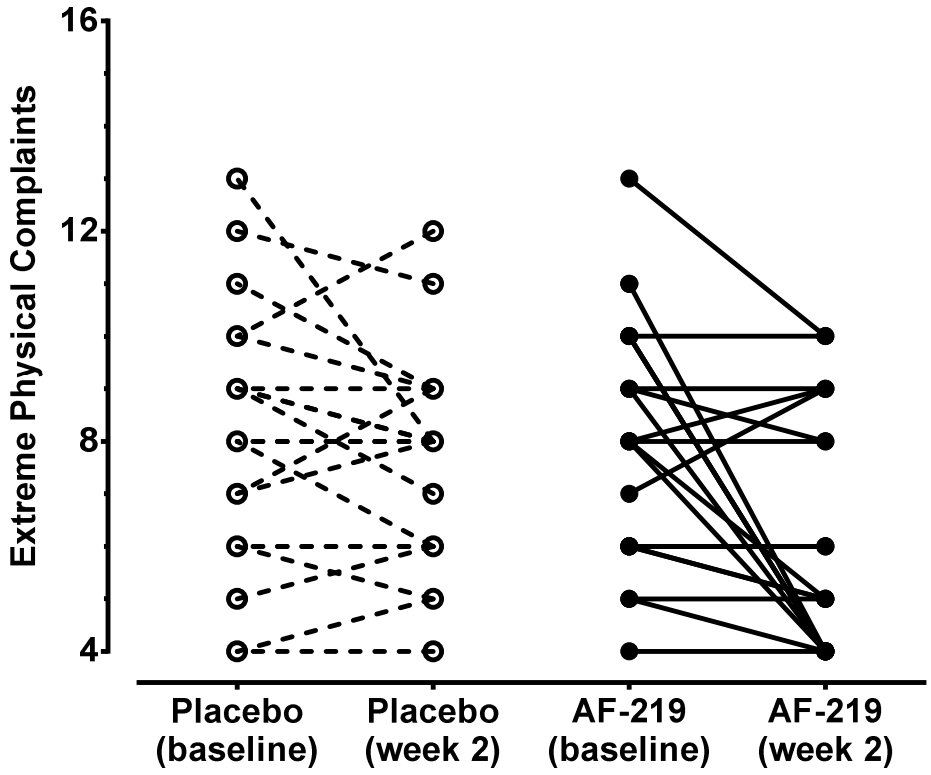


Table 72: Model estimated difference (AF-219 vs. Placebo) in change in extreme physical complaints domain of CQLQ

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	df	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
AF-219	Placebo	-1.6	0.553	37	0.007	-2.7	-0.5

The extreme physical complaints domain improved significantly with AF-219 compared with placebo.

Personal Safety Fears:

The personal safety fears domain has 3 items; scores range between 3 and 12.

Table 73: Personal safety fears

	All baselines	Baseline	Week 2
Placebo			
Number	22	21	21
Mean (SD)	5.2 (2.1)	5.3 (2.1)	5.3 (2.5)
AF-219			
Number	24	22	22
Mean (SD)	5.5 (2.2)	5.4 (2.1)	4.6 (1.9)

Figure 38: Personal safety fears domain scores for both placebo and AF-219 at baseline and week 2

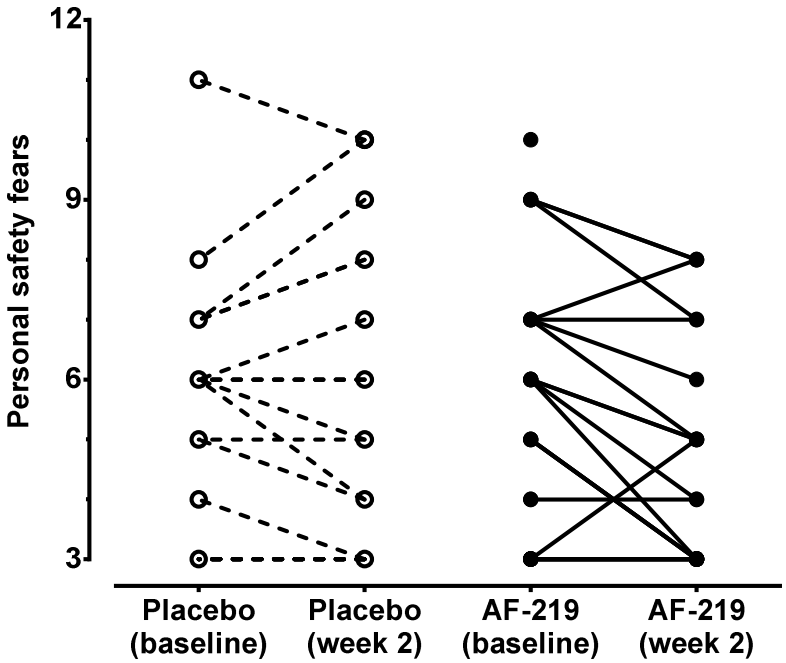


Table 74: Model estimated difference (AF-219 vs. Placebo) in change in personal safety domain of CQLQ

(I) Intervention	(J) Intervention	Mean Difference (I-J)	Std. Error	df	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
AF-219	Placebo	-0.6	0.29	20	0.075	-1.2	+0.1

The personal safety domain did not change significantly.

Global Rate of Change

Table 75: Global Rate of Change

Change in cough severity (ITT)	AF-219 N=24		Placebo N=22	
	n	%	n	%
Better	13	54%	2	9%
Same	5	46%	16	91%
Worse	6		4	

In the table above, I grouped all better or worse responses together.

Table 76: Better rating

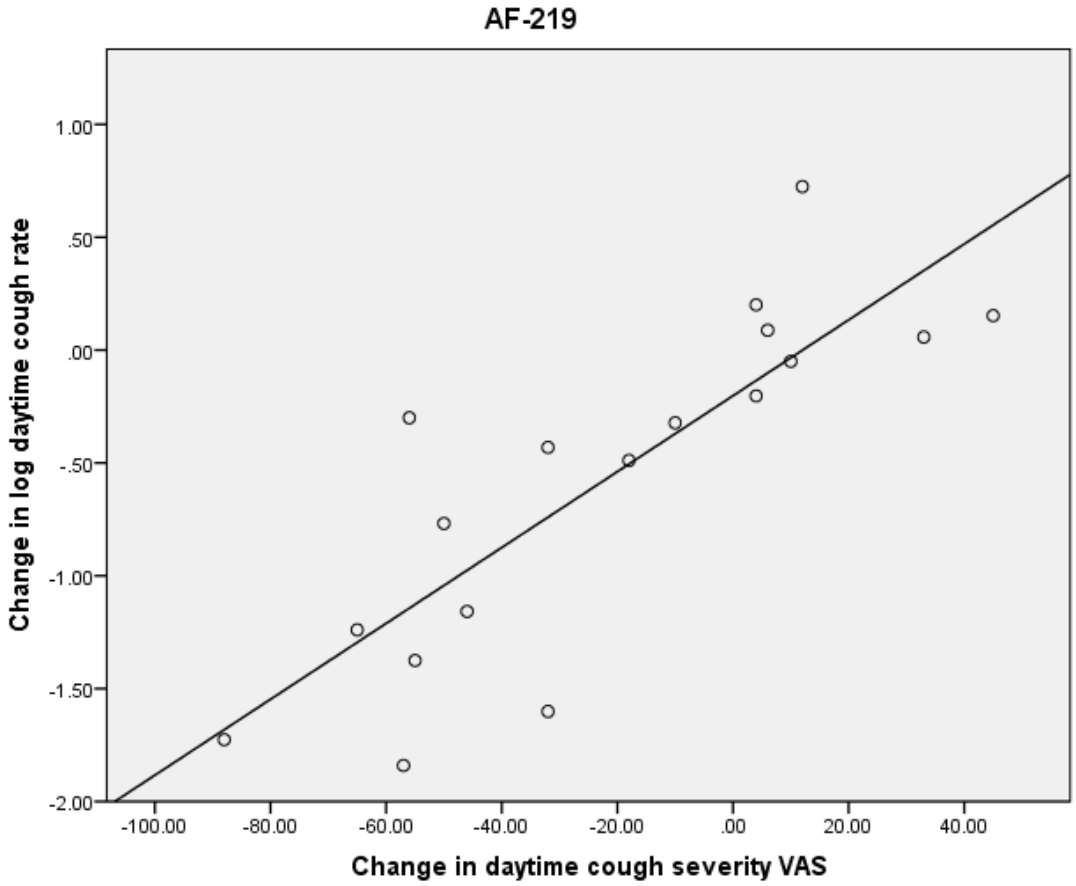
	Better ratings AF-219 ITT (N=24)	Better ratings Placebo ITT (N=22)
A very great deal better	6	1
A great deal better	4	1
A good deal better	2	
Moderately better	1	
A little better		

54% of patients rated their cough as better with AF-219 compared with only 9% with placebo. The majority of patients who rated their cough as better said that their cough was a very great deal better.

3.7.7 Correlation Between Change in Objective and Subjective Cough Assessment Tools

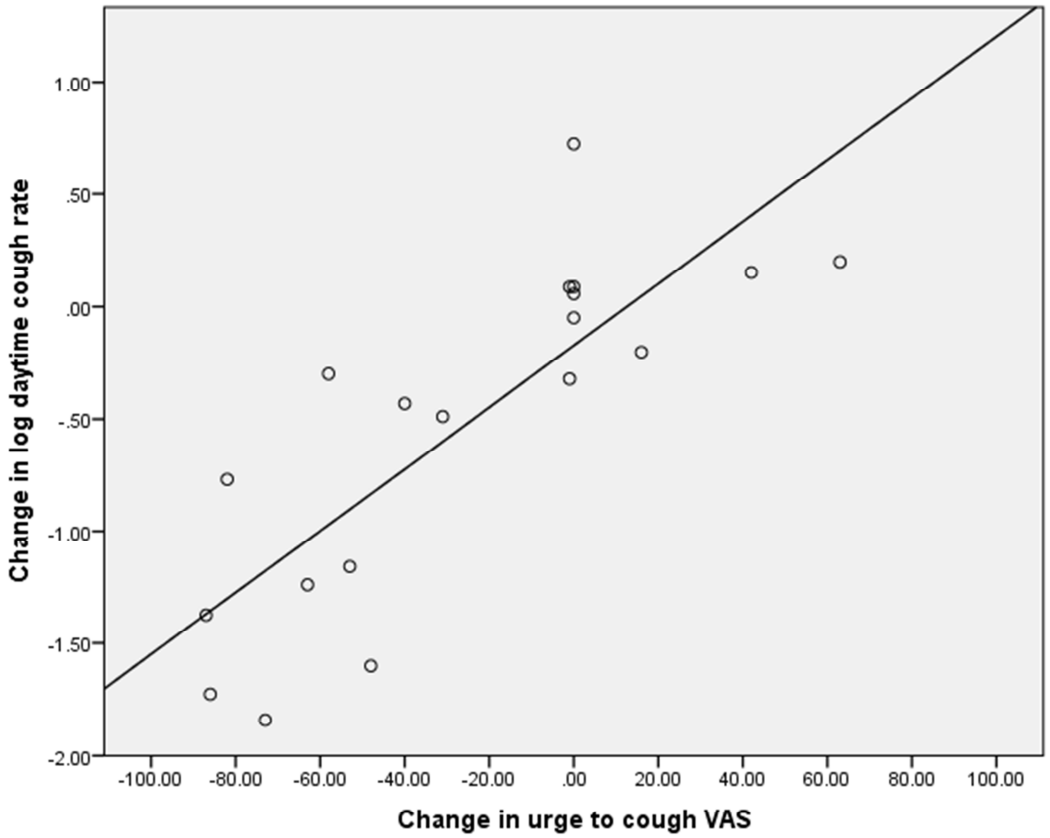
There is a significant strong positive correlation (Pearson correlation coefficient = 0.823, $p < 0.001$) between the change in daytime cough severity VAS and daytime cough rate with AF-219.

Figure 39: Correlation between the change in daytime cough VAS and change in daytime cough rate with AF-219



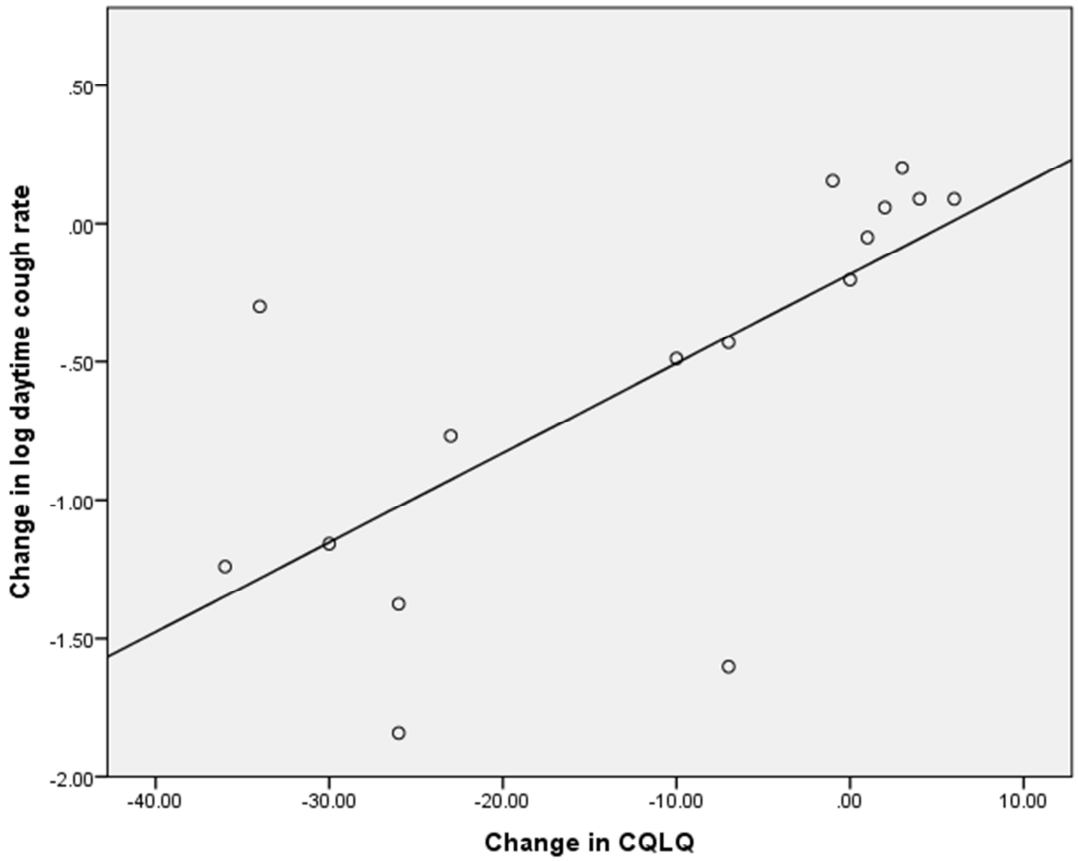
The correlation between urge to cough VAS and daytime cough rate is also strongly significant with AF-219 (Pearson correlation coefficient = 0.808, $p < 0.001$).

Figure 40: Correlation between the change in urge to cough VAS and change in daytime cough rate with AF-219



Similarly, there is a positive significant relationship between the changes in CQLQ and change in daytime cough rate (Pearson correlation coefficient = 0.709, p = 0.002).

Figure 41: Correlation between the change in CQLQ and change in daytime cough rate



3.7.8 Adverse Events

Adverse events observed in more than one subject during AF-219 treatment are summarised in Table 77. No serious adverse events occurred during the study. Reported adverse events were generally mild to moderate. All subjects experienced taste disturbances while taking AF-219; those were reduction/loss of taste sensation or abnormal taste sensation.

Table 77: Adverse events

Adverse Event	AF-219 (N=24)		Placebo (N=22)	
	n	%	n	%
Taste Disturbance	24	100	0	0
Nausea	9	37.5	1	4.5
Oropharyngeal pain	5	20.8	1	4.5
Headache	3	12.5	1	4.5
Salivary hypersecretion	3	12.5	1	4.5
Cough	3	12.5	0	0
Anosmia	2	8.3	0	0
Constipation	2	8.3	0	0
Gastroesophageal reflux	2	8.3	0	0
Painful tongue	2	8.3	0	0
Depressed mood	2	8.3	0	0
Vision blurred	2	8.3	0	0

n = number of subjects reporting adverse events (not number of adverse events).

3.7.9 Summary of Results

In summary (see Table 78 and Table 79), I have demonstrated that with the P2X3 antagonism (AF-219) compared with placebo:

- Daytime and 24 hour cough frequency improved markedly and significantly.
- Patients who responded (> 30 % reduction) had a significantly higher cough rate, and there was a strong positive relationship between the baseline day cough rate and the reduction..
- The change in night time cough frequency did not change significantly.
- Daytime cough severity VAS reduced significantly.
- Night time cough severity VAS had a trend to a significant reduction in the intent-to-treat analysis, but significant reduction in the per protocol analysis.
- Urge to cough reduced significantly.
- CQLQ improved significantly.
- Psychosocial and extreme physical complaints CQLQ domains improved significantly but other domains of CQLQ did not change significantly.
- Missing data has not significantly affected the outcome measures. Intent-to-treat using all available data, intent-to-treat worst case analysis and per protocol complete-case analysis all yielded significant change in the primary endpoint daytime cough.
- There was a positive, strong and significant correlation between changes in objective cough frequency and patient-reported outcomes (cough severity VAS, urge to cough VAS and CQLQ).

