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XEN-D0501, a novel TRPV1 Antagonist, does not reduce Cough in Refractory Cough Patients

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What is the current scientific knowledge on this subject?

Conclusive data regarding the importance of TRPV1 as a treatment target in chronic cough has been lacking due the absence of a safe, potent and efficacious tool compound for use in clinical studies. In a previous study, a TRPV1 antagonist (SB-705498) failed to improve spontaneous cough frequency in treatment resistant chronic cough patients but the reduction in capsaicin-evoked cough was only small.

What does this study add to the field?

This study rules out TRPV1 as an effective therapeutic target in refractory chronic cough patients. It also highlights the importance of pharmacodynamic pre-clinical and clinical models in the interpretation of negative clinical trial data, but questions the use of cough challenge models in target identification.

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ABSTRACT

RATIONALE: Heightened cough responses to inhaled capsaicin, a TRPV1 agonist, are characteristic of patients with chronic cough. However, previously a TRPV1 antagonist (SB-705498) failed to improve spontaneous cough frequency in these patients despite small reductions in capsaicin-evoked cough.

OBJECTIVES: XEN-D0501 (potent TRPV1 antagonist) was compared with SB-705498 in pre-clinical studies to establish whether an improved efficacy profile would support a further clinical trial of XEN-D0501 in refractory chronic cough.

METHODS: XEN-D0501 and SB-705498 were profiled against capsaicin in a sensory nerve activation assay and *in vivo* potency established against capsaicin-induced cough in the guinea pig.

Twenty patients with refractory chronic cough participated in a double-blind, randomised, placebo-controlled, crossover study evaluating the effect of 14 days XEN-D0501 (oral, 4mg bd) versus placebo on awake cough frequency (primary outcome), capsaicin-evoked cough and patient reported outcomes.

MEASUREMENTS AND MAIN RESULTS: XEN-D0501 was more efficacious and 1000-fold more potent than SB-705498 at inhibiting capsaicin-induced depolarization of guinea pig and human isolated vagus. *In vivo*, XEN-D0501 completely inhibited capsaicin-induced cough whereas 100-times more SB-705498 was required to achieve the same effect. In patients, XEN-D0501 substantially reduced maximal cough responses to capsaicin (mean change from baseline XEN-D0501 -19.3(±16.4) coughs vs. placebo -1.8(±5.8), $p<0.0001$), but not spontaneous awake cough frequency (mean change from baseline XEN-D0501 6.7c/h(±16.9) vs. placebo 0.4c/h(±13.7), $p =0.41$).

CONCLUSIONS: XEN-D0501 demonstrated superior efficacy and potency in pre-clinical and clinical capsaicin challenge studies; despite this improved pharmacodynamic profile, spontaneous cough

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frequency did not improve, ruling out TRPV1 as an effective therapeutic target for refractory cough.

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INTRODUCTION

Chronic cough (>8 weeks duration) is thought to affect approximately 12% of the population(1). Patients presenting with isolated chronic cough (with normal chest radiography and spirometry) frequently report that coughing is associated sensations of throat irritation and an urge-to-cough, which are triggered by trivial exposures to environmental irritant chemicals, temperature changes and use of their voice(2,3). These clinical features are suggestive of a hyper-excitability of the neuronal pathways responsible for controlling cough. Consistent with this hypothesis, heightened cough responses to inhaled irritants, most commonly capsaicin, have been shown to be a characteristic feature in such patients(4-6).

The cough reflex is regulated by vagal afferent nerves which innervate the airway. Some are more mechanically sensitive, and some are more chemosensitive; C fibres and Aδ nociceptors(7). Ion channels present on these vagal nerve termini can be activated by a wide variety of stimuli to elicit cough. The main family of ion channels implicated in the initiation of sensory reflexes are transient receptor potential (TRP) channels(7). Transient receptor potential vanilloid 1 (TRPV1) is a polymodal ion channel and was the first to be identified. It is activated by diverse stimuli including the direct activators capsaicin (constituent of chilli peppers from piquant Capsicum spp. Plants), noxious heat $>42^{o}$C and acidic conditions/protons which interact directly with the channel to cause a lowering of its voltage dependency, leading to opening of the pore domain(8). It can also be activated indirectly by endogenous “disease relevant” mediators including bradykinin and PGE_2(9,10).

Capsaicin reliably activates the cough reflex in several species including humans, and is a specific TRPV1 agonist(10-13); TRPV1 is expressed by vagal afferent C- and Aδ nociceptive fibres innervating the airways(7). Capsaicin is the most frequently used agent for experimentally inducing cough in pre-

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clinical and clinical studies, where the concentration of capsaicin evoking at least five coughs (C5) is the traditional endpoint. Numerous studies have demonstrated lower capsaicin C5 in chronic cough compared with healthy controls (i.e. the cough reflex is more sensitive), suggesting TRPV1 could be an important treatment target(4,5). However, from clinical cough challenges, it cannot be determined whether this lowering of the cough threshold is related to changes in TRPV1 at the sensory nerve terminals or in central pathways.

Only one previous clinical study has evaluated the anti-tussive effects of a single dose of a TRPV1 antagonist (SB-705498) in patients with refractory chronic cough(12). However, disappointingly there was no effect on spontaneous cough frequency although there was a small but statistically significant impact on the capsaicin C5 (increased by approximately one doubling dose over placebo), giving some evidence of target engagement. These data therefore questioned the role of TRPV1 mediated mechanisms in patients with refractory chronic cough, however it remained unclear whether more potent antagonists and/or more prolonged dosing might still be efficacious. XEN-0501 is a potent and safe TRPV1 antagonist. It has demonstrated sub-nanomolar potency for TRPV1 receptors in human and rat recombinant cell systems and rat cultured dorsal root ganglia neurons and its selectivity has been confirmed against panels of enzymes, ion channels and G-protein coupled receptors(14). Thus the aims of this study were firstly to compare a potent TRPV1 antagonist (XEN-D0501) with SB-705498 in pre-clinical studies to establish whether an improved efficacy profile would support a further clinical trial. Secondly, to evaluate the anti-tussive effect of XEN-D0501 in a double blind randomised controlled trial in patients with refractory chronic cough. Some of the results of these studies have been previously reported in the form of abstracts (15).

METHODS

For detailed methods see Online Repository.

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PRE-CLINICAL STUDIES

Animals

In vivo and ex vivo experiments were conducted in male Dunkin-Hartley guinea pigs (300-500g) (Harlan and B&K Universal, UK). The experiments were performed in accordance with the UK Home Office guidelines for animal welfare based on the Animals (Scientific Procedures) act 1986 and the ARRIVE guidelines(16).

Guinea-pig and human isolated vagus nerve recording

Recording of depolarisation (as a measure of vagal, afferent sensory nerve activity) of isolated guinea-pig and human vagus was carried out as described previously(9,17,18). Briefly, nerves were stimulated twice with capsaicin (1μM) for 2min before vehicle or XEN-D0501 (0.1-100nM) or SB-705498 (1-10μM) was perfused for 10min. Response to capsaicin (1μM) was then re-assessed in presence of vehicle or test compounds. After a 10min washout, nerves were finally stimulated again with capsaicin (1μM) to confirm viability.

Human en bloc lungs unsuitable for transplantation were obtained from International Institute for the Advancement of Medicine (New Jersey, USA), and vagal tissue was obtained (N=4, 36-57 years old, 3 male). In all cases tissue was consented for use in scientific research. Ethics approval was obtained from the Royal Brompton & Harefield Trust.

Capsaicin evoked cough in guinea-pigs

Vehicle (0.5% methylcellulose, 0.1% Tween80, 5ml/kg, i.p.) or XEN-D0501 (1-10mg/kg, i.p.) or SB-705498 (10-100mg/kg, i.p.) were administered 1 hour prior to cough recording. Conscious, unrestrained guinea-pigs were placed in individual plastic transparent whole-body plethysmograph chambers (Buxco, Wilmington, NC, USA) and cough assessed to an aerosol of capsaicin (60μM in 1%

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ethanol, 1% Tween 80 in 0.9% sterile saline for 5mins) and coughs counted for 10mins as previously described(9, 18). Studies were also performed to establish if the XEN-D0501 compound retained efficacy after repeat dosing. In these studies guinea-pigs were dosed with vehicle (0.5% methylcellulose, 0.1% Tween80, 5ml/kg, i.p.) or compound (1 or 3mg/kg, bd) for 13 days and then 1 hour prior to cough assessment. Capsaicin-induced cough was then assessed as previously.

CLINICAL STUDY

Study Design

A double-blind, randomised, placebo-controlled, crossover study was performed to evaluate the anti-tussive efficacy of 14 days treatment with XEN-D0501 (oral 4mg bd) compared with matched placebo; the two treatment periods were separated by a 14 day washout, Figure 1A. Participants were recruited from two specialist cough clinics (University Hospital of South Manchester, Manchester and Belfast City Hospital, Belfast) between July 2014 and June 2015. Cough was assessed prior to dosing and at the end of each treatment period using an ambulatory cough monitor and patient reported outcomes. Capsaicin evoked cough responses were included as a pharmacodynamic endpoint.

Participants

We recruited subjects with refractory chronic cough, as determined by national cough guidelines (BTS Thorax 2006(19). An awake cough frequency of >1.5c/h and a maximal capsaicin cough response over 4 inhalations (E_{max}) of >10 coughs were required for inclusion, to avoid patients with very low cough frequency/capsaicin-evoked cough responses, where a treatment effect would be difficult to demonstrate. Current smokers and ex-smokers with a smoking history of >20 pack years were ineligible, as were women of child-bearing potential and those taking treatments that may modulate cough (e.g. morphine, gabapentin). The study was approved by an independent Research Ethics

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Committee (NRES Committee North West - Liverpool East Ref: 14/NW/0211) and all patients gave written informed consent.