Table 78: Summary of results

Analysis Population	Outcome	Mean Difference (AF-219 vs. Placebo), 95% CI	P Value
ITT Observed Case	Daytime Cough	-75% (-50% to -88%)	<0.001
	Night Time Cough	-62% (+3% to -86%)	0.057
	24hr Cough	-74% (-46% to -87%)	0.001
	Daytime Cough VAS	-25.6 mm (-9.6 to -41.5)	0.003
	Night Time Cough VAS	-8.5 mm (+3.9 to -20.9)	0.172
	Urge to Cough VAS	-21.3 mm (-1.5 to -41)	0.035
	CQLQ	-9.2 (-1.7 to -16.8)	0.018
Per Protocol	Daytime Cough	-84% (-60% to -94%)	<0.001
	Night Time Cough	-69% (+7% to -91%)	0.061
	24hr Cough	-89% (-67% to -97%)	0.001
	Daytime Cough VAS	-30.0 mm (-10.3 to -49.6)	0.004
	Night Time Cough VAS	-15.5 mm (-2.3 to -28.8)	0.023
	Urge to Cough VAS	-34.6 mm (-11.4 to -57.9)	0.005
	CQLQ	-10.7 (-0.6 to -20.8)	0.04
ITT Worst case	Daytime Cough	-65% (-29% to -82%)	0.005

Table 79: ITT and PP Populations – Summary

Population	Total (N)	Daytime Cough Frequency	Night Time Cough Frequency	24hr Cough Frequency	Daytime Cough Severity VAS	Night Time Cough Severity VAS	UTC VAS	CQLQ							GROCS
								Overall	P	PS	F	E	EP	PS	
ITT Period 1	24	19	18	18	22	22	24	20	21	23	22	22	22	22	24
ITT Period 2	24	21	20	20	19	19	20	16	17	21	19	21	21	21	22
PP Period 1	16	14	12	12	15	15	14	12	13	15	14	14	14	14	16
PP Period 2	16	14	12	12	15	15	14	12	13	15	14	14	14	14	16
ITT (WC) Period 1	22	22													
ITT (WC) Period 2	22	22													

VAS = Visual Analogue Scale; P = Physical, PS = Psychosocial; F = Functional, E = Emotional well-being; EP = Extreme Physical; PS=Personal Safety

3.8 Discussion

This is the first clinical study evaluating a P2X3 antagonist in any human disease and additionally the first implicating a mechanistic role for P2X3-containing receptors in chronic cough. I have demonstrated an unprecedented reduction in 24 hour and daytime cough frequency with P2X3 antagonism compared with placebo. Furthermore, patient-reported cough outcome measures; daytime cough severity VAS, urge to cough VAS, and CQLQ all have significantly improved with AF-219 compared with placebo. Cough is naturally suppressed during sleep and hence cough rates are low and there is little power to detect changes in cough frequency overnight. Consistent with this, night time cough frequency and cough VAS scores did not change significantly.

Site of Action of P2X3 Antagonism

The site of anti-tussive action of AF-219 and the relative contribution of P2X3 receptors in the peripheral vs. central terminals cannot be determined from this study. Furthermore, P2X3 channels are expressed by both oesophageal and airway afferent C-fibres [87] (Figure 42). Due to the medication being systemically administered, both of these peripheral targets were potential sites for the action of AF-219. In a recent study, almost 50% of an unselected group of patients with chronic cough had their coughs preceded by reflux, indicating an important role for reflux in chronic cough regardless of the underlying trigger [156].

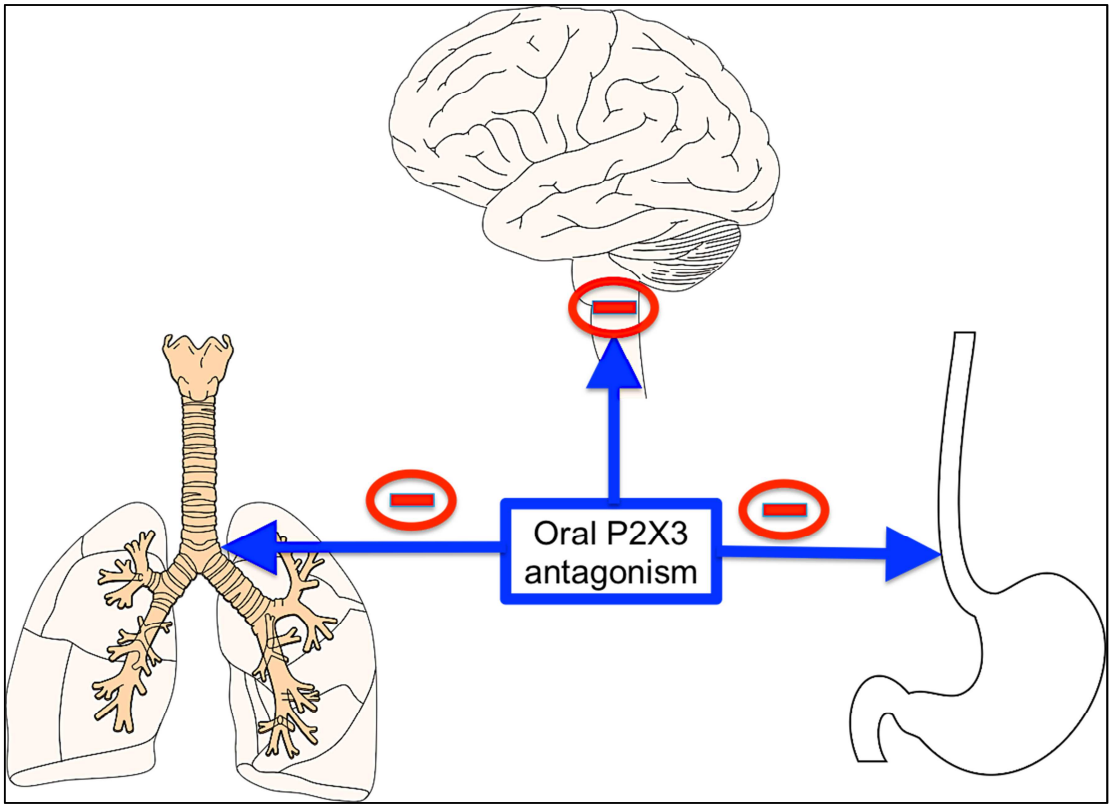
Central Nervous System

In the central nervous system, pre-synaptic terminals of primary afferent nerves have been shown to express P2X3 receptors [233, 234]. Activation of presynaptic P2X3 receptors is thought to enhance the release of excitatory neurotransmitters and modulators such as glutamate and substance P into the central synapse [233, 235] (Figure 43). There is some evidence that would suggest central nervous system upregulation of the cough reflex in patients suffering from cough. Firstly, patient report sensitivity to a broad range of environmental exposures (chemical and temperature) and physiological events (e.g. talking, eating), which would seem more likely explained by a CNS mechanism that increased expression/sensitisation of a range of peripheral nerve receptors. Secondly, we have recently demonstrated that when tussive challenges are performed using a range of irritants, chronic cough

patients tend to exhibit heightened cough responses, irrespective of the agent used [236]. Finally the observation that in a proportion of patients with chronic cough, coughing that tends to follow reflux events is most readily explained by crosstalk between vagal afferents from the airway and oesophagus where they converge in the brainstem (nucleus tractus solitarius).

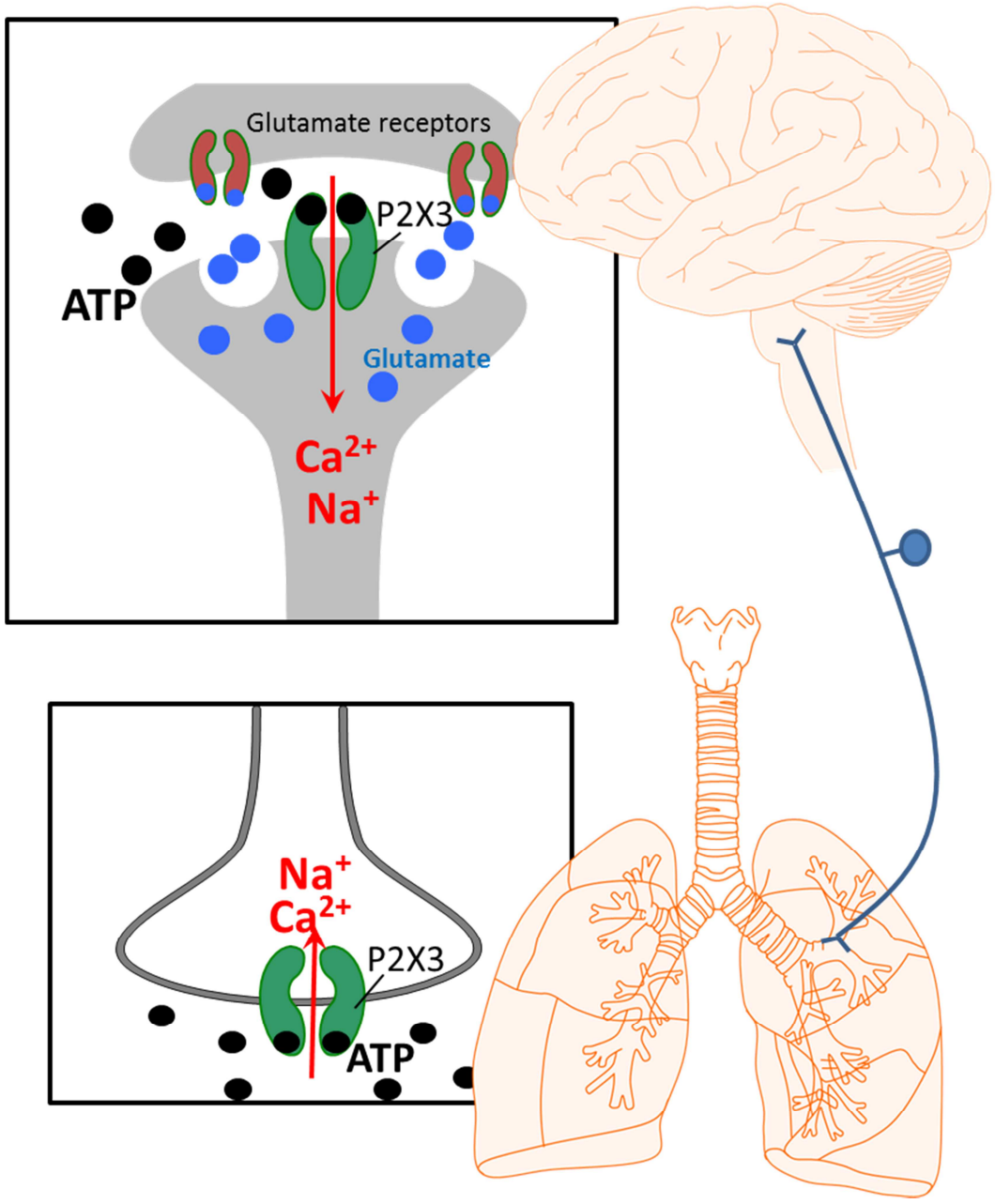
AF-219 has poor (<10%) penetration of the blood brain barrier in animal studies; therefore, a central action of AF-219 seems to be less likely. Nevertheless, central mechanisms of action cannot be ruled out; vagal afferents terminate in the NTS of the brainstem. This is adjacent to the circumventricular organs such as the area postrima (vomiting centre) which lack a full blood-brain barrier (BBB) [237]. Therefore, the effectiveness of the BBB to prevent the influence of drugs on the NTS is debatable. AF-219 might have inhibited the release of glutamate into the NTS. If central sensitisation is an important contribution to chronic cough, such a central effect of AF-219 is likely to be beneficial.

Figure 42: Sites of action of AF-219



P2X3 receptors are expressed by vagal afferents from both the oesophagus and airways and their presynaptic terminals in the NTS. Peripheral site of action is more likely than central action since the medication had poor penetration of the blood brain barrier, but cannot be ruled out.

Figure 43: P2X3 in the airway nerve endings and presynaptic terminal in the brain stem



The bottom insert represents the peripheral nerve terminals in the airways, whereas the top insert is the central synapse in the NTS. P2X3 receptors are present in both the nerve endings and the central presynapse.

Systemic administration (s.c) of the selective P2X3 & P2X2/3 antagonist, A-317491, was effective in reducing pain sensitivity in a rat model of inflammatory pain. However, selective application to the nociceptive site (locally to the hindpaw) was less effective than application to the dorsal horn of the spinal cord (intrathecally given) with neither as effective as the systemic administration [238]. This suggests that more than one site of action may be important and results in greater positive outcome [68].

To determine whether the anti-tussive effect of the P2X3 antagonist is through a peripheral or a central mechanism, the use of a topical route of administration such as a nebuliser or an inhaler should be contemplated. This would also determine if the observed effect in this study was mediated by blocking the P2X3 receptors in the airways or in the oesophagus.

An alternative possible explanation for the anti-tussive effect of AF-219 might have been the result of the profound sensations of taste disturbance. Such a substantial sensation may have overcome the urge to cough sensation, which drives cough in some patients. In other words, since cough is, to some degree, under conscious control, taste disturbance may have suppressed patients' attention to the urge to cough. However, the anti-tussive effect was not observed in all patients despite the fact that every participant had taste disturbance with AF-219.

Peripheral Nervous System

The findings from this study raise an interesting question regarding the role played by the different vagal fibre subtypes in mediating cough and ATP in the airways. Guinea pig studies indicate that only nodose C-fibres and RARs were activated by ATP [30, 49]. It has been demonstrated previously that nodose C-fibres project only to the intrapulmonary airways and not to the extra-pulmonary airways [49]. Stimulation of pulmonary C-fibres is thought to result in inhibition of cough in cats [50], and the role of RARs in mediating cough is uncertain [26]. Consistent with these findings several studies have reported that the inhalation of ATP in conscious guinea pigs does not evoke coughing. On the other hand, activation of jugular C fibres evokes coughing in conscious animals and humans (section 1.2) and Jugular C-fibres have been shown to express P2X3 receptors [87]. However, ATP fails to activate jugular C-fibres [49]. Nonetheless, it might have a priming effect, *i.e.* lowering the threshold for activation. Whether the role of these different vagal

afferent fibre subtypes are different in the sensitised state or whether there is a change in the type of fibres expressing P2X3 receptors is unclear and questions the predictive value and applicability of data obtained from animal cough studies for human chronic cough symptom.

ATP concentration in bronchoalveolar lavage (BAL) fluid has been found to be significantly higher in COPD patients compared with healthy persons [239]. The authors in that study suggested that the elevated ATP concentration in the BAL fluid played a role in modulating the inflammatory cells in the lung and as a result contributes to COPD symptoms, but no suggestion has been made about the involvement in airway neuronal sensitisation. Unpublished work from our group showed that ATP level in BAL fluid of patients with chronic cough was not different from healthy volunteers. However, in an experimental animal model of neuropathic pain, levels of tissue ATP were not elevated despite the increase in pain related behaviours and allodynia, which significantly improved after treatment with a P2X antagonist [73]. Models of neuropathic and inflammatory pain indicate an enhanced function and increased expression, possibly via trafficking and/ or increased gene expression, of P2X3 receptors in the sensitised state rather than increases in ATP [73, 83, 240]. So it is possible to speculate that either increased expression or sensitisation of the function of P2X3 receptors are present in chronic cough patients given the significant antitussive effect observed with AF-219. No studies of P2X3 immunoreactivity, mRNA or protein level have been done in patients with chronic cough.

Responders and Non-Responders

It is clear from the results that we are dealing with 2 distinct groups: responders vs. non-responders. Approximately half of the AF-219 group had 30 % or more reduction in cough frequency. Similarly, in the study by Morice et al investigating the effect of morphine in patients with chronic cough, authors reported that there was a group of responders and non-responders [19]. Responders had a significantly higher baseline cough rate than non-responders and there was a significant strong negative relationship between baseline daytime cough and the change seen. In a cohort analysis of gabapentin in chronic cough, 20/35 patients reported an improvement in cough subjectively; responders had a significantly higher pre-treatment cough severity scores than non-responders. This highlights an important issue that patients with chronic cough are a heterogeneous group. It is possible to

speculate that patients with higher cough rate had more P2X3 expression in their airways and therefore with P2X3 antagonism there was a therapeutic benefit. Pharmacological tools such as this novel channel antagonist may help in characterising patients based on the role played by different membrane receptors in mediating neuronal excitability of the vagal afferents involved in the cough reflex. In contrast the mechanism of action of other treatments thought to have some effect in chronic cough, such as thalidomide, gabapentin and morphine are less specific and not well understood and therefore they provide less insight into mechanisms.

Two previous clinical trials investigating the effect of two centrally acting agents, morphine [19] and gabapentin [20] in chronic cough reported an improvement in a cough-specific quality of life measure; the Leicester cough questionnaire (LCQ). The study by Morice *et al* did not objectively measure coughs and although Ryan and colleagues reported an improvement in cough frequency, this was based on one hour data only including the time patients were inhaling an irritant, capsaicin, to measure the cough reflex sensitivity. A recent study of a novel, selective oral antagonist of TRPV1 ion channel failed to significantly reduced objective cough frequency or CQLQ [59]. My study demonstrated an improvement in both subjective and objective measures of coughs with a novel P2X3, P2X2/3 antagonist. The magnitude of reduction in cough frequency has never been demonstrated for any treatment for chronic cough before.

With regard to the use of CQLQ in therapeutic trials of cough treatment, a RCT of thalidomide in IPF-related coughs was published recently [241]. CQLQ was the primary endpoint of the study and there was a significant improvement in CQLQ (thalidomide vs. placebo mean difference -11.4 [95% CI, −15.7 to −7.0]; $P < 0.001$). Our study showed an improvement of -9.2 (95 CI -16.8 to - 1.7) in the ITT analysis but similar change of -10.7 (-20.8 to - 0.6) in the PP analysis. Some of the CQLQ data is missing from our analysis due to some patients missing questions, which may have affected the results. The improvement in CQLQ may have been more significant had this missing data been included, particularly as some missing scores were for patients with significantly reduced cough frequency after treatment with AF-219. Also, compared with IPF, patients with chronic cough may respond differently in terms of quality of life.

The same group that developed the CQLQ has recently published a work estimating the minimal important difference (MID) for CQLQ; this was estimated to be 10.58

using GRCS (retrospectively assessing overall change) and 21.89 using a prospective tool for assessing change, Punum ladder [193]. The authors argue that predictable MID should be based on the Punum ladder tool as it is more accurately correlating with the both baseline and current state, whereas GROCS is mainly correlated with the current situation but not with the baseline one. In comparison, the MID for the LCQ was calculated using the GROCS [194].

Cough improved significantly in this study; however, the improvement in CQLQ score is lower than the estimated MID. This could be explained by the study population, which is patients with refractory chronic cough in contrast with chronic cough patients who are undergoing initial assessment and treatment as in the study by Fletcher et al [193]. In addition, we have a group of responders and non-responders and therefore the improvement in CQLQ on average is lower.

It is a very interesting finding of the analysis of CQLQ domains to find that psychosocial items, such as *"Family and or close friends can't tolerate it anymore"*, and extreme physical items, such as *"I wet my pants"*, mainly drove the significant change in the total CQLQ scores. It highlights the issue of how pre-treatment cough affected patients in their social life and extreme physical consequences of chronic cough. For example, chronic cough patients have similar total CQLQ scores to acute cough patients but differ significantly in two subscales: psychosocial and emotional well-being [192]. In the thalidomide study for IPF-related cough, psychosocial, extreme physical complaints, physical complains and personal safety fears domains significantly improved but the emotional and functional domains did not change [241]. Our patients have been investigated thoroughly over the years and know that there is no sinister cause of their cough but people around them may not understand this and it is therefore embarrassing for patients when they are in social situations.

This is the first study showing a significant improvement in ambulatory objective cough frequency and subjective assessments with an anti-tussive medication. Therefore, it has been possible to examine the relationship between changes in ambulatory objectively recorded cough frequency and how patients perceived those changes. There was a significant and strong correlation between the change in cough frequency and subjective measures of cough. In this study, the change in daytime cough was marked (75% reduction), which suggests that substantial reduction in cough is necessary for patients to reliably appreciate. Less marked

change (33% reduction) in cough frequency, associated with the placement of an oesophageal catheter, in patients with chronic cough was not correlated with the reduction in cough severity VAS scores [222]. Despite the strong correlation between changes in cough frequency and VAS scores seen in this study, clinical trials of anti-tussive medications should use both objective and subjective tools because they measure different aspects of cough.

All participants had taste disturbance, which is an effect of blocking the P2X3-containing receptors in the primary afferents relaying signals from the taste buds [242, 243]. There is also some evidence of P2X3 receptor distribution in retina and olfactory nerve as well, explaining the adverse events of blurred vision and loss of smell [244]. Some subjects reported feeling low in mood and attributed this to loss of taste and as a result not able to enjoy food.

3.8.1 Study Limitations: Measures Taken to Minimize and Compensate

Blinding of the study may not have been achieved given the prominent taste issues associated with AF-219. Chronic refractory cough patients have low expectations for treatment success and therefore are less likely to be affected by the unblinding factor. In addition, not all patients improved despite that all of them experienced the taste disturbance. The primary end point of the study was objectively recorded cough frequency, which limits potential bias compared with subjective tools.

6/24 subjects discontinued the study medication early (AF-219). If the treatment period was shorter, for example one week, this may have enhanced the ability to retain subjects in the study. At the time of designing the study, it was unknown whether any anti-tussive effect of AF-219 would be immediate or would take a longer time to detect. In this study subjects who have appreciated improvement in their cough reported such within a day or so.

Although missing data, because of early withdrawals in the AF-219 group, could potentially undermine the inferences drawn from the study, I performed three different analyses to understand the influences of the missing data: intention-to-treat using all available data, intention-to-treat imputing worst change for informatively missing data, and the per protocol complete-case analysis. The results from all the

analyses were still robustly significant, suggesting that missing data did not alter substantially the overall significance of the change in cough outcome measures.

The majority of subjects were female. This generally reflects the population of patients attending chronic cough clinics.

3.9 Conclusion

The following conclusions can be drawn from this proof-of-concept study:

- This study has shown that antagonising P2X3, P2X2/3 has resulted in an unprecedented improvement in cough as evident by the reduction in ambulatory cough count, cough severity and urge to cough VAS scores, and cough-specific health-related quality of life.
- The study unravels a mechanistic role played by the ATP-gated P2X3-containing receptors in chronic cough; it suggests these receptors contribute to the hypersensitivity of the afferent pathways mediating the cough reflex.
- P2X3, P2X2/3 appears to be a promising novel anti-tussive target.

CHAPTER 4

An Open Label Feasibility Study of Memantine in Patients with Chronic Cough

4.1 Background and Rationale

Vagal afferents of the cough reflex are believed to terminate in the NTS area of the brain stem. Activation of cough neurons results in the release of glutamate, a major excitatory neurotransmitter, into the central synapse. Pre-clinical studies indicate that glutamate-gated NMDA receptors mediate cough centrally. NMDAR antagonists microinjected into the NTS have been shown to significantly reduce experimentally evoked coughs in anaesthetised animals [24, 245]. In the introduction chapter, I described how central sensitisation could provide a plausible explanation for chronic cough. I also provided details about the molecular mechanisms leading to central sensitisation and how NMDARs are involved in its establishment. Taken together, blocking NMDARs could be an effective therapy in chronic cough.

The NMDA receptor is comprised of four different subunits. The NR1 subunit is essential for the receptor to function. NMDARs in different neuronal tissues selectively express the other subunits (NR2 A-D and NR3 A-B). For example, genes for NR2A subunits have been found, at least in guinea pigs, to be present in jugular ganglia, but seldom in the NTS or nodose ganglia [147]. Assuming those subunits are also expressed by airway nerve terminals and they have functional activity in cough, they could be a target for the treatment of chronic cough. NMDARs on airway nerve terminals are suggested to modulate the release of neurotransmitters into the central synapse [147].

Clinically, dextromethorphan, a weak NMDAR antagonist [246], is widely used as an anti-tussive compound in many of the cough medicines available over the counter. In healthy volunteers, pre-treatment with oral dextromethorphan successfully attenuates evoked cough [247-249]. However, its efficacy in suppressing cough is debatable as it reduced objectively counted cough by no more than 17% over placebo in patients with acute cough [250]. Furthermore, the exact mechanism of action of dextromethorphan is uncertain. In addition to blocking NMDARs, it is also an agonist of sigma receptors [251] and an antagonist of voltage-gated calcium channels [252].

Memantine is another NMDAR antagonist, which is licensed for the treatment of Alzheimer's disease. Unlike dextromethorphan, it has no action on sigma or voltage-gated calcium channels receptors and targets primarily those NMDARs that are

highly active [146]. Both of these features could explain the reported good tolerability and safety of memantine [253]. Importantly, memantine has been proven to be efficacious in substantially blocking cough provoked by inhalation of citric acid and bradykinin in conscious guinea pigs [147]. There are not yet any reported clinical trials of memantine in chronic cough patients.

4.2 Hypothesis

NMDARs-dependent hyper excitability of central neurons in the NTS is a key mechanism in chronic cough and, therefore, memantine would significantly improve cough.

4.3 Aim

This is a feasibility study designed to inform future randomised clinical trials of memantine in treating chronic cough.

The aims of this study are:

- To explore the efficacy of escalating doses (10 mg OD titrated to a maximum of 40 mg OD) of memantine, taken orally, in a group of refractory chronic cough patients.
- To explore the tolerability of escalating doses (10 mg OD titrated to a maximum of 40 mg OD) of memantine, taken orally, in a group of refractory chronic cough patients.
- To generate data for estimating the sample size for a future randomised controlled trial of memantine compared with matched placebo.

4.4 Objectives

Primary Objective:

- To evaluate the change from baseline to end of treatment in daytime cough frequency for the maximum tolerated dose of memantine in patients with refractory chronic cough.

Secondary Objective:

- To evaluate the tolerability of memantine in patients with chronic cough.
- To evaluate the change in CQLQ with memantine treatment
- To assess patients reported global rating of change in cough frequency and severity

4.5 Methodology

4.5.1 Study Subjects

Patients with idiopathic chronic cough or chronic cough resistant to treatment of specific triggers were recruited from our tertiary cough clinic (University Hospitals of South Manchester, UK) over a 6-month period from February 2013 to August 2013. Patients were investigated according to a diagnostic algorithm [211, 212]. The diagnostic algorithm enabled patients to be investigated thoroughly for specific triggers of chronic cough. All patients had full lung function testing, methacholine/histamine challenge testing, nasoendoscopy, high resolution computerised tomography of the chest, and bronchoscopy (with lavage for differential cell count, and endobronchial biopsies). All patients were treated for any detected triggers of chronic cough (inhaled corticosteroid for asthma/eosinophilic bronchitis, corticosteroid nasal spray and anti-histamines for post-nasal drip syndrome, and proton pump inhibitor twice a day and nocturnal ranitidine for gastro-oesophageal reflux). Patients with cough refractory to treatment of underlying triggers were considered eligible for the study.

Approvals from Haydock North West Research and Ethics Committee (reference: 11/NW/0840) and the Medicines and Healthcare Products Regulatory Agency (reference number 35030/0003/001) were obtained prior to the start of the study. The trial was registered at ISRCTN (ISRCTN99941214). All patients signed an informed consent form and the study was conducted according to the Declaration of Helsinki.

4.5.2 Inclusion and exclusion criteria

Inclusion Criteria

- Male and female subjects, age 18 years and over
- Normal CXR and spirometry
- Chronic idiopathic cough or chronic cough resistant to treatment of specific triggers
- Women of child-bearing potential must have had a negative pregnancy test and agreed to use one of the following acceptable birth control methods: true

complete abstinence, surgical sterilisation of either the female subject in study or of her male partner, established hormonal contraception, an intrauterine device (IUD) or intrauterine system (IUS), double barrier method.

Exclusion Criteria

- Recent upper respiratory tract infection (< 4 weeks)
- Pregnancy/breast feeding
- Current smoker, individuals who had given up smoking within the past 6 months, or those with > 20 pack year smoking history
- Current treatment with ACEI
- Drug or alcohol abuse
- Uncontrolled hypertension (i.e. > 160/100 mmHg despite adequate treatment)
- Recent myocardial infarction, or history of congestive cardiac failure
- Any clinically significant neurological disorder
- Prior renal transplant, current renal dialysis, creatinine clearance < 30 ml/min
- Severe hepatic impairment
- Fructose intolerance
- Any clinically significant or unstable medical or psychiatric condition
- History of seizure disorder, recent head trauma that resulted in loss of consciousness
- Prohibited medications:
    - Medications that may affect the cough reflex such as opioids, anticonvulsants or tricyclic antidepressants (should had stopped taking such medications for at least 2 weeks to allow entry into the study)
    - Other NMDAR antagonists (*e.g.* dextromethorphan, ketamine, amantadine)
    - Medications that may interact with memantine (*e.g.* cimetidine, ranitidine, systemic anti-cholinergics, warfarin)

- Clinically significant abnormal laboratory test results

4.5.3 Study Design

This was an open-label, uncontrolled, feasibility study of escalating doses (10 – 40 mg OD taken orally) of memantine to assist in designing a future randomised controlled trial.

4.5.4 Study Procedures and Visits

See Figure 44

Visit 1

- Informed written consent
- Checking eligibility criteria
- Measuring blood pressure and pulse (both standing and lying)
- FBC, U&Es, LFTs, serum βHCG (for women of child bearing age)
- Baseline CQLQ
- Baseline 24 hour ambulatory cough monitor

Visit 2

- Patients were initiated on memantine 10 mg per day for one week then 20 mg per day for another week.
- At the end of week 1, patients were contacted by phone to ensure that they were well and if so they were reminded to increase the dose to 20 mg.

Visit 3

- Measuring blood pressure and pulse (both standing and lying)
- Patients completed a global rating of change scale (GROCS).
- If memantine was tolerated well then the dose was increased to 30 mg OD for one week.

- If subjects did not tolerate 20 mg of memantine, the dose was decreased to 10 mg and they remained on this dose for 4 weeks until the end of study visits.

Visit 4

- Measuring blood pressure and pulse (both standing and lying)
- Patients completed a global rating of change scale.
- If memantine was tolerated well, the dose was increased to 40 mg OD for one week
- If subjects did not tolerate 40 mg of memantine, the dose was decreased to 30 mg and they remained on this dose for 4 weeks until end of study visits

Visit 5

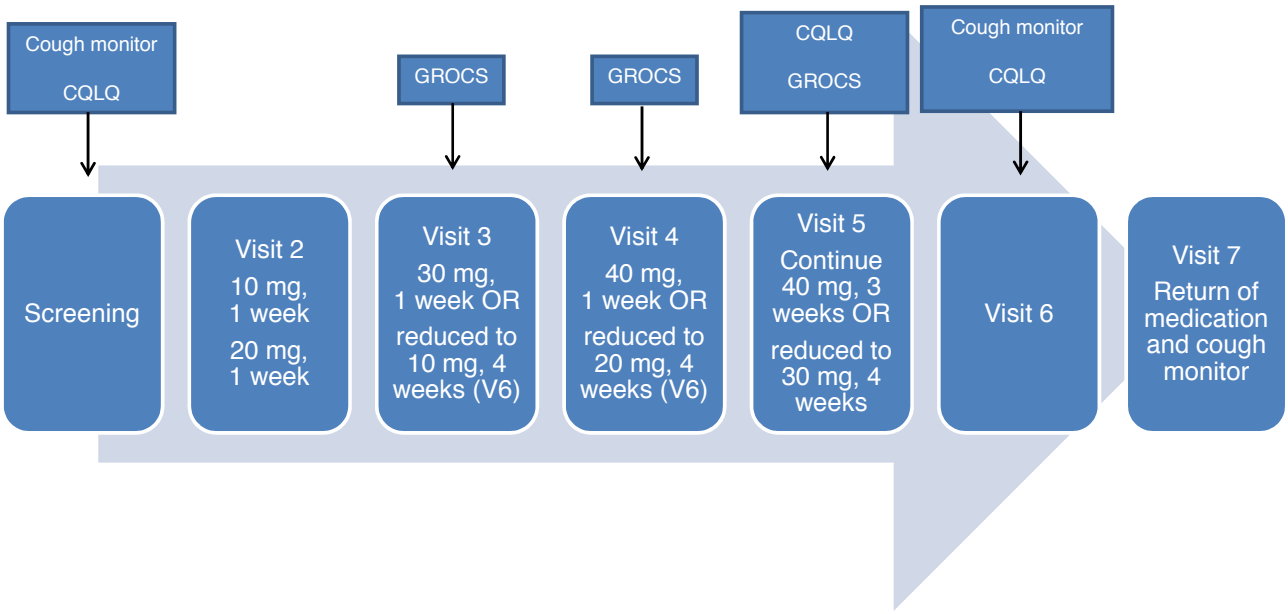
- Measuring blood pressure and pulse (both standing and lying)
- Patients completed a global rating of change scale and CQLQ.
- If memantine was still well-tolerated, 40 mg was continued for another 3 weeks.
- If 40 mg was not tolerated, dose was reduced to 30 mg and subjects remained on this dose for 4 weeks.

Visits 6 and 7

- Measuring blood pressure and pulse (both standing and lying)
- End of treatment outcome measures:
    - 24 hour ambulatory cough monitor
    - CQLQ
    - global rating of change

- 24 hours later, unused tablets and cough monitor were returned.

Figure 44: Memantine study procedures



4.5.5 Detailed Description and Justification of Methodology

Rationale for the Dose Escalation and Length of Treatment

Memantine is licensed to treat moderate to severe Alzheimer's disease in doses of 10–20 mg. In trials of neuropathic pain, doses of up to 80 mg a day were used [254-257]. However, doses greater than 40 mg were associated with significant adverse events. Memantine has not previously been administered to patients with chronic cough; therefore this study aimed to examine the tolerability of doses from 10 to 40 mg (Table 80). Subjects who experienced intolerable adverse effects were allowed to reduce the dose by 10 mg decrements to find the best tolerated dose and then remain on that dose for 4 weeks.

Central sensitisation is a process that results in long lasting hyper excitability of central neurons. It is unknown how long it might take to reverse, but it is likely to be a slow process that takes weeks rather than days. Arbitrarily, the duration of maintenance dose (40 mg per day or the maximum tolerated dose) was chosen to be four weeks. End of study efficacy measures were performed earlier than four weeks if a subject felt unable to tolerate the memantine for the full study period.

Table 80: Dose titration

Week	Dose
Week 1	10 mg/day (1 tablet)
Week 2	20 mg/day (2 tablets)
Week 3	30mg/day (3 tablets)
Week 4	40mg/day (4 tablets)
Week 5	40mg/day (4 tablets)
Week 6	40mg/day (4 tablets)
Week 7	40mg/day (4 tablets)

Ambulatory Objective Cough Recording

For the purpose of obtaining objective cough frequency, the VitaloJAK™ cough monitor (Vitalograph Ltd, UK), a custom-built digital recording device, was used. VitaloJAK™ features an air microphone attached to the subject's lapel and an adhesive sensor attached to the chest wall over the sternum. The device records all sounds (8 kHz, 16 bit wave format) continuously over 24 hours. Data was written to a 4 Gigabyte compact flash data card, which was then downloaded onto a personal computer and archived on a digital versatile disc. A validated custom-written software was then used to compress the recording from 24 hours to a shorter file by detecting all potential cough sounds and cutting out non-cough sounds such as silence, background noise and speech [232]. Trained staff then manually listened to the compressed file and counted the number of explosive cough sounds using an audio editing software package (Adobe® Audition® 3.0). The results were expressed as explosive cough sounds per hour. This is a validated and repeatable objective measure of cough frequency [186], with excellent intra-and inter-observer agreement [179].

CQLQ

This is described in the introduction chapter (section 1.6.2)

Global Rating of Change Scale

This is a 15-point questionnaire to retrospectively assess the overall change in cough frequency and severity after intervention. Patients were asked to score the change in their cough frequency and severity on a scale ranging from "a very great deal better" to "a very great deal worse" (Appendix 3).

4.5.6 Sample Size and Statistical Analysis

No sample size estimation was done because of the pilot nature of this study. Cough rate was log transformed to obtain a normal distribution. A paired t-test (SPSS, version 20.0) was used to compare mean cough frequency (coughs/hour) and CQLQ scores before and after treatment. A conventional two-sided 5% significance level was used. Graphs were generated by Prism (version 6, GraphPad Software Inc., CA, USA).

4.6 Results

4.6.1 Subjects

Seventeen subjects were screened and 14 received memantine, but only 12 subjects had remained on the medication at the time of efficacy visit (visit 6), see Figure 45. Demographic data of the enrolled subjects is summarised in Table 81.