Study Treatment, Randomisation and Masking

Patients were assigned to one of the two possible treatment sequences by a computer-generated randomisation schedule with a ratio of 1:1 for XEN-D0501/placebo or placebo/XEN-D0501. Patients, health-care providers, investigators and the sponsor were all masked to the treatment sequence assignment. The dose of XEN-D0501 used in this study (4mg bid) was considered to be the maximum tolerable dose in the older age group of patients typically presenting with chronic cough.

Procedures

Patient demographics, treatments and co-morbidities were collected at screening. Cough was assessed at four time points; for both treatment periods this was prior to dosing and at the end of the treatment period, Figure 1A. Safety was assessed through monitoring of vital signs, ECGs, body temperature, haematology, biochemistry and adverse events.

Outcomes

The primary endpoint was awake objective cough frequency collected using a 24h ambulatory cough monitor commenced at day 0 and day 13 of each treatment period; sleep and 24h cough frequency were also determined. The secondary endpoints included changes from baseline in a 100mm cough severity visual analogue scale (VAS), cough specific quality of life questionnaire, and also a 15-point global rating of change scale for cough severity and frequency, completed at the end of each treatment period. Cough responses to inhaled capsaicin provided a pharmacodynamic endpoint and were assessed on day 1 and 14, following completion of each 24h cough recording.

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Ambulatory Cough Monitoring: Twenty-four hour acoustic recordings were made using the VitaloJAK™ cough monitor (Vitalograph Ltd, Buckinghamshire, UK) with cough sounds per hour quantified by a semi-automated method using validated custom-written software(12,20,21).

Cough Challenges: Four breaths of doubling concentrations of capsaicin (0.97-1000µM, Stockport Pharmaceuticals Ltd, Stockport, UK) were inhaled at one minute intervals from a dosimeter (Koko® dosimeter, De Vilbiss Health Care Inc., Somerset, PA). The number of coughs in the 15 seconds following each inhalation was counted The challenge continued until the maximum tolerated dose or the final concentration was inhaled(6, 22). The maximum number of coughs evoked at any concentration (E_{max}) was the main endpoint, but the concentration evoking 50% of the maximal response (ED_{50}) was also reported. To allow comparison with previous studies the concentration of capsaicin evoking two (C2) and five (C5) endpoints were also extracted from the cough challenge data for analysis.

Patient Reported Cough Measures: Subjects rated cough severity and urge-to-cough on separate 100mm VAS scales for day and night times. Cough specific quality of life was assessed by the Leicester Cough Questionnaire (LCQ); higher scores indicated better quality of life(23).

DATA ANALYSIS

Vagal depolarisation: Inhibition of depolarisation was analysed utilising a paired t-test, given observations were made using the tissue as its own control. Data presented are means ± S.E.M with statistical significance set at a value of $p<0.05$.

Cough: Inhibition of cough by the TRPV1 antagonists *in vivo* was analysed initially utilising a Bartlett's Test in order to determine whether the variances were equal and then either a parametric test (Analysis of Variance followed by a Bonferroni's Multiple Comparison Test) or a non-parametric test

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(Kruskal-Wallis followed by Dunn's Multiple Comparison Test) to compare responses from the antagonist group with those from the vehicle control group. Data presented are means ± S.E.M with statistical significance set at a value of $p<0.05$.

Clinical Study: Sample size was estimated from previous data, (awake cough frequency ~25c/h, $\log_{10}$ standard deviation 0.23-0.41). Based on these assumptions, 16 patients would be needed to detect a 56% reduction in awake cough frequency (90% power, 5% significance level). The change from baseline in the awake objective cough frequency was analysed with a mixed effects linear model, with baseline and treatment period as fixed effects and patient as a random effect. Awake cough frequency was log-transformed for analysis and the standard $p<0.05$ level of significance applied. Similar models were applied to the secondary endpoints, with or without log-transformation as appropriate.

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RESULTS

PRE-CLINICAL STUDIES

Guinea-pig and Human Vagus nerve depolarisation

XEN-D0501 (0.1-100nM) inhibited capsaicin-induced depolarisation of the guinea pig vagus in the nanomolar range in a concentration dependent manner with a maximal inhibition of 87.2 ± 4.4% at 10nM (Figure 2A). The maximally effective concentration of XEN-D0501 (10nM) had no effect on responses to the TRPA1 agonist, acrolein (300μM) indicating no off target activity on another TRP channel expressed on C-fibre afferents, which when activated is also known to evoke cough in guinea-pigs and humans(24). SB-705498 (1-10μM) also evoked a concentration dependent inhibition of capsaicin-induced depolarisation of guinea pig vagus that was in the micromolar range with a maximal inhibition of 52.4 ± 5.5% at 10μM (Figure 2B). Higher concentrations could not be formulated for *in*

vitro experiments as solubility was compromised. Similarly, XEN-D0501 (0.1-100nM) also inhibited capsaicin-induced depolarisation of the human vagus in the nanomolar range in a concentration dependent manner with 80.54 ± 10.4% at 100nM (Figure 2C). Furthermore, consistent with data generated in the guinea-pig, SB-705498 inhibited depolarisation in the micromolar range.

Capsaicin Evoked Cough in a Conscious Guinea Pig Model

XEN-D0501 significantly inhibited capsaicin-induced cough at all doses tested (Vehicle: 8.63 ± 2.3 versus XEN-D0501 (1mg/kg): 2.13 ±0.95 coughs/10min, n=8) (Figure 3A). SB-705498 only significantly inhibited cough at the highest dose tested (Vehicle: 6.71 ± 1.9 versus XEN-D0501 (100mg/kg): 0.29 ± 0.29 coughs/10min, n=7) (Figure 3B). XEN-D0501 retained its efficacy following 13 days dosing bd, inhibiting capsaicin evoked cough to the same extent as in guinea pigs only receiving a single dose 1 hour prior to the cough challenge (Figure 3C).

CLINICAL STUDY

Subjects

Twenty patients with refractory chronic cough (mean age 63.1yrs, 15 female) were recruited, see Table 1 and Figure 1B. Patients were typical of those attending specialist cough clinics and those randomised to each treatment sequence were well matched. Two patients discontinued study treatment and withdrew from the study. One patient was withdrawn during XEN-D0501 treatment due to a treatment-emergent adverse event of fatigue, considered related to study treatment. A further patient was withdrawn during placebo dosing as they did not meet all the inclusion criteria and were included in error. A tablet count suggested that compliance with study treatment was high and almost identical for XEN-D0501 and placebo. Over the two week treatment periods, this

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suggested subjects consumed a mean of 26.7 XEN-D0501 tablets (95%CI 26.5-27.5) and 27.0 (25.9-27.5) placebo tablets (p=0.56).

Cough Frequency and Capsaicin Evoked Cough Responses

Consistent with the pre-clinical data, XEN-D0501 dramatically reduced maximal cough responses to capsaicin [E_{max} mean difference XEN-D0501 -19.3 coughs (±16.4) vs. placebo -1.8 coughs (±5.8), p<0.0001], Figure 4. The logC2 and logC5 endpoints also significantly improved from baseline with XEN-D0501; increases in logC2 of +1.10µM (SEM ±0.34), p=0.005 and logC5 of +1.22µM (±0.40), p=0.007 over placebo, equivalent to increases of +3.7 and +4.1 doubling doses of capsaicin respectively. However, there was no difference in the change from baseline in spontaneous daytime cough frequency for treatment with XEN-D0501 compared with placebo [awake cough frequency (mean change XEN-D0501 6.7c/h (±16.9) vs. placebo 0.4c/h (±13.7), p=0.41), Table 2. Similarly there was no significant change in 24h or sleep cough frequency; hourly cough counts on day 14 of treatment with placebo and XEN-D0501 are shown in Figure 5. Treatment sequence and period had no significant effect in any of these analyses.

Patient Reported Outcomes

Overall, the patient reported endpoints did not suggest patients perceived significant improvements in their cough. The only exception was awake cough severity VAS which improved with placebo treatment compared to XEN-D0501, although the magnitude of the difference was very small indeed [mean difference 2.8mm (SD ±21.4), p=0.03]. Again, treatment sequence and period had no significant effect in any of the analyses.

Safety and Adverse Events

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No serious adverse events (SAEs) were reported during the study. In total, 103 treatment emergent adverse events (TEAEs) were reported by 18 (94.7%) patients receiving XEN-D0501; 89 events were considered to be treatment-related. Fourteen AEs were reported by 9 (47.4%) patients receiving placebo, of which 5 events were considered treatment-related. The most frequently reported AEs were related to temperature sensations or mouth related events and were consistent with those seen in previous studies(14). The most common AEs, reported by ≥3 patients taking XEN-D0501 were: thermohypoaesthesia (n=8), dysgeusia (n=9), and feeling of body temperature change (n=6), feeling hot (n=3), oral paraesthesia (n=3), and hyperhidrosis (n=3). Headache was also a frequently reported TEAE (n=3 taking XEN-D0501 and n=1 taking placebo). No concerns were raised by other measures of safety i.e. ECGs, vital signs including body temperature, physical examination findings, and routine haematology and coagulation, clinical chemistry, and urinalysis assessments.

DISCUSSION

Cough is the most common condition for which patients seek medical attention but currently there are no licensed treatments for refractory chronic cough and evidence is lacking to suggest over-the-counter (OTC) cough medications are effective(25). The cough reflex is regulated by vagal afferent nerves which innervate the airway, some are more mechanically sensitive; the Rapidly Adapting Receptors (RARs), Slowly Adapting Receptors (SARs), and the subtype known as the 'cough' receptor, and some are more chemosensitive; C fibres and Aδ nociceptors(7). Ion channels present on these vagal nerve termini can be activated by a wide variety of stimuli to elicit cough and other reflexes. The main family of ion channels implicated in the initiation of sensory reflexes are TRP channels(7).