Figure 45: Flow diagram

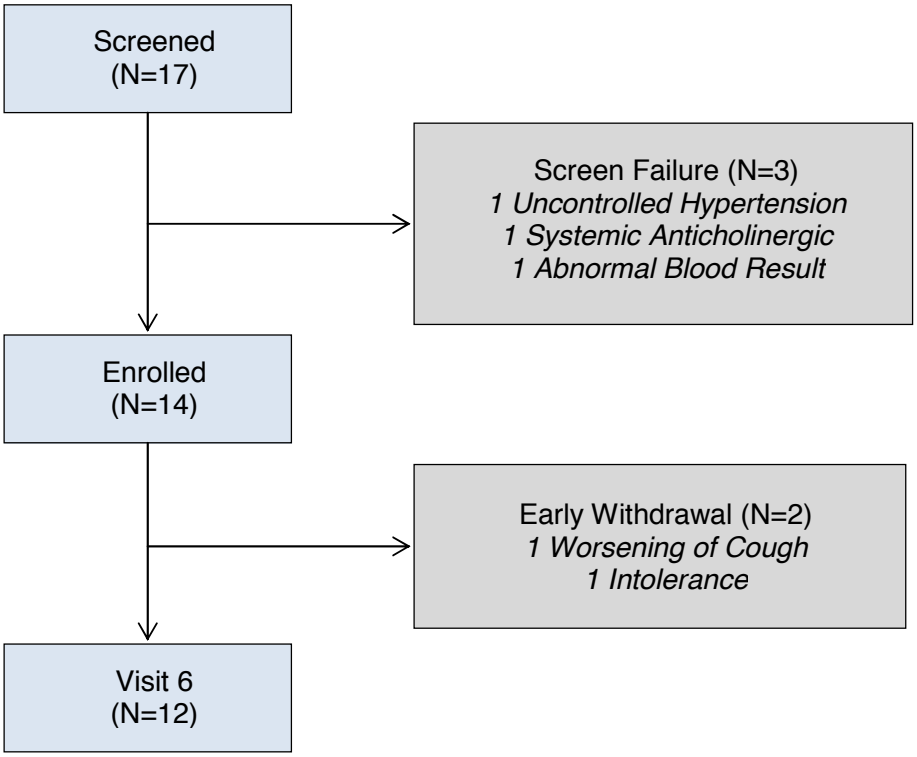


Table 81: Demographics of participants

Variable	Description
Participants, n	14
Age, mean years (SD)	57.9 (SD 11.8)
Gender, female: male	13:1
Smoking history	
Never smoker, n	11
Ex-smoker, n	3
Cough duration, mean years (SD)	13.7 (SD 6.8)
Type of cough	
Dry, n	11
Productive, n	3

4.6.2 Dosing and Duration of Treatment

Of the 14 subjects enrolled into the study, the number (%) of patients who, during the study, reached memantine dose of 10 mg, 20 mg, 30 mg and 40 mg were 14 (100%), 12 (85.7%), 6 (42.9%), and 1 (7.1%) respectively. Median (min, max) duration of the treatment period including dose escalation was 38.5 days (7–49).

At the end of the study, the majority of subjects (n=10, 71.4%) were on a maximum dose of 10 mg per day. Two subjects (14.3%) were on 20 mg/day and two (14.3%) on 30 mg/day. No subject remained on 40 mg per day (Table 83).

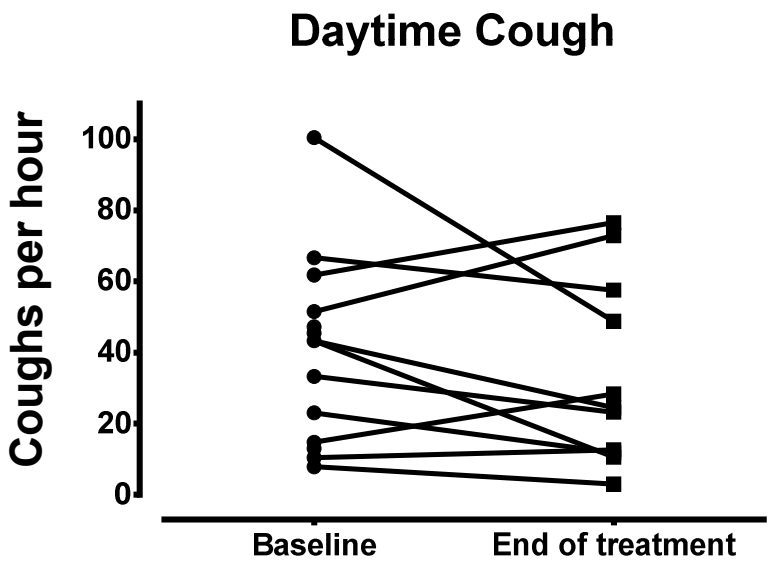
Nine of the 14 subjects (64.3%) remained on the maximum tolerated dose for four weeks (n=6 on 10 mg, n=2 on 20 mg, n=1 on 30 mg). The reasons for less than four weeks of treatment with the maximum tolerated dose were: intolerance (n=3), worsening of cough (n=1), and going on holiday (n=1). The median duration of the maximum tolerated dose*, i.e.* maintenance period, was 28 days (4 – 28)**.**

4.6.3 Effect of Memantine on Daytime Cough Frequency

Eleven subjects had cough recordings at both baseline and visit 6. One subject had a URTI on visit 6; therefore cough recording was not carried out.

Daytime cough rate was not significantly different with memantine treatment (geometric mean 30.9 coughs/hr [95% CI, 15.6 – 61.2]) compared with baseline (41.1 coughs/hr [95% CI, 22.9 – 73.8]), paired-sample t (10) = -1.601, P = 0.141 (Figure 46). The median (min, max) percentage change in daytime cough frequency with memantine treatment was -17.3% (-73.6% to +115%).

Figure 46: Daytime cough rate before and after memantine

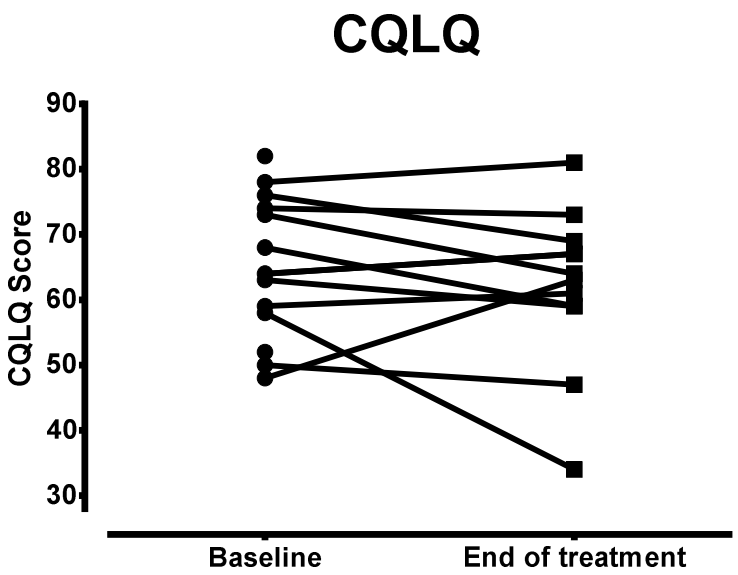


4.6.4 Effect of Memantine on Cough Quality of Life

One subject did not completely answer all the questions on visit 6. Therefore, CQLQ scores for both baseline and end of study visits were available for 13 subjects. Those who discontinued the treatment early (n=2) completed the questionnaire for the period they were on the medication.

Cough quality of life scores did not change significantly with memantine treatment (mean 62.0 [95% CI, 54.3 – 69.7] vs. 64.6 [95% CI, 58.4 – 70.8]; paired-sample t (11) = -0.944; P= 0.366) (Figure 47). The mean difference in CQLQ scores with memantine treatment was -2.6 (95% CI -8.6 to +3.4).

Figure 47: CQLQ scores before and after memantine



Note: lower scores indicate a better quality of life.

4.6.5 Global Rating of Change

Fifty percent of the subjects who remained on 10 mg of treatment did not feel a change in their cough frequency and 80% did not think that the severity of their cough was different. The two subjects who stayed on 20 mg felt that their cough had improved. However, both subjects who continued on 30 mg of memantine rated their cough as worse than before treatment. The relationship between the rating of change and dose is difficult to determine given the small number of subjects in each dose category and hence no testing of statistical significance was performed. Three subjects altered their rating of change in cough from "better" (n=2 moderately better, n=1 a little better) while taking 20 mg on visit 3 to "about the same" on visit 6 when they were taking 10 mg. On the contrary, one subject did not perceive a change in her cough on visit 3 (20 mg) but thought her cough was moderately better on visit 6 (10 mg). Details of the global rating of change are shown in Table 82 and Table 84.

Table 82: Summary of global rating of change for the different maximum tolerated doses

Dose	Cough Frequency	Cough Severity
10 mg	Better (n=3)	Better (n=1)
	A very great deal better (n=1)	*A very great deal better (n=1)*
	Moderately better (n=2)	
	Same (n=5)	Same (n=8)
	Worse (n=2)	Worse (n=2)
	Moderately worse (n=1)	*Moderately worse (n=1)*
	Hardly any worse (n=1)	
20 mg	Better (n=2)	Better (n=2)
	A little better (n=1)	*A little better (n=1)*
	Moderately better (n=1)	*Moderately better (n=1)*
30 mg	Worse (n=2)	Worse (n=2)
	A very great deal worse (n=1)	*A very great deal worse (n=1)*
	A little worse (n=1)	*A little worse (n=1)*

Table 83: Summary of study findings

Demographics				End of Study Outcome Measures			
Subject ID	Uninterrupted Treatment Time	Maximum Tolerated Dose	Maximum Dose Duration	% Change Daytime Cough	Change in CQLQ	GROCS Cough Frequency	GROCS Cough Severity
301	6 weeks	10 mg	4 weeks	-50.1%	+3	About the same	About the same
302[a]	5 weeks	10 mg	2 weeks, 4 days	+13.2%	-7	Moderately worse	Moderately worse
303[b]	3 weeks, 2 days	10 mg	3 weeks, 2 days	-17.3%	-4	About the same	About the same
306[c]	4 weeks, 5 days	10 mg	2 weeks, 5 days	+115%	+3	Moderately better	About the same
307[d]	1 week	10 mg	1 week	Withdrew		About the same	About the same
308	6 weeks	10 mg	4 weeks	+34.9%	-1	About the same, hardly any worse at all	About the same
309[e]	4 weeks	10 mg	4 weeks	+3%	-3	Moderately better	About the same
310	6 weeks	10 mg	4 weeks	-15.5%	+3	About the same	About the same
311	7 weeks	20 mg	4 weeks	-49.3%	-24	Moderately better	Moderately better
312[f]	7 weeks	30 mg	4 weeks	Not Done	+2	A little worse	A little worse
313	6 weeks	10 mg	4 weeks	-34.5%	-9	About the same	About the same
314	4 weeks	10 mg	4 weeks	-58.6%	-9	A very great deal better	A very great deal better
316[g]	2 weeks, 4 days	30 mg	4 days	Withdrew	+15	A very great deal worse	A great deal worse
317	7 weeks	20 mg	4 weeks	-73.6%	Missing	A little better	A little better

[a]Dose escalated to 30 mg but subject had to reduce to 10 mg and shorten duration of treatment due to feeling tired.

[b]Subject had 10 mg for one week, 20 mg one week, and severe AEs after one dose of 30 mg. Following that, the treatment was interrupted for 4 weeks because of URTI then restarted at 10 mg (frightened to have higher doses) for around 3 weeks (did not complete 4 weeks because of planned holiday).

[c]Shorter duration of treatment because of AE (sensation of “increased pressure inside the head”),

[d]Withdrew because of drowsiness and tiredness

[e]Tried 20 mg for 4 days but did not tolerate it (lightheadedness & tiredness), also had URTI in week 2. Dose was interrupted for 6 weeks than restarted at 10 mg for 4 weeks.

[f]Had URTI on V6.

[g]Withdrew because of lack of effect and worsening of cough

Table 84: Global ratings of change in cough frequency/severity since the start of treatment

Subject	Visit 3	Dose	Visit 4	Dose	Visit 5	Dose	Visit 6	Dose
301	About the same	10 mg	NA		NA		About the same	10 mg
302	A good deal worse	20mg	A good deal worse		NA		Moderately worse	10 mg
303	Moderately better	20 mg	NA		NA		About the same	10 mg
306	About the same	20 mg	NA		NA		Moderately better	10 mg
307	About the same	10 mg	NA		NA		NA	
308	A little better	20 mg	NA		NA		Almost the same, hardly any worse at all	10 mg
309	NA		NA		NA		Moderately better, about the same	10 mg
310	About the same	10 mg	NA		NA		About the same	10 mg
311	A good deal better	20 mg	A good deal better	25 mg	NA		Moderately better	20 mg
312	A little worse	20 mg	A little worse	30 mg	A little worse	30 mg	A little worse	30 mg
313	Moderately better	20 mg	NA		NA		About the same	10 mg
314	A great deal better	10 mg	NA		NA		A very great deal better	10 mg
316	Somewhat worse/ about the same	20 mg	NA		NA		A very great deal worse/ a great deal worse	30 mg
317	About the same/ moderately better	20 mg	About the same/ a little better	30 mg	NA		A little better	20 mg

NA= not available because the patient did not have the visit. Subject 311 did not tolerate dose escalation to 30 mg and, therefore, took two and a half tablets (25 mg) between visits 3 and 4. Subject 312 did not tolerate 40 mg so reduced the dose to 30 mg before visit 5.

4.6.6 Adverse Events and Tolerability

There were no serious adverse events during the study. The most common adverse events were dizziness, tiredness, and drowsiness (Table 85). Even at a daily dose of 10 mg, 8 of the 14 subjects (57%) experienced adverse events related to memantine (mainly drowsiness n=4, tiredness n=3, dizziness n=3, headache n=3) (Table 86). Taking 20 mg of memantine a day (n=12) was associated with related adverse events in 9 (75%) subjects that tended to be more bothersome than the ones associated with taking 10 mg.

Adverse Events Reported with 30 mg

The dose was escalated to 30 mg in 6 subjects. One subject had severe symptoms of dizziness, slurred speech, perception of “funny sensation” on her right side, and feeling spaced out and moderately severe nausea. Another subject felt spaced out which affected her ability to work and was not able to drive. The other two subjects were moderately lightheaded. No adverse events were reported with taking 30 mg in two subjects.

Adverse Events Reported with 40 mg

Only one subject could have had her dose increased to 40 mg, but she did not tolerate this dose well enough to remain on it because of lightheadedness.

There were no observed changes in blood pressure (i.e. hypertension or postural drop).

Table 85: Adverse events during memantine treatment

Adverse event	Number of subjects (n=14)
Dizziness[¥]	10 (71.4%)
Tiredness	6 (42.9%)
Drowsiness[±]	5 (35.7%)
Headache	4 (28.6%)
URTI*	4 (28.6%)
Nausea	3 (21.4%)
Spaced out	3 (21.4%)
Constipation	2 (14.3%)
Slurred speech	1 (7.1 %)
Unilateral funny sensation	1 (7.1%)
Pressure sensation inside the head	1 (7.1%)
Haemoptysis	1 (7.1%)
Loose stools	1 (7.1%)
Worsening of cough	1 (7.1%)
Chest infection	1 (7.1%)

[¥] Dizziness complaint included lightheadedness and unsteadiness.

[±] including adverse events of sleepiness

*When URTIs occurred, treatment was interrupted and then recommenced after 4-6 weeks starting at 10 mg and escalated if appropriate.

Table 86: Memantine-related adverse events for the various study doses

Subject ID	10 mg (n=14)	20 mg (n=12)	30 mg (n=6)	40 mg (n=1)
301	Mild headache	Spaced out & unsteady both moderate	NA	NA
302	Mild tiredness, had to shorten duration of treatment	Moderate tiredness	Spaced out which affected work and ability to drive	NA
303	Mild nausea & constipation, moderate drowsiness (avoided driving) & dizziness but then tolerated well.	Mild nausea	Severe AEs: unilateral funny sensation, slurred speech, dizziness and moderate nausea	
306	Mild headache & tiredness, moderate dizziness, sensation of increased pressure inside the head	Mild headache, moderate dizziness	NA	NA
307	Mild nausea, moderate drowsiness & tiredness which made her withdrew	NA	NA	NA
308	Mild lightheadedness & sleepiness, Nausea requiring cyclizine	Moderate lightheadedness & sleepiness, nausea	NA	NA
309	None	Moderate lightheadedness & tiredness	NA	NA
310	None	Severe dizziness & drowsiness. Mild headache	NA	NA

Subject ID	10 mg (n=14)	20 mg (n=12)	30 mg (n=6)	40 mg (n=1)
311	None	Mild lightheadedness & tiredness	Moderate lightheadedness	NA
312	None	None	None	lightheadedness
313	None	Moderate dizziness and tiredness. Mild constipation	NA	NA
314	Mild headache, mild-moderate sleepiness	NA	NA	NA
316	Worsening of cough	None	None	NA
317	None	None	Moderate lightheadedness	NA

NA: not applicable because the subject did not receive this dose.

4.7 Discussion

4.7.1 Summary of Main Findings

The main goal of this feasibility study was to explore the optimal dose of memantine in patients with chronic cough based upon estimates of efficacy and tolerability. The study showed that memantine, a licensed treatment for Alzheimer's disease, is poorly tolerated by patients with chronic cough. The maintenance memantine dose was not higher than 10 mg in the majority of participants and only 60% of the subjects who remained on 10 mg tolerated memantine treatment for four weeks. The most common adverse experiences were dizziness, drowsiness, and tiredness. Half of the subjects experienced at least one of these adverse events even at the lowest dose of 10 mg per day. Doses higher than 10 mg were associated with more severe and troublesome adverse events.

The efficacy of memantine as an anti-tussive was estimated using both subjective and objective methods. The mean daytime cough rate and CQLQ scores did not change significantly with memantine treatment; however, this is not unexpected as the study was not powered to detect statistically significant differences. The median improvement in cough frequency however was small at just 17% and improvements in cough related quality of life were minimal. Of note, four of the 11 subjects who had cough recordings had reduction in cough frequency by ~ 50% or more, implying a minority of subjects may have responded. Nonetheless, it should be kept in mind that there was no comparison to placebo, and so caution should be applied in interpreting these results.

4.7.2 Tolerability

Memantine is a low affinity, uncompetitive, use-dependent blocker of NMDA receptors. Therefore, it is believed to target predominantly the open NMDA channels involved in pathological conditions, where there is a sustained activation of the channels, rather than disturbing their normal physiological functions [258]. For example, in Alzheimer's disease, dizziness is reported in less than 10 % of subjects taking memantine 20 mg per day [257]. In contrast, dizziness was troublesome and experienced by half of the patients taking the same dose in our study. Patients with

moderate-severe Alzheimer's disease probably have less independent activities of daily living and poorer recall, therefore, are less likely to report adverse symptoms such as dizziness and lightheadedness.

Two other potent NMDA antagonists (ketamine and a novel molecule) were investigated previously by our cough research group. Ketamine, administered intravenously, did not demonstrate an anti-tussive effect over placebo in patients with chronic cough [259]. It resulted in significant CNS side effects; all subjects experienced lightheadedness. A novel NMDA antagonist, V3381 taken orally, reduced cough (did not reach a statistical significance) after 8 weeks of treatment in an open-label pilot study in 12 patients with chronic cough [260]. However, it was associated with intolerable adverse events; 80% reported dizziness. In our study, memantine also resulted in a similar incidence of dizziness. It seems that the use of readily available NMDA antagonists, including memantine, disappointingly has unacceptable adverse events in patients with chronic cough.

4.8 Conclusion

In conclusion, the findings of this study do not favour the design of a larger placebo-controlled trial to evaluate the effectiveness of memantine in patients with chronic cough as the reported side effects would seem to outweigh the small estimated treatment effect. Nonetheless, future studies could use lower memantine doses (5 to 10 mg).

Identifying NMDA receptor subtype(s) that are specific to the cough reflex could still provide novel targets for treatment and targeted NMDA receptor subtype antagonists might improve the tolerability. If such a treatment were available, long treatment periods would be advised, not to exclude a slow effect on the long-term structural and functional changes that potentially occur with central sensitisation.

CHAPTER 5
Final Discussion

Patients with chronic cough suffer significantly impaired quality of life. Currently available treatments such as morphine and gabapentin lack evidence of their ability to reduce 24 hour objective cough frequency. In addition, they are not well-tolerated and their mechanisms of action are uncertain. Therefore, there is an unmet need to develop therapeutic strategies for medications with clear mechanisms of action that significantly improve cough, are better tolerated by patients, and importantly, shed light on possible mechanistic explanations for chronic cough.

Chronic cough is a heterogeneous entity. For example, patients attending our specialist clinic vary in terms of what triggers their cough, the sensations experienced and locations of the urge to cough, and preceding insults such as viral infections. Thus, one therapeutic strategy is not likely to be successful in all patients and possibly combination of strategies could be better than single ones. This thesis has evaluated the clinical effect of three ion channel antagonists (voltage-gated sodium, purinergic P2X3 and NMDA) using validated and reliable objective (VitaloJAK^{TM}) and subjective measures of cough. Furthermore, the findings of this thesis have helped to expand the current understanding of the mechanisms contributing to the pathophysiology of chronic cough.

5.1 Summary of Thesis Studies

5.1.1 Chapter 2: Effect of Lidocaine

Voltage-gated sodium channels are essential for the generation and propagation of neuronal action potentials encoding cough. Case-series reports have indicated an anti-tussive effect of nebulised lidocaine (unselective NaV channel blocker). For example, Howard et al claimed that cough had subjectively improved for up to 6 weeks after a single treatment with nebulised lidocaine in four patients [224]. However, objective evidence of the clinical efficacy and its duration is lacking.

In chapter two, I examined the effect of a single dose of nebulised lidocaine in 26 patients with chronic cough enrolled into a randomised, double dummy (nebulised normal saline and lidocaine throat spray), double blind, three-way crossover study. The study displayed a trend towards a significant difference in cough counts among the three treatments. This was the result of lower cough count after spraying lidocaine into the throat compared to placebo, particularly in the first hour. Nebulised

lidocaine did not significantly inhibit cough, which is in disagreement with the previous case reports and experimental cough models in both animals and humans.

5.1.2 Chapter 3: Effect of P2X3 Antagonism

Twenty four chronic cough patients were recruited into a randomised, double blind, placebo controlled, crossover study evaluating a novel target, P2X3 receptors, in the treatment of chronic cough. Airway vagal afferents have been found to express the ATP-gated ion channels, P2X3 receptors. Experimental animal models of pain have implicated a key role of these channels in increasing the excitability of sensory neurons. In the introduction chapter, I described the concept of sensitisation of afferent neurons and how it could provide reasonable explanations for the cause of chronic cough. Therefore, the first in class oral P2X3 antagonist, AF-219, was hypothesised to lead to clinically meaningful improvement in cough.

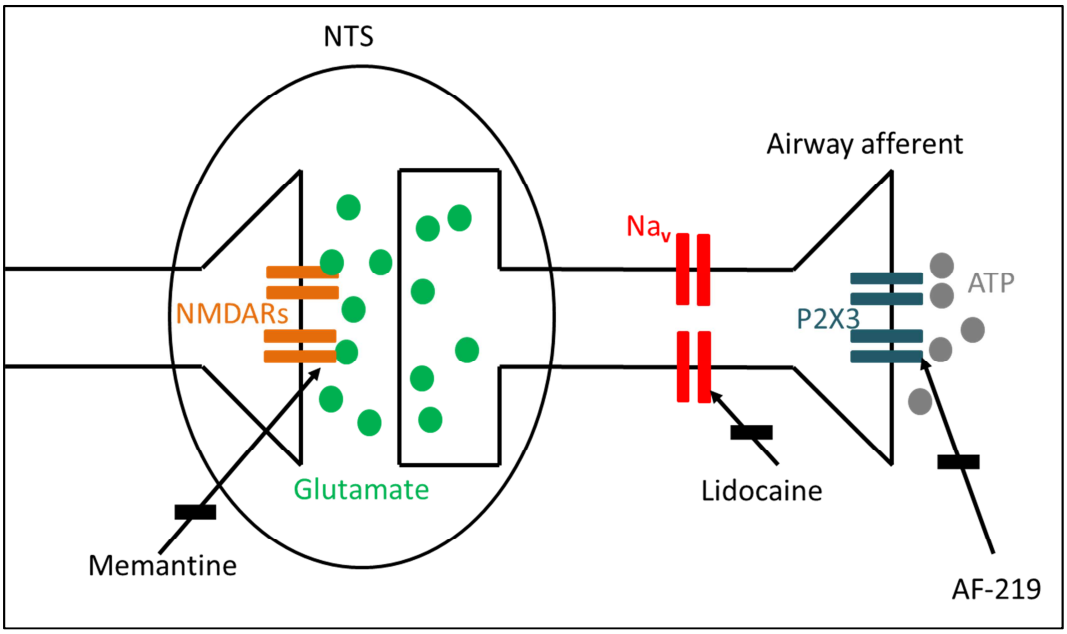
The results of the study supported our theory. Both objective cough frequency and patients reported outcomes improved significantly. The primary end point, the daytime cough rate, fell markedly by 75% over placebo in the intention-to-treat analysis. In the per protocol analysis, the reduction with treatment compared to placebo was even larger at 84%. There was a significantly strong correlation between the change in objective cough rate and both VAS scores and CQLQ. Psychosocial issues and extreme physical complaints were the two CQLQ domains that mostly drove the improvement in quality of life.

5.1.3 Chapter 4: The Tolerability of Memantine (NMDA Antagonist)

Sensitisation of central neurons is thought to be mediated via the glutamate-gated NMDA receptors. NMDAR antagonists such as dextromethorphan and ketamine have narrow therapeutic windows, which substantially limit their tolerability. In contrast, memantine is considered to have good tolerability given its preference for open NMDA channels. The aims of the study described in chapter 4 were to explore the efficacy and tolerability of escalating doses (10 mg titrated to 40 mg per day) of oral memantine. This was an open-label non-randomised feasibility study in 14 patients with chronic cough.

The results were inconsistent with the use-dependent activity of memantine and the reported well tolerability in both its licensed indication, Alzheimer's disease, and in trials in chronic pain. Dizziness, tiredness, and drowsiness were experienced in 71.4%, 42.9% and 35.7% of subjects, respectively. The maximum tolerated dose was 10 mg in the majority of subjects. The median reduction in daytime cough frequency was not statistically or clinically meaningful at 17%.

Figure 48 Summary of treatment targets in the thesis



A diagram showing the treatment targets in the cough reflex as studied in this thesis. In addition to the expression of P2X3 receptors on peripheral nerve endings, P2X3 receptors are also expressed on central pre-synaptic membranes (not shown) where they may modulate the release of glutamate into the synapse. AF-219 has poor blood brain barrier penetration and therefore its mechanism of action is not thought to be central. Memantine blocks NMDARs on post synaptic membranes. Lidocaine inhibits voltage-gated sodium channels.

5.2 Thesis Limitation

Inadequate blinding was the main limitation in both the trial of nebulised lidocaine and P2X3 antagonist. In chapter 2, my primary aim was to compare nebulised lidocaine to placebo (normal saline). However, I also added a third treatment of lidocaine throat spray. This was an attempt to enhance the blinding of the study by adding a control treatment that causes oropharyngeal numbness but was not predicted to change cough. Despite this effort, most patients were able to identify correctly the different treatments they received. Arguably, this unblinding of the study is not relevant here since nebulised lidocaine was not different from placebo.

In chapter three, all patients experienced taste disturbance with AF-219 treatment, which meant that blinding of the study was an inadequate. Nevertheless, the substantial decrease in cough frequency with AF-219 is unlikely to be explained by this unblinding issue. Firstly, the studied subjects had suffered from chronic cough for many years, which was refractory to multiple open-label clinical treatments. Some of the study subjects had previously participated in other research clinical trials including a phase 2 study of a TRPV1 antagonist and my previous study of lidocaine, which were both ineffective. Therefore, they generally had low expectations of any treatment benefit. Secondly, nebulised lidocaine was unsuccessful in reducing cough even though patients were faced with a similar blinding issue and differentiated lidocaine from placebo correctly. Thirdly, there was a group of AF-219 responders and a group of non-responders despite that everyone experienced the taste disturbance. Finally, the degree of reduction in cough frequency in patients with chronic cough is unprecedented at 75% over placebo.

A placebo effect has been shown to contribute to the majority of anti-tussive effects in trials of cough medicines [261]. However, all of those studies were in patients with acute cough, who tend to have high expectations for a clinical benefit, with cough being recorded over only a few minutes in observed laboratory environments. In contrast, my thesis studies were in patients with chronic cough using ambulatory 24 hour objective cough monitoring.

5.3 Discussion of Main Findings

5.3.1 Nebulised Lidocaine is not an Effective Anti-tussive

The study in chapter 2 highlights the need for better voltage-gated sodium channel blockers that are able to inhibit cough more effectively. The site of delivery of inhaled cough treatment might also be crucial. Nebulised lidocaine particles were of a small size (mainly, less than 5 microns), which suggests that they were primarily deposited in the distal small airways. Pulmonary C-fibres originating from the nodose ganglia innervate the intrapulmonary airways, and are thought to have an inhibitory effect on cough. This could explain the lack of cough reduction after nebulised lidocaine treatment because lidocaine may have blocked an inhibitory pathway of the cough reflex. On the other hand, larger lidocaine particles delivered topically to the throat and/or the larynx via a spray reduced the cough count, albeit a modest reduction.

Designing future studies of inhaled cough therapy should take into account these findings. Particle sizes that result in drug deposition in the laryngopharynx and proximal large airways, rather than the small distal airways, appear to be ideal sites to block cough afferents. This needs to be balanced against the potential risks of upper airway compromise associated with anaesthesia. Novel blockers of NaV channels that are selective for sensory neurons could allow safely the use of higher doses and more potent agents.

5.3.2 ATP-gated P2X3 Channels Contribute to the Hyper Excitability of Cough Afferents and their Antagonists Represent a Promising New Class of Effective Anti-tussives

The unprecedented 75% reduction in ambulatory objective cough frequency with the P2X3 antagonist, AF-219, is an exciting novel finding in the field of chronic cough. It implies an important role for the purinergic P2X3 cation channels in mediating hyper excitability of sensory afferents regulating the cough reflex. Small amounts of ATP may be able to partially depolarise the neuronal membranes, but in a sustained

manner; therefore, cough threshold would be lowered for subsequent stimuli (personal communication, Prof. Alan North).

5.3.3 Unselective NMDAR Antagonism is not a Suitable Therapeutic Strategy in Chronic Cough

So far, the NMDA antagonists ketamine, V3381, and memantine failed in their tolerability. Dextromethorphan has no clinically meaningful anti-tussive effect. Consequently, NMDARs do not currently represent an attractive target in the treatment of chronic cough. However, future cough-specific NMDA antagonists (currently unavailable) could have better tolerability, which would enable them to be evaluated for clinical efficacy.

5.4 Implications for Mechanisms in Chronic Cough

Patients with chronic cough are sensitive to a broad range of environmental airway stimuli and possibly to oesophageal events. Therefore, central sensitisation would seem to be a plausible mechanistic explanation for chronic cough. Central sensitisation of pain is thought to be initiated and maintained by NMDA receptors. However, the evidence from the study described in chapter 4 does not support a significant therapeutic benefit of the centrally acting NMDA antagonist, memantine. NMDA-independent mechanisms of central sensitisation cannot be ruled out. On the other hand, peripheral P2X3 antagonism resulted in a marked anti-tussive effect. This indicates that peripheral sensitisation is probably the key mechanism in the majority of patients. Central sensitisation could provide an alternative explanation in those who did not respond to the P2X3 antagonist, albeit not proven yet. One would have expected that blocking the voltage-gated sodium channels in the airway nerves by inhaling aerosolised lidocaine to reduce the cough rate compared with placebo. Possible explanations for its ineffectiveness are discussed above.

5.5 Directions for Future Work

5.5.1 Novel Voltage-Gated Sodium Channel Blockers

Currently available voltage-gated sodium channel antagonists such as local anaesthetics are pan NaV blockers. NaV Subtypes 1.7, 1.8, and 1.9 have been shown to be expressed primarily on sensory afferents including vagal neurons innervating the airways. Selectively blocking those subtypes could provide safe antitussive agents with reduced risk of cardiac and CNS toxicity. However, such selective blockers have not been developed yet. GSK 2339345 is a novel voltage-gated sodium channel blocker that has been demonstrated to be more potent than lidocaine and significantly inhibited cough elicited by citric acid inhalation in both guinea pigs and dogs [262]. Although the molecule is not subtype specific, it displays preference for the active NaV channels involved in excessive activity, i.e. use- and frequency-dependent activity. Therefore, its inhalation may not cause oropharyngeal numbness, which was a limitation of using lidocaine in my study presented in chapter 2. The evaluation of the effectiveness of its inhalation in humans with chronic cough is upcoming (ClinicalTrials.gov Identifier: NCT01899768).

5.5.2 ATP Challenges

Given the substantial improvement in cough with blocking the ATP-gated P2X3 channels, further work is needed to investigate role of P2X3 channels and the effect of ATP on the cough reflex. Several studies in animal models of cough have not supported a tussive effect of ATP inhalation, whereas in humans the picture is less clear. To my knowledge, there are only two studies of ATP inhalation in humans; both of them were designed to assess bronchoconstriction, not cough. One study reported evoked cough with ATP inhalation, but the other study did not. In addition, inhalation of ATP has been demonstrated to lower the threshold for citric acid in a single study of guinea pigs. This implies a sensitising effect of ATP on cough afferent pathways. Suggestions for future work in this area include ATP inhalation in humans to investigate whether or not cough could be provoked by ATP and also to assess the modulatory effect of ATP on subsequent inhalation of agents such as capsaicin and citric acid.

5.5.3 P2X3 Antagonist Dose-Response

In the study presented in chapter 3, the maximum therapeutic dose of the P2X3 antagonist was used. Future work is needed to decide the optimal dosing in a dose-escalation study and whether this will obviate the experience of taste disturbances. Furthermore, the antagonist had activity at both the homotrimeric P2X3 and the heterotrimeric P2X2/3 channels. It is believed that P2X2/3 are responsible for the transduction of taste sensation, but it is still unknown if the anti-tussive effect is through antagonising the homotrimeric or the heterotrimeric channels. It would improve the tolerability of the medication if the anti-tussive effect could be separated from the transmission of taste. Further clinical trials to explore the therapeutic potential of P2X3 antagonism to cough in other disease are also worthy of study.

5.6 Conclusion

It is hoped that the continuation of the work presented in this thesis to identify the role of various receptors and signalling pathways, both peripherally and centrally, would result in improving the understanding of the pathogenic mechanisms contributing to chronic cough. Ultimately, the goal is to offer patients effective and well-tolerated treatments that bring a relief of their daily suffering from chronic cough. It is essential that any future medications are able to preferentially target the receptors/pathways contributing to the hyper excitability of the cough reflex, but to leave defensive cough mechanisms intact.

References

1. Morice, A.H., *Recommendations for the management of cough in adults.* Thorax, 2006. **61**(suppl_1): p. i1-i24.

2. Schappert, S.M. and C.W. Burt, *Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 2001-02.* Vital and Health Statistics. Series 13, Data from the National Health Survey, 2006(159): p. 1-66.

3. Irwin, R.S., W.M. Corrao, and M.R. Pratter, *Chronic persistent cough in the adult: the spectrum and frequency of causes and successful outcome of specific therapy.* The American Review of Respiratory Disease, 1981. **123**(4 Pt 1): p. 413-417.

4. McGarvey, L.P.A., et al., *Evaluation and outcome of patients with chronic non-productive cough using a comprehensive diagnostic protocol.* Thorax, 1998. **53**(9): p. 738-743.

5. Brightling, C.E., et al., *Eosinophilic Bronchitis Is an Important Cause of Chronic Cough.* American Journal of Respiratory and Critical Care Medicine, 1999. **160**(2): p. 406-410.

6. Irwin, R.S., *Diagnosis and management of cough : Accp evidence-based clinical practice guidelines.* CHEST Journal, 2006. **129**(1_suppl): p. 24S-24S.