A significant amount of information has been gathered regarding the biology of TRPV1 from clinical, capsaicin challenge studies. Interestingly, the threshold for provoking cough by capsaicin (C2 or the C5

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concentration of capsaicin required to elicit at least 2 or 5 coughs, respectively) has been found to be lowered in various populations of patients with Chronic Obstructive Pulmonary(4,26), asthma(22,26), Idiopathic Pulmonary Fibrosis(27) and also refractory chronic cough patients(4,5,28) compared to healthy controls. These data imply that TRPV1 function is frequently increased in a disease setting. However, it is still not certain if increased sensitivity to various challenge agents acting on particular ion channels informs us regarding whether a particular intervention will be useful in a certain disease setting. In one of the studies above, different patient groups (COPD, healthy smokers, refractory chronic cough, and asthma) were compared and exhibited different patterns of modulation of cough responses to a range of inhaled irritants. This data supports the concept of disease-specific neurophenotypes in airway disease suggesting that chronic cough associated with different aetiologies may not be sensitive to similar interventions(4).

Of all the TRP channels, antagonists for TRPV1 are the most advanced with regard to drug development and clinical trials. However, development of these inhibitors has not been straightforward due to the activity of TRPV1 as a thermosensor for hot temperatures. This has seen several TRPV1 antagonists fail to progress due to adverse effects such as increased body temperature and latent withdrawal to noxious hot stimuli. For instance AMG 517 caused significant and long lasting increases in body temperature in phase 1 clinical trials in patients following molar extractions. In this case the trial was terminated before any efficacy, in relation to possible analgesic effects, could be determined(29). Other compounds, also trialled in dental pain studies exhibited similar adverse events with increases in body temperature and altered noxious heat sensation. In all cases these adverse effects were correlated with target engagement. More recently compounds have been identified that are not limited because of adverse events.

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SB-705498 has been reported to be a potent, selective, orally bioavailable competitive TRPV1 receptor antagonist which does not exhibit significant disturbances in temperature perception or control(30). The availability of safe, "clinical ready" TRPV1 antagonists has already allowed the profiling of SB-705498 in a double blind randomised controlled trial in patients with refractory chronic cough(12). This trial utilised objective cough monitoring and capsaicin challenge as a pharmacodynamic marker of target engagement. In this study, a single dose of SB-705498 was reported to be well tolerated with no significant increase in tympanic temperature or reported temperature perception. However, disappointingly SB-705498 lacked efficacy in improving spontaneous cough frequency in refractory chronic cough patients, although there was a small but statistically significant shift in capsaicin-evoked cough. Pharmacodynamic modelling estimated SB-705498 receptor occupancy to be 45% at 2 hours (associated with more than a doubling dose shift in C5 values over placebo) and 25% at 24 hours (associated with a 0.7 doubling dose shift in C5 values over placebo). Therefore, it was not clear whether the lack of efficacy in reducing spontaneous cough was due to the modest receptor occupancy and whether a compound with increased potency and efficacy at reducing cough to capsaicin would be required. Furthermore, the long half-life of SB-705498 (50-60hrs) limited previous clinical studies of this drug to investigating the efficacy of single doses, and therefore the potential effects of more prolonged TRPV1 blockade could not be determined.

In these experiments we utilised a potent and selective TRPV1 receptor antagonist, XEN-D0501, which has been shown to demonstrate sub-nanomolar potency in human recombinant cellular systems and rat dorsal root ganglia neuron preparations. In a phase 1 safety study, XEN-D0501 was well tolerated with repeat dosing with no serious adverse events and a slight increase in core body temperature which attenuated over time and was not considered to be of clinical concern(14). In this study we

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demonstrate superior efficacy and potency of the TRPV1 antagonist, XEN-D0501, over SB-705498 in a range of pre-clinical *in vitro* and *in vivo* studies. XEN-D0501 (0.1-10nm) was more efficacious and 1000-fold more potent than SB705498 (1-10μM) at inhibiting capsaicin-induced depolarisation of guinea pig and human isolated vagus. The *in vitro* potency of SB-705498 in this study was slightly less than reported in a FLIPR assay on the guinea-pig and human recombinant TRPV1 receptor expressed in HEK293 cells (apparent pKi values of 7.3±0.1 7.6±0.2, respectively). However, one might expect a 10-fold or more drop off in potency when one looks at the activity against the native ion channel in a tissue based assay. In vivo, XEN-D0501 (1mg/kg) completely inhibited capsaicin-induced cough whereas 100 times more SB705498 (100mg/kg) was required to achieve the same effect which was somewhat consistent with previous data demonstrating some inhibition with SB-705498 (at 10, 30mg/kg) in a capsaicin induced secondary hyperalgesia model in the rat(31).

In this study, target engagement was demonstrated in pre-clinical and clinical studies with XEN-D0501 blocking the cough to capsaicin observed in both models; highlighting the translational capacity of the guinea-pig model. Rather than the traditional clinical C5 endpoint which is somewhat arbitrary, we utilised novel cough challenge methodology which captures the dose-response relationship between capsaicin and cough, using the pharmacodynamic endpoints E_{max} and ED_{50}(6). We have recently shown that E_{max} better discriminates chronic cough patients from healthy controls and mild asthma patients, and it is more readily compared to pre-clinical cough studies. E_{max} exhibited substantial improvements in this study. However, this did not translate into efficacy for XEN-D0501 on spontaneous coughing in chronic cough patients.

This study has some limitations; as a proof of concept study, the sample size was small. Nonetheless significant changes in capsaicin cough responses were still clearly demonstrated. Furthermore, both our power calculations and previous studies demonstrate that objective cough monitoring is a

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powerful tool for demonstrating efficacy, and large studies are not required(21). It should also be acknowledged that whilst no serious adverse events or changes in body temperature occurred in this study, TRPV1 antagonism was still associated with frequent non-severe adverse effects, mainly relating to temperature sensing, perceived body temperature and alterations in taste.

In summary, that potent TRPV1 antagonism, producing considerable reductions in capsaicin evoked cough, was not associated with any improvements in objective cough frequency suggests that TRPV1 is not an effective target in refractory chronic cough. This is in stark contrast to recent demonstrations of the efficacy of P2X3 antagonism, implicating this ion channel and its endogenous ligand, ATP, in the mechanisms under pinning chronic cough (20). The current study may also provide an indication that increased sensitivity to one mechanism (e.g. TRPV1), demonstrated by challenge studies (e.g. utilising capsaicin) in one patient group (refractory chronic cough), does not necessarily implicate the utility of said target in a particular patient group. However, this may not be the case for TRPV1 in other conditions that exhibit chronic cough or with other TRP channels involved in chronic cough. What this study does illustrate is that challenge paradigms across pre-clinical and clinical models can deliver effective pharmacodynamic models which can inform dosing regimens in future clinical trials across any therapeutic area.

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TABLES

Table 1. Characteristics of randomised participants. Data are mean and standard deviations.

		Treatment Sequence		Overall
		XEN-D0501/ Placebo (n=11)	**Placebo/ XEN-D0501** (n=9)	
Age	(years)	64.9 (7.8)	60.8 (11.0)	63.1 (9.4)
Sex	Male (n, %)	3 (27.3%)	2 (22.2%)	5 (25.0%)
	Female (n, %)	8 (72.7%)	7 (77.8%)	15 (75.0%)
Race (n, %)	White	11 (100.0%)	9 (100.0%)	20 (100.0%)
Height (cm)		161.9 (6.8)	164.1 (8.4)	162.9 (7.4)
Weight (kg)		77.0 (15.5)	71.7 (17.9)	74.6 (16.4)
BMI (kg/m2)		29.3 (4.9)	26.3 (4.8)	28.0 (5.0)
FEV1 % predicted		105.5% (13.1)	104.8% (15.6)	105.2% (13.9)
Former smoker		3 (27.3)	1 (11.1)	4 (20.0)
Never smoked		8 (72.7)	8 (88.9)	16 (80.0)
Current smoker		0	0	0
Pack years (ex-smokers)		4.7 (4.0)	4.0	4.5 (3.3)

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Table 2. Measures of Cough at baseline and after 2 weeks treatment with XEN-0501 and matched placebo. Data are means and standard deviations unless otherwise stated.

		XEN-D0501 (n=18)		**Placebo** (n=19)		**p**
		Baseline	Day 14	Baseline	Day 14	
Objective Cough Frequency	Awake	34.4c/h (±41.9)	41.1c/h (±52.7)	40.9c/h (±63.2)	41.3c/h (±58.3)	0.42
	24h	24.2c/h (±29.0)	29.5c/h (±36.8)	29.2c/h (±46.0)	30.5c/h (±44.0)	0.43
	Night *median (IQR)*	0.7c/h (0.3-2.3)	1.7c/h (0.8-4.5)	2.3c /h (0.9-3.3)	1.4c/h (0.7-4.4)	0.29
Cough Severity VAS	Day	40.6mm (±26.6mm)	39.7mm (±32.5mm)	34.3mm (±23.0mm)	31.5mm (±27.8mm)	**0.03**
	Night	21.9mm (±25.8mm)	16.4mm (±22.3mm)	20.2mm (±17.5mm)	14.7mm (±18.2mm)	0.12
Urge to Cough VAS	Day	42.9mm (±26.2mm)	34.6mm (±30.9mm)	31.1mm (±21.5mm)	27.2mm (±25.3mm)	0.25
	Night	22.2mm (±23.8mm)	16.3mm (±24.3mm)	18.4mm (±17.0mm)	14.6mm (±22.5mm)	0.58
Cough Quality of Life (LCQ)		13.05 (4.09)	13.80 (4.03)	13.70 (4.53)	14.43 (4.43)	0.82
Capsaicin Challenge	E_{max}	32.3 coughs (±17.1)	13.1 coughs (±11.3)	27.7 coughs (±15.2)	25.9 coughs (±12.3)	**<0.001**

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FIGURE LEGENDS:

Figure 1. Clinical Study Design and Consort Diagram.

(A) Summary of the double blind randomised crossover design of the clinical study including timing of efficacy and pharmacodynamics endpoints. (B) Patient recruitment and flow; one patient withdrew* after 5 days treatment with XEN-0501 due to intolerance to the study drug and a second patient was withdrawn† during placebo dosing as they were included in the study in error.

Figure 2. Inhibition of Vagal Sensory Nerve activation.

The effect of vehicle (0.1% DMSO v/v) or (A) XEN-D0501 (0.1-100nM.) or (B) SB705498 (1-10 μM) on depolarization of the guinea-pig vagus induced by capsaicin (1μM). A similar effect was produced in human vagus (C); typical example traces (D). Data expressed as mean ± S.E.M, n=4 and n=3 observations per treatment group in guinea-pig and human studies, respectively.

Figure 3. Inhibition of Capsaicin-induced Cough in a Conscious Guinea-pig model

The effect of vehicle (0.5% methylcellulose, 0.1% Tween80, 5ml/kg, i.p.) or (A) XEN-D0501 (1, 3 or 10 mg/kg, i.p., n=8) or (B) SB705498 (10, 30 or 100 mg/kg, i.p., n=7) administered 1 hour prior to cough recording on capsaicin (60 μM in 1% ethanol, 1% Tween 80 in 0.9% sterile saline for 5 mins, coughs counted for 10mins) -induced cough in a conscious guinea-pig model. (C) In repeat dose studies capsaicin-induced cough was assessed after acute and chronic dosing of XEN-D0501 (1 or 3 mg/kg, i.p., bd, n=8) or vehicle. Data expressed as mean ± S.E.M. Inhibition of cough by the TRPV1 antagonists in vivo was analysed initially utilising a Bartlett's Test in order to determine whether the variances were equal and then either a parametric test (Analysis of Variance followed by a Bonferroni's Multiple Comparison Test-data set in A) or a non-parametric test (Kruskal-Wallis followed by Dunn's Multiple Comparison Test-data sets B & C) to compare responses from the antagonist

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group with those from the vehicle control group. Data presented are means ± S.E.M with statistical significance set at a value of $P < 0.05$.

Figure 4. Capsaicin Evoked Cough Responses.

Individual patient maximal cough responses to capsaicin (E_{max}), at baseline and after 2 weeks of treatment with placebo and XEN-D0501. XEN-D0501 significantly reduced Emax compared with placebo, $p<0.001$.

Figure 5. Hourly Cough Frequency.

Comparison of cough frequency hour by hour on day 14 of treatment with XEN-0501 compared with placebo; data shown are medians.

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XEN-D0501, a novel TRPV1 Antagonist, does not reduce Cough in Refractory Cough Patients

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What is the current scientific knowledge on this subject?

Conclusive data regarding the importance of TRPV1 as a treatment target in chronic cough has been lacking due the absence of a safe, potent and efficacious tool compound for use in clinical studies. In a previous study, a TRPV1 antagonist (SB-705498) failed to improve spontaneous cough frequency in treatment resistant chronic cough patients but the reduction in capsaicin-evoked cough was only small.

What does this study add to the field?

This study rules out TRPV1 as an effective therapeutic target in refractory chronic cough patients. It also highlights the importance of pharmacodynamic pre-clinical and clinical models in the interpretation of negative clinical trial data, but questions the use of cough challenge models in target identification.

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ABSTRACT

RATIONALE: Heightened cough responses to inhaled capsaicin, a TRPV1 agonist, are characteristic of patients with chronic cough. However, previously a TRPV1 antagonist (SB-705498) failed to improve spontaneous cough frequency in these patients despite small reductions in capsaicin-evoked cough.

OBJECTIVES: XEN-D0501 (potent TRPV1 antagonist) was compared with SB-705498 in pre-clinical studies to establish whether an improved efficacy profile would support a further clinical trial of XEN-D0501 in refractory chronic cough.

METHODS: XEN-D0501 and SB-705498 were profiled against capsaicin in a sensory nerve activation assay and *in vivo* potency established against capsaicin-induced cough in the guinea pig.

Twenty patients with refractory chronic cough participated in a double-blind, randomised, placebo-controlled, crossover study evaluating the effect of 14 days XEN-D0501 (oral, 4mg bd) versus placebo on awake cough frequency (primary outcome), capsaicin-evoked cough and patient reported outcomes.

MEASUREMENTS AND MAIN RESULTS: XEN-D0501 was more efficacious and 1000-fold more potent than SB-705498 at inhibiting capsaicin-induced depolarization of guinea pig and human isolated vagus. *In vivo*, XEN-D0501 completely inhibited capsaicin-induced cough whereas 100-times more SB-705498 was required to achieve the same effect. In patients, XEN-D0501 substantially reduced maximal cough responses to capsaicin (mean change from baseline XEN-D0501 -19.3(±16.4) coughs vs. placebo -1.8(±5.8), $p<0.0001$), but not spontaneous awake cough frequency (mean change from baseline XEN-D0501 6.7c/h(±16.9) vs. placebo 0.4c/h(±13.7), $p =0.41$).

CONCLUSIONS: XEN-D0501 demonstrated superior efficacy and potency in pre-clinical and clinical capsaicin challenge studies; despite this improved pharmacodynamic profile, spontaneous cough

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frequency did not improve, ruling out TRPV1 as an effective therapeutic target for refractory cough.

(250 words)

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INTRODUCTION

Chronic cough (>8 weeks duration) is thought to affect approximately 12% of the population(1). Patients presenting with isolated chronic cough (with normal chest radiography and spirometry) frequently report that coughing is associated sensations of throat irritation and an urge-to-cough, which are triggered by trivial exposures to environmental irritant chemicals, temperature changes and use of their voice(2,3). These clinical features are suggestive of a hyper-excitability of the neuronal pathways responsible for controlling cough. Consistent with this hypothesis, heightened cough responses to inhaled irritants, most commonly capsaicin, have been shown to be a characteristic feature in such patients(4-6).

The cough reflex is regulated by vagal afferent nerves which innervate the airway. Some are more mechanically sensitive, and some are more chemosensitive; C fibres and Aδ nociceptors(7). Ion channels present on these vagal nerve termini can be activated by a wide variety of stimuli to elicit cough. The main family of ion channels implicated in the initiation of sensory reflexes are transient receptor potential (TRP) channels(7). Transient receptor potential vanilloid 1 (TRPV1) is a polymodal ion channel and was the first to be identified. It is activated by diverse stimuli including the direct activators capsaicin (constituent of chilli peppers from piquant Capsicum spp. Plants), noxious heat $>42^{o}$C and acidic conditions/protons which interact directly with the channel to cause a lowering of its voltage dependency, leading to opening of the pore domain(8). It can also be activated indirectly by endogenous “disease relevant” mediators including bradykinin and PGE_2(9,10).

Capsaicin reliably activates the cough reflex in several species including humans, and is a specific TRPV1 agonist(10-13); TRPV1 is expressed by vagal afferent C- and Aδ nociceptive fibres innervating the airways(7). Capsaicin is the most frequently used agent for experimentally inducing cough in pre-

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clinical and clinical studies, where the concentration of capsaicin evoking at least five coughs (C5) is the traditional endpoint. Numerous studies have demonstrated lower capsaicin C5 in chronic cough compared with healthy controls (i.e. the cough reflex is more sensitive), suggesting TRPV1 could be an important treatment target(4,5). However, from clinical cough challenges, it cannot be determined whether this lowering of the cough threshold is related to changes in TRPV1 at the sensory nerve terminals or in central pathways.

Only one previous clinical study has evaluated the anti-tussive effects of a single dose of a TRPV1 antagonist (SB-705498) in patients with refractory chronic cough(12). However, disappointingly there was no effect on spontaneous cough frequency although there was a small but statistically significant impact on the capsaicin C5 (increased by approximately one doubling dose over placebo), giving some evidence of target engagement. These data therefore questioned the role of TRPV1 mediated mechanisms in patients with refractory chronic cough, however it remained unclear whether more potent antagonists and/or more prolonged dosing might still be efficacious. XEN-0501 is a potent and safe TRPV1 antagonist. It has demonstrated sub-nanomolar potency for TRPV1 receptors in human and rat recombinant cell systems and rat cultured dorsal root ganglia neurons and its selectivity has been confirmed against panels of enzymes, ion channels and G-protein coupled receptors(14). Thus the aims of this study were firstly to compare a potent TRPV1 antagonist (XEN-D0501) with SB-705498 in pre-clinical studies to establish whether an improved efficacy profile would support a further clinical trial. Secondly, to evaluate the anti-tussive effect of XEN-D0501 in a double blind randomised controlled trial in patients with refractory chronic cough. Some of the results of these studies have been previously reported in the form of abstracts (15).

METHODS

For detailed methods see Online Repository.

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PRE-CLINICAL STUDIES

Animals

In vivo and ex vivo experiments were conducted in male Dunkin-Hartley guinea pigs (300-500g) (Harlan and B&K Universal, UK). The experiments were performed in accordance with the UK Home Office guidelines for animal welfare based on the Animals (Scientific Procedures) act 1986 and the ARRIVE guidelines(16).

Guinea-pig and human isolated vagus nerve recording

Recording of depolarisation (as a measure of vagal, afferent sensory nerve activity) of isolated guinea-pig and human vagus was carried out as described previously(9,17,18). Briefly, nerves were stimulated twice with capsaicin (1μM) for 2min before vehicle or XEN-D0501 (0.1-100nM) or SB-705498 (1-10μM) was perfused for 10min. Response to capsaicin (1μM) was then re-assessed in presence of vehicle or test compounds. After a 10min washout, nerves were finally stimulated again with capsaicin (1μM) to confirm viability.

Human en bloc lungs unsuitable for transplantation were obtained from International Institute for the Advancement of Medicine (New Jersey, USA), and vagal tissue was obtained (N=4, 36-57 years old, 3 male). In all cases tissue was consented for use in scientific research. Ethics approval was obtained from the Royal Brompton & Harefield Trust.