7. Haque, R.A., O.S. Usmani, and P.J. Barnes, *Chronic Idiopathic Cough*.* Chest, 2005. **127**(5): p. 1710-1713.

8. Birring, S.S., et al., *Idiopathic chronic cough and organ-specific autoimmune diseases: a case–control study.* Respiratory Medicine, 2004. **98**(3): p. 242-246.

9. Morice, A.H. and J.A. Kastelik, *Cough • 1: Chronic cough in adults.* Thorax, 2003. **58**(10): p. 901-907.

10. Fujimura, M., et al., *Female Gender as a Determinant of Cough Threshold to Inhaled Capsaicin.* European Respiratory Journal, 1996. **9**(8): p. 1624-1626.

11. Kastelik, J.A., et al., *Sex-related Differences in Cough Reflex Sensitivity in Patients with Chronic Cough.* Am. J. Respir. Crit. Care Med., 2002. **166**(7): p. 961-964.

12. Kelsall, A., et al., *Sex differences and predictors of objective cough frequency in chronic cough.* Thorax, 2009. **64**(5): p. 393-398.

13. Ford, A.C., et al., *Cough in the community: a cross sectional survey and the relationship to gastrointestinal symptoms.* Thorax, 2006. **61:**: p. 975-979.

14. French, C.L., et al., *Impact of chronic cough on quality of life.* Arch Intern Med, 1998. **158**(15): p. 1657-61.

15. Everett, C.F., et al., *Chronic persistent cough in the community: a questionnaire survey.* Cough, 2007. **3**: p. 5.

16. Dicpinigaitis, P.V., R. Tso, and G. Banauch, *Prevalence of depressive symptoms among patients with chronic cough.* Chest, 2006. **130**(6): p. 1839-43.

17. PAGB. *Annual Review 2012: Securing our Future Health - Time for Real Engagement*. 2012 [cited 2013 23/09/2013]; 2011 UK OTC market summary].

18. Smith, S., K. Schroeder, and T. Fahey, *Over-the-counter medications for acute cough in children and adults in ambulatory settings.* Cochrane Database of Systematic Reviews

, 2008.

19. Morice, A.H., et al., *Opiate Therapy in Chronic Cough.* Am. J. Respir. Crit. Care Med., 2007. **175**(4): p. 312-315.

20. Ryan, N.M., S.S. Birring, and P.G. Gibson, *Gabapentin for refractory chronic cough: a randomised, double-blind, placebo-controlled trial.* Lancet, 2012. **380**(9853): p. 1583-9.

21. Karlsson, J.A., *The role of capsaicin-sensitive C-fibre afferent nerves in the cough reflex.* Pulm Pharmacol, 1996. **9**(5-6): p. 315-21.

22. Higenbottam, T., et al., *The cough response to ultrasonically nebulized distilled water in heart-lung transplantation patients.* Am Rev Respir Dis, 1989. **140**(1): p. 58-61.

23. Dicpinigaitis, P.V., D.R. Grimm, and M. Lesser, *Cough Reflex Sensitivity in Subjects with Cervical Spinal Cord Injury.* Am. J. Respir. Crit. Care Med., 1999. **159**(5): p. 1660-1662.

24. Mutolo, D., et al., *The role of excitatory amino acids and substance P in the mediation of the cough reflex within the nucleus tractus solitarii of the rabbit.* Brain Research Bulletin, 2007. **74**(4): p. 284-293.

25. Mazzone, S.B., et al., *Representation of Capsaicin-evoked Urge-to-Cough in the Human Brain Using Functional Magnetic Resonance Imaging.* Am. J. Respir. Crit. Care Med., 2007. **176**(4): p. 327-332.

26. Canning, B.J., *Anatomy and Neurophysiology of the Cough Reflex.* Chest, 2006. **129**(1 suppl): p. 33S-47S.

27. Widdicombe, J., *Functional morphology and physiology of pulmonary rapidly adapting receptors (RARs).* Anat Rec A Discov Mol Cell Evol Biol, 2003. **270**(1): p. 2-10.

28. Mazzone, S.B., *Sensory regulation of the cough reflex.* Pulmonary Pharmacology & Therapeutics, 2004. **17**(6): p. 361-368.

29. Jonzon, A., et al., *Rapidly adapting receptor activity in dogs is inversely related to lung compliance.* J Appl Physiol, 1986. **61**(5): p. 1980-7.

30. Canning, B.J., et al., *Identification of the tracheal and laryngeal afferent neurones mediating cough in anaesthetized guinea□pigs.* The Journal of Physiology, 2004. **557**(2): p. 543-558.

31. Ho, C.Y., et al., *Sensitivity of vagal afferent endings to chemical irritants in the rat lung.* Respir Physiol, 2001. **127**(2-3): p. 113-24.

32. Sant'Ambrogio, G. and J. Widdicombe, *Reflexes from airway rapidly adapting receptors.* Respir Physiol, 2001. **125**(1-2): p. 33-45.

33. Canning, B.J., *Anatomy and neurophysiology of the cough reflex: ACCP evidence-based clinical practice guidelines.* Chest, 2006. **129**(1 Suppl): p. 33S-47S.

34. Tatar, M., G. Sant'Ambrogio, and F.B. Sant'Ambrogio, *Laryngeal and tracheobronchial cough in anesthetized dogs.* J Appl Physiol, 1994. **76**(6): p. 2672-2679.

35. Barnes, N.C., P.J. Piper, and J.F. Costello, *Comparative effects of inhaled leukotriene C4, leukotriene D4, and histamine in normal human subjects.* Thorax, 1984. **39**(7): p. 500-4.

36. Fujimura, M., et al., *Effects of methacholine induced bronchoconstriction and procaterol induced bronchodilation on cough receptor sensitivity to inhaled capsaicin and tartaric acid.* Thorax, 1992. **47**(6): p. 441-5.

37. Widdicombe, J.G., *Afferent receptors in the airways and cough.* Respir Physiol, 1998. **114**(1): p. 5-15.

38. Schelegle, E.S. and J.F. Green, *An overview of the anatomy and physiology of slowly adapting pulmonary stretch receptors.* Respir Physiol, 2001. **125**(1-2): p. 17-31.

39. Matsumoto, S., *The activities of lung stretch and irritant receptors during cough.* Neurosci Lett, 1988. **90**(1-2): p. 125-9.

40. Hanacek, J., A. Davies, and J.G. Widdicombe, *Influence of lung stretch receptors on the cough reflex in rabbits.* Respiration, 1984. **45**(3): p. 161-8.

41. Agostoni, E., et al., *Functional and histological studies of the vagus nerve and its branches to the heart, lungs and abdominal viscera in the cat.* The Journal of Physiology, 1957. **135**(1): p. 182-205.

42. Lee, L.-Y. and T.E. Pisarri, *Afferent properties and reflex functions of bronchopulmonary C-fibers.* Respir Physiol, 2001. **125**(1–2): p. 47-65.

43. Lundberg, D.J.M., et al., *Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man.* Cell and Tissue Research, 1984. **235**(2): p. 251-261.

44. Ricco, M.M., et al., *Interganglionic segregation of distinct vagal afferent fibre phenotypes in guinea-pig airways.* The Journal of Physiology, 1996. **496**(Pt 2): p. 521-530.

45. Hunter, D.D. and B.J. Undem, *Identification and substance P content of vagal afferent neurons innervating the epithelium of the guinea pig trachea.* American Journal of Respiratory and Critical Care Medicine, 1999. **159**(6): p. 1943-1948.

46. Myers, A.C., R. Kajekar, and B.J. Undem, *Allergic inflammation-induced neuropeptide production in rapidly adapting afferent nerves in guinea pig airways.* American Journal of Physiology - Lung Cellular and Molecular Physiology, 2002. **282**(4): p. L775-L781.

47. Coleridge, H.M. and J.C.G. Coleridge, *Impulse activity in afferent vagal C-fibres with endings in the intrapulmonary airways of dogs.* Respir Physiol, 1977. **29**(2): p. 125-142.

48. Coleridge, J.C.G. and H.M. Coleridge, *Afferent vagal C fibre innervation of the lungs and airways and its functional significance*, in *Reviews of Physiology, Biochemistry and Pharmacology, Volume 99*. 1984, Springer Berlin Heidelberg. p. 1-110.

49. Undem, B.J., et al., *Subtypes of vagal afferent C-fibres in guinea-pig lungs.* The Journal of Physiology, 2004. **556**(3): p. 905-917.

50. Tatar, M., S.E. Webber, and J.G. Widdicombe, *Lung C-Fibre Receptor Activation and Defensive Reflexes in Anaesthetized Cats.* The Journal of Physiology, 1988. **402**(1): p. 411-420.

51. Groneberg, D.A., et al., *Increased Expression of Transient Receptor Potential Vanilloid-1 in Airway Nerves of Chronic Cough.* Am. J. Respir. Crit. Care Med., 2004. **170**(12): p. 1276-1280.

52. Widdicombe, J.G., *Neurophysiology of the cough reflex.* European Respiratory Journal, 1995. **8**(7): p. 1193-1202.

53. Collier, J.G. and R.W. Fuller, *Capsaicin inhalation in man and the effects of sodium cromoglycate.* British Journal of Pharmacology, 1984. **81**(1): p. 113-117.

54. Choudry, N.B., R.W. Fuller, and N.B. Pride, *Sensitivity of the human cough reflex: effect of inflammatory mediators prostaglandin E2, bradykinin, and histamine.* The American Review of Respiratory Disease, 1989. **140**(1): p. 137-141.

55. Kummer, W., et al., *The sensory and sympathetic innervation of guinea-pig lung and trachea as studied by retrograde neuronal tracing and double-labelling immunohistochemistry.* Neuroscience, 1992. **49**(3): p. 715-737.

56. Bolser, D.C., et al., *Central antitussive activity of the NK1 and NK2 tachykinin receptor antagonists, CP-99,994 and SR 48968, in the guinea-pig and cat.* British Journal of Pharmacology, 1997. **121**(2): p. 165-170.

57. Lalloo, U.G., et al., *Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs.* Journal of applied physiology (Bethesda, Md.: 1985), 1995. **79**(4): p. 1082-1087.

58. Khalid, S., et al., *A double blind, placebo controlled, randomised, study to assess the effects of placebo, codeine and Talnetant, on citric acid cough threshold in healthy subjects* Thorax, 2010. **65**: p. A52-A53.

59. Smith, J., et al., *P152 The Impact of a Selective oral TRPV1 Antagonist in Patients with Chronic Cough.* Thorax, 2012. **67**(Suppl 2): p. A128

.

60. Pascoe S, Knight H, and C. K, *A dual NK1/NK2 receptor antagonist, does not inhibit cough in COPD.* Am J Respir Crit Care Med 2007. **175**: p. A451.

61. Choudry, N.B. and R.W. Fuller, *Sensitivity of the cough reflex in patients with chronic cough.* European Respiratory Journal, 1992. **5**(3): p. 296-300.

62. Canning, B.J., D.G. Farmer, and N. Mori, *Mechanistic studies of acid-evoked coughing in anesthetized guinea pigs.* Am J Physiol Regul Integr Comp Physiol, 2006. **291**(2): p. R454-463.

63. Kollarik, M. and B.J. Undem, *Mechanisms of acid-induced activation of airway afferent nerve fibres in guinea-pig.* The Journal of Physiology, 2002. **543**(2): p. 591-600.

64. Lieu, T. and B.J. Undem, *Neuroplasticity in vagal afferent neurons involved in cough.* Pulmonary Pharmacology & Therapeutics, 2011. **24**(3): p. 276-279.

65. Mazzone, S.B., et al., *Selective expression of a sodium pump isozyme by cough receptors and evidence for its essential role in regulating cough.* Journal of Neuroscience, 2009. **29**(43).

66. Canning, B.J. and N. Mori, *An essential component to brainstem cough gating identified in anesthetized guinea pigs.* The FASEB Journal, 2010. **24**(10): p. 3916-3926.

67. Undem, B.J. and M.J. Carr, *Targeting Primary Afferent Nerves for Novel Antitussive Therapy.* Chest, 2010. **137**(1): p. 177-184.

68. Ford, A.P., *In pursuit of P2X3 antagonists: novel therapeutics for chronic pain and afferent sensitization.* Purinergic Signal, 2012. **8**(Suppl 1): p. 3-26.

69. Khakh, B.S. and R.A. North, *P2X receptors as cell-surface ATP sensors in health and disease.* Nature, 2006. **442**(7102): p. 527-532.

70. Burnstock, G., *Purinergic nerves.* Pharmacological Reviews, 1972. **24**(3): p. 509-581.

71. Brederson, J.-D. and M.F. Jarvis, *Homomeric and heteromeric P2X3 receptors in peripheral sensory neurons.* Current opinion in investigational drugs (London, England: 2000), 2008. **9**(7): p. 716-725.

72. Undem, B.J. and C. Nassenstein, *Airway nerves and dyspnea associated with inflammatory airway disease.* Respiratory Physiology & Neurobiology, 2009. **167**(1): p. 36-44.

73. Chen, Y., et al., *Mechanisms underlying enhanced P2X receptor-mediated responses in the neuropathic pain state.* Pain, 2005. **119**(1–3): p. 38-48.

74. Yegutkin, G.G., *Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade.* Biochim Biophys Acta, 2008. **1783**(5): p. 673-94.

75. Hamilton, S.G., A. Wade, and S.B. McMahon, *The effects of inflammation and inflammatory mediators on nociceptive behaviour induced by ATP analogues in the rat.* British Journal of Pharmacology, 1999. **126**(1): p. 326-332.

76. Bleehen, T. and C.A. Keele, *Observations on the algogenic actions of adenosine compounds on the human blister base preparation.* Pain, 1977. **3**(4): p. 367-377.

77. Hamilton, S.G., et al., *ATP in human skin elicits a dose-related pain response which is potentiated under conditions of hyperalgesia.* Brain, 2000. **123**(6): p. 1238-1246.

78. Cockayne, D.A., et al., *P2X2 knockout mice and P2X2/P2X3 double knockout mice reveal a role for the P2X2 receptor subunit in mediating multiple sensory effects of ATP.* The Journal of Physiology, 2005. **567**(2): p. 621-639.

79. Barclay, J., et al., *Functional Downregulation of P2X3 Receptor Subunit in Rat Sensory Neurons Reveals a Significant Role in Chronic Neuropathic and Inflammatory Pain.* The Journal of Neuroscience, 2002. **22**(18): p. 8139-8147.

80. Dorn, G., et al., *siRNA relieves chronic neuropathic pain.* Nucleic Acids Research, 2004. **32**(5): p. e49-e49.

81. Xiang, Z., et al., *Functional up-regulation of P2X3 receptors in the chronically compressed dorsal root ganglion.* Pain, 2008. **140**(1): p. 23-34.

82. Novakovic, S.D., et al., *Immunocytochemical localization of P2X3 purinoceptors in sensory neurons in naive rats and following neuropathic injury.* Pain, 1999. **80**(1–2): p. 273-282.

83. Xu, G.Y. and L.Y. Huang, *Peripheral inflammation sensitizes P2X receptor-mediated responses in rat dorsal root ganglion neurons.* J Neurosci, 2002. **22**(1): p. 93-102.

84. Jarvis, M.F., et al., *A-317491, a novel potent and selective non-nucleotide antagonist of P2X3 and P2X2/3 receptors, reduces chronic inflammatory and neuropathic pain in the rat.* Proceedings of the National Academy of Sciences, 2002. **99**(26): p. 17179-17184.

85. McGaraughty, S., et al., *Effects of A-317491, a novel and selective P2X3/P2X2/3 receptor antagonist, on neuropathic, inflammatory and chemogenic nociception following intrathecal and intraplantar administration.* British Journal of Pharmacology, 2003. **140**(8): p. 1381-1388.

86. Pelleg, A. and C.M. Hurt, *Mechanism of action of ATP on canine pulmonary vagal C fibre nerve terminals.* The Journal of Physiology, 1996. **490**(Pt 1): p. 265-275.

87. Kwong, K., et al., *P2X2 receptors differentiate placodal vs. neural crest C-fiber phenotypes innervating guinea pig lungs and esophagus.* Am J Physiol Lung Cell Mol Physiol, 2008. **295**(5): p. L858-865.

88. Kamei, J., et al., *Involvement of P2X receptor subtypes in ATP-induced enhancement of the cough reflex sensitivity.* European Journal of Pharmacology, 2005. **528**(1–3): p. 158-161.

89. Muroi, Y., et al., *Selective inhibition of vagal afferent nerve pathways regulating cough using NaV1.7 shRNA silencing in guinea pig nodose ganglia.* American journal of physiology. Regulatory, integrative and comparative physiology, 2013.

90. Basoglu, O.K., et al., *Effects of Aerosolized Adenosine 5'-Triphosphate Vs Adenosine 5'-Monophosphate on Dyspnea and Airway Caliber in Healthy Nonsmokers and Patients With Asthma*.* Chest, 2005. **128**(4): p. 1905-1909.

91. Pellegrino, R., et al., *Lung mechanics during induced bronchoconstriction.* J Appl Physiol, 1996. **81**(2): p. 964-75.

92. Kamei, J. and Y. Takahashi, *Involvement of ionotropic purinergic receptors in the histamine-induced enhancement of the cough reflex sensitivity in guinea pigs.* European Journal of Pharmacology, 2006. **547**(1–3): p. 160-164.

93. Caterina, M.J., et al., *The capsaicin receptor: a heat-activated ion channel in the pain pathway.* Nature, 1997. **389**(6653): p. 816-824.

94. Clapham, D.E., et al., *International Union of Pharmacology. XLIX. Nomenclature and Structure-Function Relationships of Transient Receptor Potential Channels.* Pharmacological Reviews, 2005. **57**(4): p. 427-450.

95. Szallasi, A. and P.M. Blumberg, *Vanilloid (Capsaicin) receptors and mechanisms.* Pharmacological Reviews, 1999. **51**(2): p. 159-212.

96. Tominaga, M., et al., *The Cloned Capsaicin Receptor Integrates Multiple Pain-Producing Stimuli.* Neuron, 1998. **21**(3): p. 531-543.

97. Davis, J.B., et al., *Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia.* Nature, 2000. **405**(6783): p. 183-187.