Capsaicin evoked cough in guinea-pigs

Vehicle (0.5% methylcellulose, 0.1% Tween80, 5ml/kg, i.p.) or XEN-D0501 (1-10mg/kg, i.p.) or SB-705498 (10-100mg/kg, i.p.) were administered 1 hour prior to cough recording. Conscious, unrestrained guinea-pigs were placed in individual plastic transparent whole-body plethysmograph chambers (Buxco, Wilmington, NC, USA) and cough assessed to an aerosol of capsaicin (60μM in 1%

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ethanol, 1% Tween 80 in 0.9% sterile saline for 5mins) and coughs counted for 10mins as previously described(9, 18). Studies were also performed to establish if the XEN-D0501 compound retained efficacy after repeat dosing. In these studies guinea-pigs were dosed with vehicle (0.5% methylcellulose, 0.1% Tween80, 5ml/kg, i.p.) or compound (1 or 3mg/kg, bd) for 13 days and then 1 hour prior to cough assessment. Capsaicin-induced cough was then assessed as previously.

CLINICAL STUDY

Study Design

A double-blind, randomised, placebo-controlled, crossover study was performed to evaluate the anti-tussive efficacy of 14 days treatment with XEN-D0501 (oral 4mg bd) compared with matched placebo; the two treatment periods were separated by a 14 day washout, Figure 1A. Participants were recruited from two specialist cough clinics (University Hospital of South Manchester, Manchester and Belfast City Hospital, Belfast) between July 2014 and June 2015. Cough was assessed prior to dosing and at the end of each treatment period using an ambulatory cough monitor and patient reported outcomes. Capsaicin evoked cough responses were included as a pharmacodynamic endpoint.

Participants

We recruited subjects with refractory chronic cough, as determined by national cough guidelines (BTS Thorax 2006(19). An awake cough frequency of >1.5c/h and a maximal capsaicin cough response over 4 inhalations (E_{max}) of >10 coughs were required for inclusion, to avoid patients with very low cough frequency/capsaicin-evoked cough responses, where a treatment effect would be difficult to demonstrate. Current smokers and ex-smokers with a smoking history of >20 pack years were ineligible, as were women of child-bearing potential and those taking treatments that may modulate cough (e.g. morphine, gabapentin). The study was approved by an independent Research Ethics

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Committee (NRES Committee North West - Liverpool East Ref: 14/NW/0211) and all patients gave written informed consent.

Study Treatment, Randomisation and Masking

Patients were assigned to one of the two possible treatment sequences by a computer-generated randomisation schedule with a ratio of 1:1 for XEN-D0501/placebo or placebo/XEN-D0501. Patients, health-care providers, investigators and the sponsor were all masked to the treatment sequence assignment. The dose of XEN-D0501 used in this study (4mg bid) was considered to be the maximum tolerable dose in the older age group of patients typically presenting with chronic cough.

Procedures

Patient demographics, treatments and co-morbidities were collected at screening. Cough was assessed at four time points; for both treatment periods this was prior to dosing and at the end of the treatment period, Figure 1A. Safety was assessed through monitoring of vital signs, ECGs, body temperature, haematology, biochemistry and adverse events.

Outcomes

The primary endpoint was awake objective cough frequency collected using a 24h ambulatory cough monitor commenced at day 0 and day 13 of each treatment period; sleep and 24h cough frequency were also determined. The secondary endpoints included changes from baseline in a 100mm cough severity visual analogue scale (VAS), cough specific quality of life questionnaire, and also a 15-point global rating of change scale for cough severity and frequency, completed at the end of each treatment period. Cough responses to inhaled capsaicin provided a pharmacodynamic endpoint and were assessed on day 1 and 14, following completion of each 24h cough recording.

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Ambulatory Cough Monitoring: Twenty-four hour acoustic recordings were made using the VitaloJAK™ cough monitor (Vitalograph Ltd, Buckinghamshire, UK) with cough sounds per hour quantified by a semi-automated method using validated custom-written software(12,20,21).

Cough Challenges: Four breaths of doubling concentrations of capsaicin (0.97-1000µM, Stockport Pharmaceuticals Ltd, Stockport, UK) were inhaled at one minute intervals from a dosimeter (Koko® dosimeter, De Vilbiss Health Care Inc., Somerset, PA). The number of coughs in the 15 seconds following each inhalation was counted The challenge continued until the maximum tolerated dose or the final concentration was inhaled(6, 22). The maximum number of coughs evoked at any concentration (E_{max}) was the main endpoint, but the concentration evoking 50% of the maximal response (ED_{50}) was also reported. To allow comparison with previous studies the concentration of capsaicin evoking two (C2) and five (C5) endpoints were also extracted from the cough challenge data for analysis.

Patient Reported Cough Measures: Subjects rated cough severity and urge-to-cough on separate 100mm VAS scales for day and night times. Cough specific quality of life was assessed by the Leicester Cough Questionnaire (LCQ); higher scores indicated better quality of life(23).

DATA ANALYSIS

Vagal depolarisation: Inhibition of depolarisation was analysed utilising a paired t-test, given observations were made using the tissue as its own control. Data presented are means ± S.E.M with statistical significance set at a value of $p<0.05$.

Cough: Inhibition of cough by the TRPV1 antagonists *in vivo* was analysed initially utilising a Bartlett's Test in order to determine whether the variances were equal and then either a parametric test (Analysis of Variance followed by a Bonferroni's Multiple Comparison Test) or a non-parametric test

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(Kruskal-Wallis followed by Dunn's Multiple Comparison Test) to compare responses from the antagonist group with those from the vehicle control group. Data presented are means ± S.E.M with statistical significance set at a value of $p<0.05$.

Clinical Study: Sample size was estimated from previous data, (awake cough frequency ~25c/h, $\log_{10}$ standard deviation 0.23-0.41). Based on these assumptions, 16 patients would be needed to detect a 56% reduction in awake cough frequency (90% power, 5% significance level). The change from baseline in the awake objective cough frequency was analysed with a mixed effects linear model, with baseline and treatment period as fixed effects and patient as a random effect. Awake cough frequency was log-transformed for analysis and the standard $p<0.05$ level of significance applied. Similar models were applied to the secondary endpoints, with or without log-transformation as appropriate.

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RESULTS

PRE-CLINICAL STUDIES

Guinea-pig and Human Vagus nerve depolarisation

XEN-D0501 (0.1-100nM) inhibited capsaicin-induced depolarisation of the guinea pig vagus in the nanomolar range in a concentration dependent manner with a maximal inhibition of 87.2 ± 4.4% at 10nM (Figure 2A). The maximally effective concentration of XEN-D0501 (10nM) had no effect on responses to the TRPA1 agonist, acrolein (300μM) indicating no off target activity on another TRP channel expressed on C-fibre afferents, which when activated is also known to evoke cough in guinea-pigs and humans(24). SB-705498 (1-10μM) also evoked a concentration dependent inhibition of capsaicin-induced depolarisation of guinea pig vagus that was in the micromolar range with a maximal inhibition of 52.4 ± 5.5% at 10μM (Figure 2B). Higher concentrations could not be formulated for *in*

vitro experiments as solubility was compromised. Similarly, XEN-D0501 (0.1-100nM) also inhibited capsaicin-induced depolarisation of the human vagus in the nanomolar range in a concentration dependent manner with 80.54 ± 10.4% at 100nM (Figure 2C). Furthermore, consistent with data generated in the guinea-pig, SB-705498 inhibited depolarisation in the micromolar range.

Capsaicin Evoked Cough in a Conscious Guinea Pig Model

XEN-D0501 significantly inhibited capsaicin-induced cough at all doses tested (Vehicle: 8.63 ± 2.3 versus XEN-D0501 (1mg/kg): 2.13 ±0.95 coughs/10min, n=8) (Figure 3A). SB-705498 only significantly inhibited cough at the highest dose tested (Vehicle: 6.71 ± 1.9 versus XEN-D0501 (100mg/kg): 0.29 ± 0.29 coughs/10min, n=7) (Figure 3B). XEN-D0501 retained its efficacy following 13 days dosing bd, inhibiting capsaicin evoked cough to the same extent as in guinea pigs only receiving a single dose 1 hour prior to the cough challenge (Figure 3C).

CLINICAL STUDY

Subjects

Twenty patients with refractory chronic cough (mean age 63.1yrs, 15 female) were recruited, see Table 1 and Figure 1B. Patients were typical of those attending specialist cough clinics and those randomised to each treatment sequence were well matched. Two patients discontinued study treatment and withdrew from the study. One patient was withdrawn during XEN-D0501 treatment due to a treatment-emergent adverse event of fatigue, considered related to study treatment. A further patient was withdrawn during placebo dosing as they did not meet all the inclusion criteria and were included in error. A tablet count suggested that compliance with study treatment was high and almost identical for XEN-D0501 and placebo. Over the two week treatment periods, this

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suggested subjects consumed a mean of 26.7 XEN-D0501 tablets (95%CI 26.5-27.5) and 27.0 (25.9-27.5) placebo tablets (p=0.56).

Cough Frequency and Capsaicin Evoked Cough Responses

Consistent with the pre-clinical data, XEN-D0501 dramatically reduced maximal cough responses to capsaicin [E_{max} mean difference XEN-D0501 -19.3 coughs (±16.4) vs. placebo -1.8 coughs (±5.8), p<0.0001], Figure 4. The logC2 and logC5 endpoints also significantly improved from baseline with XEN-D0501; increases in logC2 of +1.10µM (SEM ±0.34), p=0.005 and logC5 of +1.22µM (±0.40), p=0.007 over placebo, equivalent to increases of +3.7 and +4.1 doubling doses of capsaicin respectively. However, there was no difference in the change from baseline in spontaneous daytime cough frequency for treatment with XEN-D0501 compared with placebo [awake cough frequency (mean change XEN-D0501 6.7c/h (±16.9) vs. placebo 0.4c/h (±13.7), p=0.41), Table 2. Similarly there was no significant change in 24h or sleep cough frequency; hourly cough counts on day 14 of treatment with placebo and XEN-D0501 are shown in Figure 5. Treatment sequence and period had no significant effect in any of these analyses.

Patient Reported Outcomes

Overall, the patient reported endpoints did not suggest patients perceived significant improvements in their cough. The only exception was awake cough severity VAS which improved with placebo treatment compared to XEN-D0501, although the magnitude of the difference was very small indeed [mean difference 2.8mm (SD ±21.4), p=0.03]. Again, treatment sequence and period had no significant effect in any of the analyses.

Safety and Adverse Events

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No serious adverse events (SAEs) were reported during the study. In total, 103 treatment emergent adverse events (TEAEs) were reported by 18 (94.7%) patients receiving XEN-D0501; 89 events were considered to be treatment-related. Fourteen AEs were reported by 9 (47.4%) patients receiving placebo, of which 5 events were considered treatment-related. The most frequently reported AEs were related to temperature sensations or mouth related events and were consistent with those seen in previous studies(14). The most common AEs, reported by ≥3 patients taking XEN-D0501 were: thermohypoaesthesia (n=8), dysgeusia (n=9), and feeling of body temperature change (n=6), feeling hot (n=3), oral paraesthesia (n=3), and hyperhidrosis (n=3). Headache was also a frequently reported TEAE (n=3 taking XEN-D0501 and n=1 taking placebo). No concerns were raised by other measures of safety i.e. ECGs, vital signs including body temperature, physical examination findings, and routine haematology and coagulation, clinical chemistry, and urinalysis assessments.

DISCUSSION

Cough is the most common condition for which patients seek medical attention but currently there are no licensed treatments for refractory chronic cough and evidence is lacking to suggest over-the-counter (OTC) cough medications are effective(25). The cough reflex is regulated by vagal afferent nerves which innervate the airway, some are more mechanically sensitive; the Rapidly Adapting Receptors (RARs), Slowly Adapting Receptors (SARs), and the subtype known as the 'cough' receptor, and some are more chemosensitive; C fibres and Aδ nociceptors(7). Ion channels present on these vagal nerve termini can be activated by a wide variety of stimuli to elicit cough and other reflexes. The main family of ion channels implicated in the initiation of sensory reflexes are TRP channels(7).

A significant amount of information has been gathered regarding the biology of TRPV1 from clinical, capsaicin challenge studies. Interestingly, the threshold for provoking cough by capsaicin (C2 or the C5

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concentration of capsaicin required to elicit at least 2 or 5 coughs, respectively) has been found to be lowered in various populations of patients with Chronic Obstructive Pulmonary(4,26), asthma(22,26), Idiopathic Pulmonary Fibrosis(27) and also refractory chronic cough patients(4,5,28) compared to healthy controls. These data imply that TRPV1 function is frequently increased in a disease setting. However, it is still not certain if increased sensitivity to various challenge agents acting on particular ion channels informs us regarding whether a particular intervention will be useful in a certain disease setting. In one of the studies above, different patient groups (COPD, healthy smokers, refractory chronic cough, and asthma) were compared and exhibited different patterns of modulation of cough responses to a range of inhaled irritants. This data supports the concept of disease-specific neurophenotypes in airway disease suggesting that chronic cough associated with different aetiologies may not be sensitive to similar interventions(4).

Of all the TRP channels, antagonists for TRPV1 are the most advanced with regard to drug development and clinical trials. However, development of these inhibitors has not been straightforward due to the activity of TRPV1 as a thermosensor for hot temperatures. This has seen several TRPV1 antagonists fail to progress due to adverse effects such as increased body temperature and latent withdrawal to noxious hot stimuli. For instance AMG 517 caused significant and long lasting increases in body temperature in phase 1 clinical trials in patients following molar extractions. In this case the trial was terminated before any efficacy, in relation to possible analgesic effects, could be determined(29). Other compounds, also trialled in dental pain studies exhibited similar adverse events with increases in body temperature and altered noxious heat sensation. In all cases these adverse effects were correlated with target engagement. More recently compounds have been identified that are not limited because of adverse events.

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SB-705498 has been reported to be a potent, selective, orally bioavailable competitive TRPV1 receptor antagonist which does not exhibit significant disturbances in temperature perception or control(30). The availability of safe, "clinical ready" TRPV1 antagonists has already allowed the profiling of SB-705498 in a double blind randomised controlled trial in patients with refractory chronic cough(12). This trial utilised objective cough monitoring and capsaicin challenge as a pharmacodynamic marker of target engagement. In this study, a single dose of SB-705498 was reported to be well tolerated with no significant increase in tympanic temperature or reported temperature perception. However, disappointingly SB-705498 lacked efficacy in improving spontaneous cough frequency in refractory chronic cough patients, although there was a small but statistically significant shift in capsaicin-evoked cough. Pharmacodynamic modelling estimated SB-705498 receptor occupancy to be 45% at 2 hours (associated with more than a doubling dose shift in C5 values over placebo) and 25% at 24 hours (associated with a 0.7 doubling dose shift in C5 values over placebo). Therefore, it was not clear whether the lack of efficacy in reducing spontaneous cough was due to the modest receptor occupancy and whether a compound with increased potency and efficacy at reducing cough to capsaicin would be required. Furthermore, the long half-life of SB-705498 (50-60hrs) limited previous clinical studies of this drug to investigating the efficacy of single doses, and therefore the potential effects of more prolonged TRPV1 blockade could not be determined.

In these experiments we utilised a potent and selective TRPV1 receptor antagonist, XEN-D0501, which has been shown to demonstrate sub-nanomolar potency in human recombinant cellular systems and rat dorsal root ganglia neuron preparations. In a phase 1 safety study, XEN-D0501 was well tolerated with repeat dosing with no serious adverse events and a slight increase in core body temperature which attenuated over time and was not considered to be of clinical concern(14). In this study we

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demonstrate superior efficacy and potency of the TRPV1 antagonist, XEN-D0501, over SB-705498 in a range of pre-clinical *in vitro* and *in vivo* studies. XEN-D0501 (0.1-10nm) was more efficacious and 1000-fold more potent than SB705498 (1-10μM) at inhibiting capsaicin-induced depolarisation of guinea pig and human isolated vagus. The *in vitro* potency of SB-705498 in this study was slightly less than reported in a FLIPR assay on the guinea-pig and human recombinant TRPV1 receptor expressed in HEK293 cells (apparent pKi values of 7.3±0.1 7.6±0.2, respectively). However, one might expect a 10-fold or more drop off in potency when one looks at the activity against the native ion channel in a tissue based assay. In vivo, XEN-D0501 (1mg/kg) completely inhibited capsaicin-induced cough whereas 100 times more SB705498 (100mg/kg) was required to achieve the same effect which was somewhat consistent with previous data demonstrating some inhibition with SB-705498 (at 10, 30mg/kg) in a capsaicin induced secondary hyperalgesia model in the rat(31).

In this study, target engagement was demonstrated in pre-clinical and clinical studies with XEN-D0501 blocking the cough to capsaicin observed in both models; highlighting the translational capacity of the guinea-pig model. Rather than the traditional clinical C5 endpoint which is somewhat arbitrary, we utilised novel cough challenge methodology which captures the dose-response relationship between capsaicin and cough, using the pharmacodynamic endpoints E_{max} and ED_{50}(6). We have recently shown that E_{max} better discriminates chronic cough patients from healthy controls and mild asthma patients, and it is more readily compared to pre-clinical cough studies. E_{max} exhibited substantial improvements in this study. However, this did not translate into efficacy for XEN-D0501 on spontaneous coughing in chronic cough patients.

This study has some limitations; as a proof of concept study, the sample size was small. Nonetheless significant changes in capsaicin cough responses were still clearly demonstrated. Furthermore, both our power calculations and previous studies demonstrate that objective cough monitoring is a

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powerful tool for demonstrating efficacy, and large studies are not required(21). It should also be acknowledged that whilst no serious adverse events or changes in body temperature occurred in this study, TRPV1 antagonism was still associated with frequent non-severe adverse effects, mainly relating to temperature sensing, perceived body temperature and alterations in taste.

In summary, that potent TRPV1 antagonism, producing considerable reductions in capsaicin evoked cough, was not associated with any improvements in objective cough frequency suggests that TRPV1 is not an effective target in refractory chronic cough. This is in stark contrast to recent demonstrations of the efficacy of P2X3 antagonism, implicating this ion channel and its endogenous ligand, ATP, in the mechanisms under pinning chronic cough (20). The current study may also provide an indication that increased sensitivity to one mechanism (e.g. TRPV1), demonstrated by challenge studies (e.g. utilising capsaicin) in one patient group (refractory chronic cough), does not necessarily implicate the utility of said target in a particular patient group. However, this may not be the case for TRPV1 in other conditions that exhibit chronic cough or with other TRP channels involved in chronic cough. What this study does illustrate is that challenge paradigms across pre-clinical and clinical models can deliver effective pharmacodynamic models which can inform dosing regimens in future clinical trials across any therapeutic area.

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Limited who also acted as sponsor. Employees and contractors of the company were involved in the design, medical monitoring, trial oversight, trial monitoring, data management, analysis and reporting of the study.

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TABLES

Table 1. Characteristics of randomised participants. Data are mean and standard deviations.

		Treatment Sequence		Overall
		XEN-D0501/ Placebo (n=11)	**Placebo/ XEN-D0501** (n=9)	
Age	(years)	64.9 (7.8)	60.8 (11.0)	63.1 (9.4)
Sex	Male (n, %)	3 (27.3%)	2 (22.2%)	5 (25.0%)
	Female (n, %)	8 (72.7%)	7 (77.8%)	15 (75.0%)
Race (n, %)	White	11 (100.0%)	9 (100.0%)	20 (100.0%)
Height (cm)		161.9 (6.8)	164.1 (8.4)	162.9 (7.4)
Weight (kg)		77.0 (15.5)	71.7 (17.9)	74.6 (16.4)
BMI (kg/m2)		29.3 (4.9)	26.3 (4.8)	28.0 (5.0)
FEV1 % predicted		105.5% (13.1)	104.8% (15.6)	105.2% (13.9)
Former smoker		3 (27.3)	1 (11.1)	4 (20.0)
Never smoked		8 (72.7)	8 (88.9)	16 (80.0)
Current smoker		0	0	0
Pack years (ex-smokers)		4.7 (4.0)	4.0	4.5 (3.3)

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Table 2. Measures of Cough at baseline and after 2 weeks treatment with XEN-0501 and matched placebo. Data are means and standard deviations unless otherwise stated.