98. Premkumar, L.S. and G.P. Ahern, *Induction of vanilloid receptor channel activity by protein kinase C.* Nature, 2000. **408**(6815): p. 985-990.

99. Fuller, R.W., *Pharmacology of inhaled capsaicin in humans.* Respiratory Medicine, 1991. **85**(Supplement 1): p. 31-34.

100. Prudon, B., et al., *Cough and Glottic-Stop Reflex Sensitivity in Health and Disease*.* Chest, 2005. **127**(2): p. 550-557.

101. Trevisani, M., et al., *4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1.* Proceedings of the National Academy of Sciences, 2007. **104**(33): p. 13519-13524.

102. Szallasi, A., et al., *The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept.* Nature Reviews Drug Discovery, 2007. **6**(5): p. 357-372.

103. Nassenstein, C., et al., *Expression and function of the ion channel TRPA1 in vagal afferent nerves innervating mouse lungs.* The Journal of Physiology, 2008. **586**(6): p. 1595-1604.

104. Taylor-Clark, T.E., et al., *Relative contributions of TRPA1 and TRPV1 channels in the activation of vagal bronchopulmonary C-fibres by the endogenous autacoid 4-oxononenal.* The Journal of Physiology, 2008. **586**(14): p. 3447-3459.

105. Story, G.M., et al., *ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures.* Cell, 2003. **112**(6): p. 819-829.

106. Jordt, S.-E., et al., *Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1.* Nature, 2004. **427**(6971): p. 260-265.

107. Bandell, M., et al., *Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin.* Neuron, 2004. **41**(6): p. 849-857.

108. Bautista, D.M., et al., *TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents.* Cell, 2006. **124**(6): p. 1269-1282.

109. McNamara, C.R., et al., *TRPA1 mediates formalin-induced pain.* Proceedings of the National Academy of Sciences of the United States of America, 2007. **104**(33): p. 13525-13530.

110. Birrell, M.A., et al., *TRPA1 Agonists Evoke Coughing in Guinea Pig and Human Volunteers.* Am. J. Respir. Crit. Care Med., 2009. **180**(11): p. 1042-1047.

111. Esterbauer, H., R.J. Schaur, and H. Zollner, *Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes.* Free Radical Biology and Medicine, 1991. **11**(1): p. 81-128.

112. Kanezaki, M., et al., *Effect of cigarette smoking on cough reflex induced by TRPV1 and TRPA1 stimulations.* Respiratory Medicine, 2012. **106**(3): p. 406-412.

113. Rahman, I., et al., *4-Hydroxy-2-Nonenal, a Specific Lipid Peroxidation Product, Is Elevated in Lungs of Patients with Chronic Obstructive Pulmonary Disease.* American Journal of Respiratory and Critical Care Medicine, 2002. **166**(4): p. 490-495.

114. Kwong, K., et al., *Voltage-gated sodium channels in nociceptive versus non-nociceptive nodose vagal sensory neurons innervating guinea pig lungs.* The Journal of Physiology, 2008. **586**(5): p. 1321-1336.

115. Dib-Hajj, S.D., et al., *Sodium Channels in Normal and Pathological Pain.* Annual Review of Neuroscience, 2010. **33**(1): p. 325-347.

116. Catterall, W.A., A.L. Goldin, and S.G. Waxman, *International Union of Pharmacology. XLVII. Nomenclature and Structure-Function Relationships of Voltage-Gated Sodium Channels.* Pharmacological Reviews, 2005. **57**(4): p. 397-409.

117. Cox, J.J., et al., *An SCN9A channelopathy causes congenital inability to experience pain.* Nature, 2006. **444**(7121): p. 894-898.

118. Waxman, S.G., et al., *Voltage-gated sodium channels and the molecular pathogenesis of pain: a review.* Journal of rehabilitation research and development, 2000. **37**(5): p. 517-528.

119. Renganathan, M., T.R. Cummins, and S.G. Waxman, *Contribution of Na(v)1.8 sodium channels to action potential electrogenesis in DRG neurons.* Journal of Neurophysiology, 2001. **86**(2): p. 629-640.

120. Rush, A.M. and S.G. Waxman, *PGE2 increases the tetrodotoxin-resistant Nav1.9 sodium current in mouse DRG neurons via G-proteins.* Brain Research, 2004. **1023**(2): p. 264-271.

121. Gold, M.S., J.D. Levine, and A.M. Correa, *Modulation of TTX-R INa by PKC and PKA and their role in PGE2-induced sensitization of rat sensory neurons in vitro.* The Journal of neuroscience: the official journal of the Society for Neuroscience, 1998. **18**(24): p. 10345-10355.

122. Nassar, M.A., et al., *Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain.* Proceedings of the National Academy of Sciences of the United States of America, 2004. **101**(34): p. 12706-12711.

123. Amaya, F., et al., *The Voltage-Gated Sodium Channel Nav1.9 Is an Effector of Peripheral Inflammatory Pain Hypersensitivity.* The Journal of Neuroscience, 2006. **26**(50): p. 12852-12860.

124. Jarvis, M.F., et al., *A-803467, a potent and selective Nav1.8 sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat.* Proceedings of the National Academy of Sciences, 2007. **104**(20): p. 8520-8525.

125. Muroi, Y., et al., *Selective silencing of NaV1. 7 decreases excitability and conduction in vagal sensory neurons.* The Journal of Physiology, 2011. **589**(23): p. 5663-5676.

126. Kwong, K. and L.-Y. Lee, *Prostaglandin E2 potentiates a TTX-resistant sodium current in rat capsaicin-sensitive vagal pulmonary sensory neurones.* The Journal of Physiology, 2005. **564**(2): p. 437-450.

127. O'Neill, J., S.B. McMahon, and B.J. Undem, *Chronic cough and pain: Janus faces in sensory neurobiology?* Pulmonary pharmacology & therapeutics, 2013.

128. Woolf, C.J. and M.W. Salter, *Neuronal Plasticity: Increasing the Gain in Pain.* Science, 2000. **288**(5472): p. 1765-1768.

129. Bielefeldt, K., J.A. Christianson, and B.M. Davis, *Basic and clinical aspects of visceral sensation: transmission in the CNS.* Neurogastroenterology & Motility, 2005. **17**(4): p. 488-499.

130. McAlexander, M.A. and M.J. Carr, *Peripheral mechanisms I: plasticity of peripheral pathways.* Pharmacology and Therapeutics of Cough, 2009: p. 129-154.

131. Ginty, D.D. and R.A. Segal, *Retrograde neurotrophin signaling: Trk-ing along the axon.* Current Opinion in Neurobiology, 2002. **12**(3): p. 268-274.

132. Braun, A., et al., *Cellular Sources of Enhanced Brain-Derived Neurotrophic Factor Production in a Mouse Model of Allergic Inflammation Notice to Professional Recruitment and Announcement Advertisers.* American Journal of Respiratory Cell and Molecular Biology, 1999. **21**(4): p. 537-546.

133. Leon, A., et al., *Mast cells synthesize, store, and release nerve growth factor.* Proceedings of the National Academy of Sciences, 1994. **91**(9): p. 3739-3743.

134. Nockher, W.A. and H. Renz, *Neurotrophins and asthma: Novel insight into neuroimmune interaction.* Journal of Allergy and Clinical Immunology, 2006. **117**(1): p. 67-71.

135. Virchow, J.C., et al., *Neurotrophins Are Increased in Bronchoalveolar Lavage Fluid After Segmental Allergen Provocation.* American Journal of Respiratory and Critical Care Medicine, 1998. **158**(6): p. 2002-2005.

136. Amaya, F., et al., *NGF and GDNF differentially regulate TRPV1 expression that contributes to development of inflammatory thermal hyperalgesia.* European Journal of Neuroscience, 2004. **20**(9): p. 2303-2310.

137. Ramer, M.S., E.J. Bradbury, and S.B. McMahon, *Nerve growth factor induces P2X3 expression in sensory neurons.* Journal of Neurochemistry, 2001. **77**(3): p. 864-875.

138. Chuaychoo, B., et al., *Allergen-induced substance P synthesis in large-diameter sensory neurons innervating the lungs.* Journal of Allergy and Clinical Immunology, 2005. **116**(2): p. 325-331.

139. Carr, M.J., et al., *Expression of Tachykinins in Nonnociceptive Vagal Afferent Neurons during Respiratory Viral Infection in Guinea Pigs.* American Journal of Respiratory and Critical Care Medicine, 2002. **165**(8): p. 1071-1075.

140. O'Connell, F., et al., *Abnormal Intraepithelial Airway Nerves in Persistent Unexplained Cough?* American Journal of Respiratory and Critical Care Medicine, 1995. **152**(6): p. 2068-2075.

141. Chaudhuri, R., et al., *Serum and sputum neurotrophin levels in chronic persistent cough.* Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology, 2005. **35**(7): p. 949-953.

142. Koskela, H.O., M.K. Purokivi, and J. Romppanen, *Neurotrophins in chronic cough: association with asthma but not with cough severity.* The Clinical Respiratory Journal, 2010. **4**(1): p. 45-50.

143. Woolf, C.J., *Evidence for a central component of post-injury pain hypersensitivity.* Nature, 1983. **306**(5944): p. 686-688.

144. Woolf, C.J. and Q. Ma, *Nociceptors—Noxious Stimulus Detectors.* Neuron, 2007. **55**(3): p. 353-364.

145. Woolf, C.J. and S.W.N. Thompson, *The induction and maintenance of central sensitization is dependent on N-methyl-d-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states.* Pain, 1991. **44**(3): p. 293-299.

146. Chaffey, H. and P.L. Chazot, *NMDA receptor subtypes: Structure, function and therapeutics.* Current Anaesthesia & Critical Care, 2008. **19**(4): p. 183-201.

147. Smith, J.A., et al., *Antitussive Effects of Memantine in Guinea Pigs.* Chest, 2011. **141**(4): p. 996-1002.

148. Latremoliere, A. and C.J. Woolf, *Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity.* The journal of pain : official journal of the American Pain Society, 2009. **10**(9): p. 895-926.

149. Joad, J.P., et al., *Passive Smoke Effects on Cough and Airways in Young Guinea Pigs Role of Brainstem Substance P.* American Journal of Respiratory and Critical Care Medicine, 2004. **169**(4): p. 499-504.

150. Chen, C.-Y., et al., *Selected Contribution: Neuroplasticity in nucleus tractus solitarius neurons after episodic ozone exposure in infant primates.* Journal of Applied Physiology, 2003. **94**(2): p. 819-827.

151. Chen, C.-Y., et al., *Extended allergen exposure in asthmatic monkeys induces neuroplasticity in nucleus tractus solitarius.* Journal of Allergy and Clinical Immunology, 2001. **108**(4): p. 557-562.

152. Bonham, A.C., S.i. Sekizawa, and J.P. Joad, *Plasticity of central mechanisms for cough.* Pulmonary Pharmacology & Therapeutics, 2004. **17**(6): p. 453-457.

153. Mazzone, S.B., N. Mori, and B.J. Canning, *Synergistic interactions between airway afferent nerve subtypes regulating the cough reflex in guinea-pigs.* The Journal of Physiology, 2005. **569**(2): p. 559-573.

154. Ing, A.J., M.C. Ngu, and A.B. Breslin, *Pathogenesis of chronic persistent cough associated with gastroesophageal reflux.* American Journal of Respiratory and Critical Care Medicine, 1994. **149**(1): p. 160-167.

155. javorkova, n., et al., *Acidification of the oesophagus acutely increases the cough sensitivity in patients with gastro-oesophageal reflux and chronic cough.* Neurogastroenterology & Motility, 2007: p. 071114170920003-???

156. Smith, J.A., et al., *Acoustic Cough-Reflux Associations in Chronic Cough: Potential Triggers and Mechanisms.* Gastroenterology, 2010.

157. Young, E.C., et al., *The effect of mindfulness meditation on cough reflex sensitivity.* Thorax, 2009. **64**(11): p. 993-998.

158. Davenport, P.W., *Urge-to-Cough: What Can It Teach Us About Cough?* Lung, 2007. **186**(S1): p. 107-111.

159. Woodcock, A., E.C. Young, and J.A. Smith, *New insights in cough.* British Medical Bulletin, 2010. **1**.

160. Widdicombe, J., R. Eccles, and G. Fontana, *Supramedullary influences on cough.* Respiratory Physiology & Neurobiology, 2006. **152**(3): p. 320-328.

161. Lee, L.-Y., *Respiratory sensations evoked by activation of bronchopulmonary C-fibers.* Respiratory Physiology & Neurobiology, 2009. **167**(1): p. 26-35.

162. Raj, H., et al., *Sensory origin of lobeline-induced sensations: a correlative study in man and cat.* The Journal of Physiology, 1995. **482**(Pt 1): p. 235-246.

163. Dicpinigaitis, P.V., et al., *Investigation of the urge-to-cough sensation in healthy volunteers.* Respirology, 2012. **17**(2): p. 337-341.

164. Gui, P., et al., *GEnder differences in perceptions of urge to cough and dyspnea induced by citric acid in healthy never smokers.* CHEST Journal, 2010. **138**(5): p. 1166-1172.

165. Davenport, P.W., et al., *The effect of codeine on the Urge-to-Cough response to inhaled capsaicin.* Pulmonary Pharmacology & Therapeutics, 2007. **20**(4): p. 338-346.

166. Dicpinigaitis, P.V. and K. Rauf, *The Influence of Gender on Cough Reflex Sensitivity.* Chest, 1998. **113**(5): p. 1319-1321.

167. Dicpinigaitis, P.V., et al., *Effect of viral upper respiratory tract infection on the urge-to-cough sensation.* Respiratory Medicine, 2011. **105**(4): p. 615-618.

168. Pavord, I.D. and K.F. Chung, *Management of chronic cough.* Lancet, 2008. **371**(9621): p. 1375-1384.

169. Woolf, C.R. and A. Rosenberg, *Objective Assessment of Cough Suppressants under Clinical Conditions Using a Tape Recorder System.* Thorax, 1964. **19**(2): p. 125-130.

170. Sevelius, H. and J.P. Colmore, *Objective Assessment of Antitussive Agents in Patients with Chronic Cough.* The Journal of New Drugs, 1966. **6**(4): p. 216-223.

171. Loudon, R.G. and L.C. Brown, *Cough frequency in patients with respiratory disease.* The American Review of Respiratory Disease, 1967. **96**(6): p. 1137-1143.

172. Loudon, R.G. and S.K. Spohn, *Cough frequency and infectivity in patients with pulmonary tuberculosis.* The American Review of Respiratory Disease, 1969. **99**(1): p. 109-111.

173. Hsu, J.Y., et al., *Coughing frequency in patients with persistent cough: assessment using a 24 hour ambulatory recorder.* European Respiratory Journal, 1994. **7**(7): p. 1246-1253.

174. Coyle, M.A., et al., *Evaluation of an ambulatory system for the quantification of cough frequency in patients with chronic obstructive pulmonary disease.* Cough, 2005. **1**: p. 3.

175. Barry, S.J., et al., *The automatic recognition and counting of cough.* Cough, 2006. **2**: p. 8.

176. Matos, S., et al., *Detection of cough signals in continuous audio recordings using hidden Markov models.* IEEE Transactions on Biomedical Engineering, 2006. **53**(6): p. 1078-1083.

177. McGuinness, K., et al., *Automated cough detection: a novel approach.* Am J Respir Crit Care Med, 2007. **175**: p. A381.

178. Morice, A.H., et al., *ERS guidelines on the assessment of cough.* European Respiratory Journal, 2007. **29**(6): p. 1256-1276.

179. Kelsall, A., et al., *How to quantify coughing: correlations with quality of life in chronic cough.* European Respiratory Journal, 2008. **32**(1): p. 175-179.

180. Morice, A.H., J.A. Kastelik, and R. Thompson, *Cough Challenge in the assessment of Cough Reflex.* British Journal of Clinical Pharmacology, 2001. **52**(4): p. 365-375.

181. Dicpinigaitis, P.V., *Short- and long-term reproducibility of capsaicin cough challenge testing.* Pulm Pharmacol Ther, 2003. **16**(1): p. 61-5.

182. Dicpinigaitis, P.V., *Experimentally induced cough.* Pulmonary Pharmacology & Therapeutics, 2007. **20**(4): p. 319-324.

183. Choudry, N.B. and R.W. Fuller, *Sensitivity of the cough reflex in patients with chronic cough.* Eur Respir J, 1992. **5**(3): p. 296-300.

184. Prudon, B., et al., *Cough and glottic-stop reflex sensitivity in health and disease.* Chest, 2005. **127**(2): p. 550-7.

185. Hilton, E.C., et al., *Pharmacodynamic modeling of cough responses to capsaicin inhalation calls into question the utility of the C5 end point.* J Allergy Clin Immunol, 2013.

186. Clare Decalmer, S., et al., *Chronic cough: how do cough reflex sensitivity and subjective assessments correlate with objective cough counts during ambulatory monitoring?* Thorax, 2007. **62**(4): p. 329-334.

187. Empey, D.W., et al., *Comparison of the antitussive effects of codeine phosphate 20 mg, dextromethorphan 30 mg and noscapine 30 mg using citric acid-induced cough in normal subjects.* Eur J Clin Pharmacol, 1979. **16**(6): p. 393-7.

188. Smith, J., et al., *Effect of codeine on objective measurement of cough in chronic obstructive pulmonary disease.* The Journal of Allergy and Clinical Immunology, 2006. **117**(4): p. 831-835.

189. Vernon, M., et al., *Measuring cough severity: Perspectives from the literature and from patients with chronic cough.* Cough, 2009. **5**(1).

190. Birring, S.S., et al., *Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ).* Thorax, 2003. **58**(4): p. 339-343.

191. Brightling, C.E., et al., *Induced sputum and other outcome measures in chronic obstructive pulmonary disease: safety and repeatability.* Respir Med, 2001. **95**(12): p. 999-1002.

192. French, C.T., et al., *Evaluation of a Cough-Specific Quality-of-Life Questionnaire*.* Chest, 2002. **121**(4): p. 1123-1131.

193. Fletcher, K.E., et al., *A prospective global measure, the Punum Ladder, provides more valid assessments of quality of life than a retrospective transition measure.* Journal of Clinical Epidemiology, 2010. **63**(10): p. 1123-1131.

194. Raj, A.A., D.I. Pavord, and S.S. Birring, *Clinical Cough IV: What is the Minimal Important Difference for the Leicester Cough Questionnaire?* Pharmacology and Therapeutics of Cough, 2009: p. 311-320.

195. Polley, L., et al., *Impact of cough across different chronic respiratory diseases: comparison of two cough-specific health-related quality of life questionnaires.* Chest, 2008. **134**(2): p. 295-302.

196. BTS, *British Thoracic Society guidelines on diagnostic flexible bronchoscopy*, in *Thorax*2001. p. i1-21.

197. Ahmedzai, S. and C. Davis, *Nebulised drugs in palliative care.* Thorax, 1997. **52 Suppl 2**: p. S75-7.

198. Jain, S.K., et al., *The effect of local anaesthesia of the airway on respiratory reflexes in the rabbit.* Clinical Science, 1973. **44**(6): p. 519-538.

199. Cross, B.A., et al., *The effect of anaesthesia of the airway in dog and man: a study of respiratory reflexes, sensations and lung mechanics.* Clinical Science, 1976. **50**(Pt 6): p. 439-454.

200. Dain, D.S., H.A. Boushey, and W.M. Gold, *Inhibition of respiratory reflexes by local anesthetic aerosols in dogs and rabbits.* Journal of Applied Physiology, 1975. **38**(6): p. 1045-1050.

201. Adcock, J.J., et al., *RSD931, a novel anti-tussive agent acting on airway sensory nerves.* British Journal of Pharmacology, 2003. **138**(3): p. 407-416.

202. Karlsson, J.A., *Airway anaesthesia and the cough reflex.* Bull Eur Physiopathol Respir, 1987. **23 Suppl 10**: p. 29s-36s.

203. Chong, C.F., et al., *Comparison of lidocaine and bronchodilator inhalation treatments for cough suppression in patients with chronic obstructive pulmonary disease.* Emerg Med J, 2005. **22**(6): p. 429-32.

204. Hansson, L., B. Midgren, and J.A. Karlsson, *Effects of inhaled lignocaine and adrenaline on capsaicin-induced cough in humans.* Thorax, 1994. **49**(11): p. 1166-1168.

205. Choudry, N.B., et al., *Separation of cough and reflex bronchoconstriction by inhaled local anaesthetics.* The European Respiratory Journal: Official Journal of the European Society for Clinical Respiratory Physiology, 1990. **3**(5): p. 579-583.

206. Howard, P., et al., *Lignocaine aerosol and persistent cough.* Br J Dis Chest, 1977. **71**(1): p. 19-24.

207. Trochtenberg, S., *Nebulized lidocaine in the treatment of refractory cough.* Chest, 1994. **105**(5): p. 1592-1593.

208. Udezue, E., *Lidocaine inhalation for cough suppression.* Am J Emerg Med, 2001. **19**(3): p. 206-7.

209. Sanders, R.V. and M.B. Kirkpatrick, *Prolonged suppression of cough after inhalation of lidocaine in a patient with sarcoid.* JAMA, 1984. **252**(17): p. 2456-7.

210. Davenport, P.W., *Urge-to-cough: what can it teach us about cough?* Lung, 2008. **186 Suppl 1**: p. S107-11.

211. Morice, A.H., et al., *The diagnosis and management of chronic cough.* Eur Respir J, 2004. **24**(3): p. 481-92.

212. Morice, A.H., L. McGarvey, and I. Pavord, *Recommendations for the management of cough in adults.* Thorax, 2006. **61 Suppl 1**: p. i1-24.

213. Hardaker, L.E. and R.H. Hatley, *In vitro characterization of the I-neb Adaptive Aerosol Delivery (AAD) system.* J Aerosol Med Pulm Drug Deliv. **23 Suppl 1**: p. S11-20.

214. Newnham, D.M. and B.J. Lipworth, *Nebuliser performance, pharmacokinetics, airways and systemic effects of salbutamol given via a novel nebuliser delivery system ("Ventstream").* Thorax, 1994. **49**(8): p. 762-770.

215. Clay, M.M. and S.W. Clarke, *Effect of nebulised aerosol size on lung deposition in patients with mild asthma.* Thorax, 1987. **42**(3): p. 190-194.

216. Korttila, K., J. Tarkkanen, and L. Tarkkanen, *Comparison of laryngotracheal and ultrasonic nebulizer administration of lidocaine in local anaesthesia for bronchoscopy.* Acta Anaesthesiol Scand, 1981. **25**(2): p. 161-5.

217. Gove, R.I., J. Wiggins, and D.E. Stableforth, *A study of the use of ultrasonically nebulized lignocaine for local anaesthesia during fibreoptic bronchoscopy.* Br J Dis Chest, 1985. **79**(1): p. 49-59.

218. Gianelly, R., et al., *Effect of lidocaine on ventricular arrhythmias in patients with coronary heart disease.* N Engl J Med, 1967. **277**(23): p. 1215-9.

219. Chinn, W.M., D.C. Zavala, and J. Ambre, *Plasma levels of lidocaine following nebulized aerosol administration.* Chest, 1977. **71**(3): p. 346-348.

220. LeLorier, J., et al., *Pharmacokinetics of lidocaine after prolonged intravenous infusions in uncomplicated myocardial infarction.* Ann Intern Med, 1977. **87**(6): p. 700-6.

221. Sumner H., et al., *A Semi Automatic Method To Reduce The Time Taken For Manual Cough Counting.* Am J Respir Crit Care Med 2010. **181**: p. A5555.

222. Kelsall, A., et al., *A Novel Approach to Studying the Relationship Between Subjective and Objective Measures of Cough.* Chest, 2011. **139**(3): p. 569-575.

223. Hanley, J.A., et al., *Statistical analysis of correlated data using generalized estimating equations: an orientation.* American journal of epidemiology, 2003. **157**(4): p. 364-375.

224. Howard, P., et al., *Lignocaine aerosol and persistent cough.* British Journal of Diseases of the Chest, 1977. **71**: p. 19-24.

225. Canning, B.J. and Y.L. Chou, *Cough sensors. I. Physiological and pharmacological properties of the afferent nerves regulating cough.* Handbook of Experimental Pharmacology, 2009(187): p. 23-47.

226. Tatar, M., S.E. Webber, and J.G. Widdicombe, *Lung C-fibre receptor activation and defensive reflexes in anaesthetized cats.* J Physiol, 1988. **402**: p. 411-20.

227. Chung, K.F., *Clinical cough VI: the need for new therapies for cough: disease-specific and symptom-related antitussives.* Handb Exp Pharmacol, 2009(187): p. 343-68.

228. Leffler, A., et al., *The vanilloid receptor TRPV1 is activated and sensitized by local anesthetics in rodent sensory neurons.* The Journal of Clinical Investigation, 2008. **118**(2): p. 763-776.

229. Wong, C.H., R. Matai, and A.H. Morice, *Cough induced by low pH.* Respir Med, 1999. **93**(1): p. 58-61.

230. McGarvey, L., et al., *Are there clinical features of a sensitized cough reflex?* Pulmonary Pharmacology & Therapeutics, 2009. **22**(2): p. 59-64.

231. North, R.A. and M.F. Jarvis, *P2X receptors as drug targets.* Molecular Pharmacology, 2013. **83**(4): p. 759-769.

232. McGuinness, K., et al., *Validation of the VitaloJAK 24 hour ambulatory cough monitor.* Thorax, 2012. **67**(Suppl 2): p. A131.

233. Gu, J.G. and A.B. MacDermott, *Activation of ATP P2X receptors elicits glutamate release from sensory neuron synapses.* Nature, 1997. **389**(6652): p. 749-753.

234. Vulchanova, L., et al., *P2X3 is expressed by DRG neurons that terminate in inner lamina II.* European Journal of Neuroscience, 1998. **10**(11): p. 3470-3478.

235. Nakatsuka, T., et al., *Depletion of substance P from rat primary sensory neurons by ATP, an implication of P2X receptor-mediated release of substance P.* Neuroscience, 2001. **107**(2): p. 293-300.

236. Khalid S, et al., *Cough responses to tussive agents in health and disease.* Thorax December 2011, 2011. **66**(Supp 4): p. S139.

237. Gross, P.M., et al., *Microvascular specializations promoting rapid interstitial solute dispersion in nucleus tractus solitarius.* The American Journal of Physiology, 1990. **259**(6 Pt 2): p. R1131-1138.

238. McGaraughty, S., et al., *Effects of A-317491, a novel and selective P2X(3)/P2X(2/3) receptor antagonist, on neuropathic, inflammatory and chemogenic nociception following intrathecal and intraplantar administration.* British Journal of Pharmacology, 2003. **140**(8): p. 1381-1388.

239. Lommatzsch, M., et al., *Extracellular adenosine triphosphate and chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2010. **181**(9): p. 928-34.

240. Novakovic, S.D., et al., *Immunocytochemical localization of P2X3 purinoceptors in sensory neurons in naive rats and following neuropathic injury.* Pain, 1999. **80**(1-2): p. 273-82.

241. Horton, M.R., et al., *Thalidomide for the Treatment of Cough in Idiopathic Pulmonary FibrosisA Randomized Trial.* Annals of Internal Medicine, 2012. **157**(6): p. 398-406.

242. Eddy, M.C., et al., *Double P2X2/P2X3 Purinergic Receptor Knockout Mice Do Not Taste NaCl or the Artificial Sweetener SC45647.* Chemical Senses, 2009. **34**(9): p. 789-797.

243. Finger, T.E., et al., *ATP signaling is crucial for communication from taste buds to gustatory nerves.* Science (New York, N.Y.), 2005. **310**(5753): p. 1495-1499.

244. Puthussery, T. and E.L. Fletcher, *Neuronal expression of P2X3 purinoceptors in the rat retina.* Neuroscience, 2007. **146**(1): p. 403-414.

245. Canning, B.J., N. Mori, and S.B. Mazzone, *Vagal afferent nerves regulating the cough reflex.* Respiratory Physiology & Neurobiology, 2006. **152**(3): p. 223-242.

246. Jaffe, D.B., S.S. Marks, and D.A. Greenberg, *Antagonist drug selectivity for radioligand binding sites on voltage-gated and N-methyl-D-aspartate receptor-gated Ca2+ channels.* Neurosci Lett, 1989. **105**(1-2): p. 227-32.

247. Grattan, T.J., et al., *The effect of inhaled and oral dextromethorphan on citric acid induced cough in man.* British Journal of Clinical Pharmacology, 1995. **39**(3): p. 261-263.

248. Karttunen, P., et al., *Antitussive Effect of Dextromethorphan and Dextromethorphan-Salbutamol Combination in Healthy Volunteers with Artificially Induced Cough.* Respiration, 1987. **52**(1): p. 49-53.

249. Empey, D.W., et al., *Comparison of the antitussive effects of codeine phosphate 20 mg, dextromethorphan 30 mg and noscapine 30 mg using citric acid-induced cough in normal subjects.* European Journal of Clinical Pharmacology, 1979. **16**(6): p. 393-397.

250. Pavesi, L., *Application and Validation of a Computerized Cough Acquisition System for Objective Monitoring of Acute Cough : A Meta-analysis.* Chest, 2001. **120**(4): p. 1121-1128.

251. Brown, C., et al., *Antitussive activity of sigma-1 receptor agonists in the guinea-pig.* British Journal of Pharmacology, 2004. **141**(2): p. 233-240.

252. Carpenter, C.L., et al., *Dextromethorphan and dextrorphan as calcium channel antagonists.* Brain Research, 1988. **439**(1-2): p. 372-375.

253. Jones, R.W., *A review comparing the safety and tolerability of memantine with the acetylcholinesterase inhibitors.* International Journal of Geriatric Psychiatry, 2010. **25**(6): p. 547-553.

254. Schley, M., et al., *Continuous brachial plexus blockade in combination with the NMDA receptor antagonist memantine prevents phantom pain in acute traumatic upper limb amputees.* European Journal of Pain, 2007. **11**(3): p. 299-308.

255. Wiech, K., et al., *A Placebo-Controlled Randomized Crossover Trial of the N-Methyl-d-Aspartic Acid Receptor Antagonist, Memantine, in Patients with Chronic Phantom Limb Pain.* Anesthesia & Analgesia, 2004. **98**(2): p. 408-413.

256. Maier, C., et al., *Efficacy of the NMDA-receptor antagonist memantine in patients with chronic phantom limb pain – results of a randomized double-blinded, placebo-controlled trial.* Pain, 2003. **103**(3): p. 277-283.

257. Kavirajan, H., *Memantine: a comprehensive review of safety and efficacy.* Expert Opinion on Drug Safety, 2009. **8**(1): p. 89-109.

258. Parsons, C.G., W. Danysz, and G. Quack, *Memantine is a clinically well tolerated N-methyl-d-aspartate (NMDA) receptor antagonist—a review of preclinical data.* Neuropharmacology, 1999. **38**(6): p. 735-767.

259. Hilton, E.C., *Towards an understanding of the neurophysiology of cough in humans* 2012, University of Manchester: UK.

260. Young, E.C., et al., *An open-label pilot study of V3381, a novel N-Methyl-D-Aspartate Receptor (NMDA-R) antagonist in chronic cough.* Lung, 2012. **190**: p. 66.

261. Eccles, R., *The powerful placebo in cough studies?* Pulm Pharmacol Ther, 2002. **15**(3): p. 303-8.

262. Kwong, K., et al., *Pharmacological Characterization of GSK2339345, A Novel Voltage-Gated Sodium Channel Blocker For The Symptomatic Relief Of Cough.* Am J Respir Crit Care Med, 2013. **187**: p. A4936.

Appendix 1: Urge to cough questionnaire

LOCATION OF THE URGE TO COUGH

Please indicate where you feel the urge to cough:
Researcher to describe location indicated by marking on diagram (if appropriate) and selecting locations from list:

☐ Nose

☐ Mouth

☐ Throat/Pharynx

☐ Neck

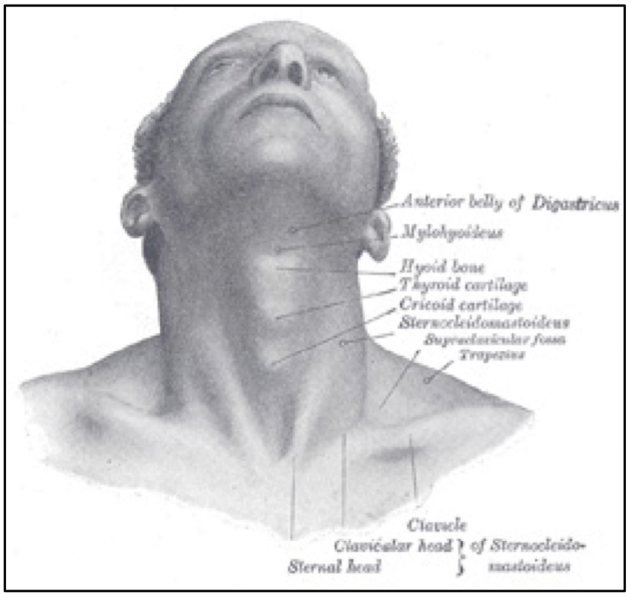
☐ Supraclavicular notch

☐ Sternum

☐ Chest

☐ Abdomen

☐ Other



Is the urge to cough located on the:

☐ LEFT ☐ RIGHT ☐ CENTRALLY ☐ BOTH SIDES

Is the urge to cough :

☐ LOCALISED (a point sensation that you could put your finger on)

☐ DIFFUSE (more generalised and spread over a larger area)

Appendix 2: Cough quality of life questionnaire (CQLQ)

NAME (OPTIONAL): _____ STUDY RECORD:_____ DATE:_____

Please indicate below how your <u>cough</u> affects you. Circle the answer that best describes your agreement with each item. Please respond to every item. Thank you for your help.

1. **Family and/or close friends can't tolerate it any more.**	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
2. I have experienced prolonged absences from important activities such as work, school, daily duties or volunteer work.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
3. **I have been completely prevented from engaging in important activities such as work, school, daily duties or volunteer work.**	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
4. I have lost my appetite.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
5. **I am sick to my stomach and I vomit.**	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
6. I cough and it makes me retch (dry heaves).	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
7. **I have a fear that I might have AIDS or tuberculosis.**	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
8. I have headaches.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE

9. I am worried that I have cancer.	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
10. I am dizzy.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
11. I wet my pants.	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
12. I soil my pants.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
13. I sweat.	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
14. I am hoarse.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
15. It hurts when I breathe.	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
16. I broke a rib.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
17. I cannot sleep at night.	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
18. I have difficulty speaking on the phone.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE

19. I can no longer sing, for instance in church.	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
20. I have stopped going to social activities such as going to the cinema, plays, local meetings.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
21. I have had to change my lifestyle.	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
22. I ache all over.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
23. I am exhausted.	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
24. I am embarrassed.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
25. I am upset by people thinking that I have something wrong with me.	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
26. I want to be reassured that I do not have anything seriously the matter with me.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE
27. I am self-conscious.	**STRONGLY DISAGREE**	**DISAGREE**	**AGREE**	**STRONGLY AGREE**
28. I am worried that I have something seriously the matter with me.	STRONGLY DISAGREE	DISAGREE	AGREE	STRONGLY AGREE

Appendix 3: Global rating of change scale (GROCS)

GLOBAL RATING of CHANGE SCALE

Overall, has there been any change in your <u>cough frequency</u> since you started the new medicine? Please indicate if there has been any change in your symptoms by choosing one of the following options.

Are your symptoms:

□ Worse □ About the same □ Better

[Patients who state they are better are then asked:]

How much better are your symptoms? Are they:

1. Almost the same, hardly any better at all
2. A little better
3. Somewhat better
4. Moderately better
5. A good deal better
6. A great deal better
7. A very great deal better

[Patients who state they are worse are then asked:]

How much worse are your symptoms? Are they:

8. Almost the same, hardly any worse at all
9. A little worse
10. Somewhat worse
11. Moderately worse
12. A good deal worse
13. A great deal worse
14. A very great deal worse

Overall, has there been any change in your <u>cough severity</u> since you started the new medicine? Please indicate if there has been any change in your symptoms by choosing one of the following options. Are your symptoms:

□ Worse □ About the same □ Better

[Patients who state they are better/worse are asked to clarify as above:]