		XEN-D0501 (n=18)		**Placebo** (n=19)		**p**
		Baseline	Day 14	Baseline	Day 14	
Objective Cough Frequency	Awake	34.4c/h (±41.9)	41.1c/h (±52.7)	40.9c/h (±63.2)	41.3c/h (±58.3)	0.42
	24h	24.2c/h (±29.0)	29.5c/h (±36.8)	29.2c/h (±46.0)	30.5c/h (±44.0)	0.43
	Night *median (IQR)*	0.7c/h (0.3-2.3)	1.7c/h (0.8-4.5)	2.3c /h (0.9-3.3)	1.4c/h (0.7-4.4)	0.29
Cough Severity VAS	Day	40.6mm (±26.6mm)	39.7mm (±32.5mm)	34.3mm (±23.0mm)	31.5mm (±27.8mm)	**0.03**
	Night	21.9mm (±25.8mm)	16.4mm (±22.3mm)	20.2mm (±17.5mm)	14.7mm (±18.2mm)	0.12
Urge to Cough VAS	Day	42.9mm (±26.2mm)	34.6mm (±30.9mm)	31.1mm (±21.5mm)	27.2mm (±25.3mm)	0.25
	Night	22.2mm (±23.8mm)	16.3mm (±24.3mm)	18.4mm (±17.0mm)	14.6mm (±22.5mm)	0.58
Cough Quality of Life (LCQ)		13.05 (4.09)	13.80 (4.03)	13.70 (4.53)	14.43 (4.43)	0.82
Capsaicin Challenge	E_{max}	32.3 coughs (±17.1)	13.1 coughs (±11.3)	27.7 coughs (±15.2)	25.9 coughs (±12.3)	**<0.001**

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FIGURE LEGENDS:

Figure 1. Clinical Study Design and Consort Diagram.

(A) Summary of the double blind randomised crossover design of the clinical study including timing of efficacy and pharmacodynamics endpoints. (B) Patient recruitment and flow; one patient withdrew* after 5 days treatment with XEN-0501 due to intolerance to the study drug and a second patient was withdrawn† during placebo dosing as they were included in the study in error.

Figure 2. Inhibition of Vagal Sensory Nerve activation.

The effect of vehicle (0.1% DMSO v/v) or (A) XEN-D0501 (0.1-100nM.) or (B) SB705498 (1-10 μM) on depolarization of the guinea-pig vagus induced by capsaicin (1μM). A similar effect was produced in human vagus (C); typical example traces (D). Data expressed as mean ± S.E.M, n=4 and n=3 observations per treatment group in guinea-pig and human studies, respectively.

Figure 3. Inhibition of Capsaicin-induced Cough in a Conscious Guinea-pig model

The effect of vehicle (0.5% methylcellulose, 0.1% Tween80, 5ml/kg, i.p.) or (A) XEN-D0501 (1, 3 or 10 mg/kg, i.p., n=8) or (B) SB705498 (10, 30 or 100 mg/kg, i.p., n=7) administered 1 hour prior to cough recording on capsaicin (60 μM in 1% ethanol, 1% Tween 80 in 0.9% sterile saline for 5 mins, coughs counted for 10mins) -induced cough in a conscious guinea-pig model. (C) In repeat dose studies capsaicin-induced cough was assessed after acute and chronic dosing of XEN-D0501 (1 or 3 mg/kg, i.p., bd, n=8) or vehicle. Data expressed as mean ± S.E.M. Inhibition of cough by the TRPV1 antagonists in vivo was analysed initially utilising a Bartlett's Test in order to determine whether the variances were equal and then either a parametric test (Analysis of Variance followed by a Bonferroni's Multiple Comparison Test-data set in A) or a non-parametric test (Kruskal-Wallis followed by Dunn's Multiple Comparison Test-data sets B & C) to compare responses from the antagonist

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group with those from the vehicle control group. Data presented are means ± S.E.M with statistical significance set at a value of $P < 0.05$.

Figure 4. Capsaicin Evoked Cough Responses.

Individual patient maximal cough responses to capsaicin (E_{max}), at baseline and after 2 weeks of treatment with placebo and XEN-D0501. XEN-D0501 significantly reduced Emax compared with placebo, $p<0.001$.

Figure 5. Hourly Cough Frequency.

Comparison of cough frequency hour by hour on day 14 of treatment with XEN-0501 compared with placebo; data shown are medians.

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ONLINE REPOSITORY

XEN-D0501, a novel TRPV1 Antagonist, does not reduce Cough in Refractory Cough Patients

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SUPPLEMENTARY METHODS

PRE-CLINICAL STUDIES

Animals

In vivo and ex vivo experiments were conducted in male Dunkin-Hartley guinea pigs (300-500g) (Harlan and B&K Universal, UK) housed in a temperature controlled (21°C) room with food and water freely available for at least one week before commencing the experimentation. The experiments were performed in accordance with the UK Home Office guidelines for animal welfare based on the Animals (Scientific Procedures) act 1986 and the ARRIVE guidelines[15].

Guinea-pig and human isolated vagus nerve recording

Recording of depolarisation (as a measure of vagal, afferent sensory nerve activity) of isolated guinea-pig and human vagus was carried out as described previously[9,16,17]. Briefly, nerves were stimulated twice with capsaicin (1μM) for 2min before vehicle or XEN-D0501 (0.1-100nM) or SB-705498 (1-10μM) was perfused for 10min. Response to capsaicin (1μM) was then re-assessed in presence of vehicle or test compounds. After a 10min washout, nerves were finally stimulated again with capsaicin (1μM) to confirm viability. One concentration of the antagonist was tested per vagus preparation.

Human en bloc lungs unsuitable for transplantation were obtained from International Institute for the Advancement of Medicine (New Jersey, USA), and vagal tissue was obtained (N = 4, 36-57 years old, 3 male). In all cases tissue was consented for use in scientific research. Ethics approval was obtained from the Royal Brompton & Harefield Trust.

Capsaicin evoked cough in guinea-pigs

Vehicle (0·5% methylcellulose, 0·1% Tween80, 5ml/kg, i.p.) or XEN-D0501 (1-10mg/kg, i.p.) or SB-705498 (10-100mg/kg, i.p.) were administered 1 hour prior to cough recording. In all experiments the operator was blinded to the treatment groups and the dosing was performed by an

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independent operator. Conscious, unrestrained guinea-pigs were placed in individual plastic transparent whole-body plethysmograph chambers (Buxco, Wilmington, NC, USA) and cough assessed to an aerosol of capsaicin (60μM in 1% ethanol, 1% Tween 80 in 0.9% sterile saline for 5mins) and coughs counted for 10mins as previously described[9,17]. Studies were also performed to establish if the XEN-D0501 compound retained efficacy after repeat dosing. In these studies guinea-pigs were dosed with vehicle (0·5% methylcellulose, 0·1% Tween80, 5ml/kg, i.p.) or compound (1 or 3mg/kg, bd) for 13 days and then 1 hour prior to cough assessment. Capsaicin-induced cough was then assessed as previously.

CLINICAL STUDY

Study Design

A double-blind, randomised, placebo-controlled, crossover study was performed to evaluate the anti-tussive efficacy of 14 days treatment with XEN-D0501 (oral 4mg bd) compared with matched placebo; the two treatment periods were separated by a 14 day washout, see Figure 1A. Participants were recruited from two specialist cough clinics (University Hospital of South Manchester, Manchester and Belfast City Hospital, Belfast) between July 2014 and June 2015. Cough was assessed prior to dosing and at the end of each treatment period using an ambulatory cough monitor and patient reported outcomes included cough severity rated on a visual analogue scale (VAS), cough specific quality of life and a global rating of change scale. Capsaicin evoked cough responses were included as a pharmacodynamic endpoint.

Participants

We recruited subjects with refractory chronic cough, as determined by national cough guidelines (BTS Thorax 2006[18]). An awake cough frequency of >1·5c/h and a maximal capsaicin cough response over 4 inhalations (E_{max}) of >10 coughs were required for inclusion, to remove patients with very low cough frequency and capsaicin evoked cough responses, where a treatment effect

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would be difficult to demonstrate. The study was approved by an independent Research Ethics Committee (NRES Committee North West - Liverpool East Ref: 14/NW/0211) and all patients gave written informed consent.

Inclusion Criteria

To participate in this study, patients were required to meet all of the following criteria:

1. Male and female patients aged 18 years or over with chronic idiopathic cough, defined as:

- Attending a specialist cough clinic with a history of cough for more than 8 weeks
- Idiopathic cough (defined as a cough for which no objective evidence of an underlying trigger could be determined after investigation) or treatment-resistant cough (defined as a cough that was unresponsive to 8 weeks of targeted treatment for identified underlying triggers including reflux disease, asthma and post-nasal drip).

2. Normal (no clinically relevant findings in the opinion of the investigator) chest radiography ie, chest X-ray or CT thorax, prior to the study (within 12 months of Visit 1).

3. Normal spirometry (ie, forced expiratory volume in 1 second [FEV1] >80% predicted).

4. Day time cough frequency >1.5 coughs/hour (from 24-hour cough monitor at Visit 2).

5. Emax (maximum number of coughs following capsaicin challenge) from capsaicin challenge >10 coughs (from challenge cough monitor at Visit 2).

6. Women must be of non-child bearing potential:

- Non-child bearing potential was defined as amenorrhoeic for at least 1 year AND, if aged under 60 years, have FSH level of at least 30 IU/L or have undergone a hysterectomy or bilateral oophorectomy (tubal ligation was not acceptable). Women who were taking hormone replacement therapy (HRT) did not have to have FSH assessments, but the amenorrhea (before starting HRT) must have been naturally (spontaneously) occurring and

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have been accompanied by an appropriate clinical profile (eg, age appropriate, history of vasomotor symptoms)

- If female partners of male patients were of childbearing potential, the patient must have been willing to use contraception (eg, condoms plus spermicide) AND their female partner must also have been using contraception (eg, hormonal or intra-uterine device). This double contraception must have been used from the first dose of study drug until at least 90 days after the last dose of study drug.

7. Written informed consent.

Exclusion Criteria

Patients meeting any of the following were excluded from participation in the study. Where criteria were time-dependent, patients may have been re-screened to enable inclusion:

1. Body Mass Index (BMI) >35 kg/m2.

2. Current smokers or patients with urine cotinine ≥500 ng/mL.

3. Ex-smoker of <6 months.

4. Cumulative smoking history of ≥10 pack years.

5. Upper respiratory tract infection within the last 6 weeks prior to randomisation (Visit 3).

6. Feverish illness within the last 1 week prior to randomisation (Visit 3).

7. Concomitant medications which may have influenced cough or interact with study drugs eg, opioids, gabapentin, pregabalin, amitriptyline, ACE inhibitors, potent CYP3A4 inhibitors (such as ketoconazole, clarithromycin or human immunodeficiency virus [HIV] protease inhibitors) and systemic corticosteroids (use of local injectable, topical or inhaled corticosteroids was permitted).

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8. Patients with concomitant conditions which may have influenced the cough reflex or the patient's ability to participate in the study eg, diabetes, cerebrovascular disease, Parkinson's disease.

9. History of drug or alcohol dependency or abuse within approximately the last year prior to screening (Visit 1).

10. Regular alcohol consumption; >21 units/week for males, or >14 units/week for females, during the study (1 unit = ½ pint beer, 25 mL of 40% spirit or a 125 mL glass of wine).

11. Any known allergy to the study drugs.

12. Pregnant and/or lactating women.

13. History or evidence of urinary retention, bladder outlet obstruction or benign prostatic hypertrophy.

14. Any clinically significant abnormalities in haematology or clinical biochemistry tests prior to randomisation (Visit 3).

15. Serum alanine transaminase (ALT), aspartate transaminase (AST) or gamma-glutamyl transpeptidase (GGT) greater than twice the upper limit of normal (ULN) at Visit 1.

16. Total serum bilirubin >1.5x ULN at Visits 1.

17. History of any kind of cancer within the last 5 years unless non-invasive, in remission and approved in writing by Sponsor.

18. Evidence of any other clinically significant disease or condition which in the opinion of the investigator would have precluded the patient's participation in this study.

19. QT interval corrected for heart rate using Fredericia's formula (QTcF) value at Visit 1 (mean) of >450 msec (males) or >470 msec (females).

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20. Patients with uncontrolled hypertension, systolic blood pressure >160 mmHg or diastolic blood pressure >90 mmHg at Visit 1 (mean) or Visit 2.

21. Received investigational or marketed products as part of any other clinical study within 30 days (or 5 half-lives whichever was longer) prior to screening (Visit 1).

22. Patient was unable or unwilling to co-operate with the study procedures.

Randomisation and Masking

Patients were assigned to one of the two possible treatment sequences by a computer-generated randomisation schedule with a ratio of 1:1 for XEN-D0501/placebo or placebo/XEN-D0501. Blinded study drug supplies were provided to each site in sequentially numbered identical bottles in accordance with the randomisation schedule, and dispensed by the local hospital pharmacies. Patients, health-care providers, investigators and the sponsor were all masked to the treatment sequence assignment. A sealed code-break envelope for each patient containing details of the treatment allocated was kept in a locked safe at the study site.

Procedures

All subjects were assessed at four time points; for both treatment periods this was prior to dosing (24hr cough recording day 0, followed by capsaicin challenge day 1) and at the end of the treatment period (24hr cough recording day 13, followed by capsaicin challenge on day 14 after the final dose of medication), see Figure 1A. Patient demographics, treatments and co-morbidities were collected at screening. Randomised subjects underwent two treatment periods of 14 days duration, separated by a 14 day washout; a final follow-up evaluation was performed at 7-14 days after the final dose. Safety was assessed through monitoring of vital signs, ECGs, body temperature, haematology, biochemistry and adverse events. Concomitant medications were monitored throughout the study.

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Outcomes

The primary endpoint was awake objective cough frequency collected using a 24h ambulatory cough monitor commenced at day 0 and day 13 of each treatment period; sleep and 24h cough frequency were also determined. The secondary endpoints included changes from baseline in a 100mm cough severity (VAS), cough specific quality of life assessed by the Leicester Cough Questionnaire (LCQ) and also a 15-point global rating of change scales for both cough severity and cough frequency (seven ratings for improvement, seven ratings for worsening and one rating for unchanged), completed at the end of each treatment period. Cough responses to inhaled capsaicin provided a pharmacodynamic endpoint and were assessed on day 1 and 14, following completion of each 24h cough recording.

Ambulatory Cough Monitoring: Twenty-four hour acoustic recordings were made using the VitaloJAK™ cough monitor (Vitalograph Ltd, Buckinghamshire, UK) and the number of cough sounds per hour quantified by a semi-automated method using validated custom-written software[12,19,20].

Cough Challenges: Four breaths of doubling concentrations of capsaicin (0·97-1000µM, Stockport Pharmaceuticals Ltd, Stockport, UK) were inhaled at one minute intervals from a nebuliser pot (De Vilbiss Health Care Inc., Somerset, PA) with a built in flow regulator valve and fixed baffle assembly, controlled by a dosimeter (Koko® dosimeter, De Vilbiss Health Care Inc., Somerset, PA). The number of coughs in the 15 seconds following each inhalation was counted and recorded by the VitaloJAK. The challenge continued until the maximum tolerated dose or the final concentration was inhaled[6,21]. The maximum number of coughs evoked at any concentration (E_{max}) was the main endpoint, but the concentration evoking 50% of the maximal response (ED_{50}) was also reported.

To explore how these novel endpoints compare with traditional cough challenge endpoints, we also calculated the concentration of capsaicin evoking atleast 2 and 5 coughs, i.e. the C2 and C5.

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Our challenge protocol is slightly different from the traditional challenge as four inhalations are performed at each concentration, rather than just one. Therefore, to calculate the C2 and C5 we simply used the number coughs evoked by the first of these four inhalations. If subjects did not cough 2 or 5 times during the whole challenge then for the purposes of analysis a value of 2000µmol/L was assigned. If patients coughed 2 or 5 times on inhalation of placebo then the data was considered missing.

Patient Reported Cough Measures: Subjects rated cough severity and urge-to-cough on separate 100mm VAS scales for day and night times. Cough specific quality of life was assessed by the LCQ; higher scores indicated better quality of life[22].

DATA ANALYSIS

Vagal depolarisation: Inhibition of depolarisation was analysed utilising a paired t-test, given observations were made using the tissue as its own control. Data presented are means ± S.E.M with statistical significance set at a value of $p<0{\cdot}05$.

Cough: Inhibition of cough by the TRPV1 antagonists *in vivo* was analysed initially utilising a Bartlett's Test in order to determine whether the variances were equal and then either a parametric test (Analysis of Variance followed by a Bonferroni's Multiple Comparison Test) or a non-parametric test (Kruskal-Wallis followed by Dunn's Multiple Comparison Test) to compare responses from the antagonist group with those from the vehicle control group. Data presented are means ± S.E.M with statistical significance set at a value of $p<0{\cdot}05$.

Clinical Study: Sample size was estimated from previous data, where awake cough frequency in chronic cough was ~25c/h, with a $\log_{10}$ standard deviation of 0·23 to 0·41. Based on these assumptions, to detect a 56% reduction in awake cough frequency, 16 patients would be needed to achieve 90% power with a 5% significance level. The change from baseline in the awake objective cough frequency was analysed with a mixed effects linear model, with baseline and

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treatment period as fixed effects and patient as a random effect. The awake cough frequency was log-transformed for analysis and the standard $p<0{\cdot}05$ level of significance applied. Similar models were applied to the secondary endpoints, with or without log-transformation as appropriate.

Role of the Funding Source

This study was funded by Ario Pharma Ltd, and conducted by Xention Ltd who also acted as the study sponsor. Employees and contractors of the company were involved in the design, medical monitoring, trial oversight, trial monitoring, data management, analysis and reporting of the study.

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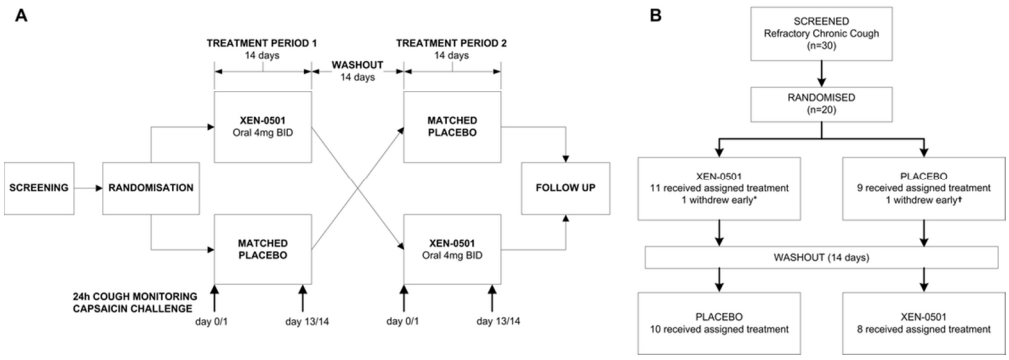


Figure 1. Clinical Study Design and Consort Diagram. (A) Summary of the double blind randomised crossover design of the clinical study including timing of efficacy and pharmacodynamics endpoints. (B) Patient recruitment and flow; one patient withdrew* after 5 days treatment with XEN-0501 due to intolerance to the study drug and a second patient was withdrawn† during placebo dosing as they were included in the study in error.

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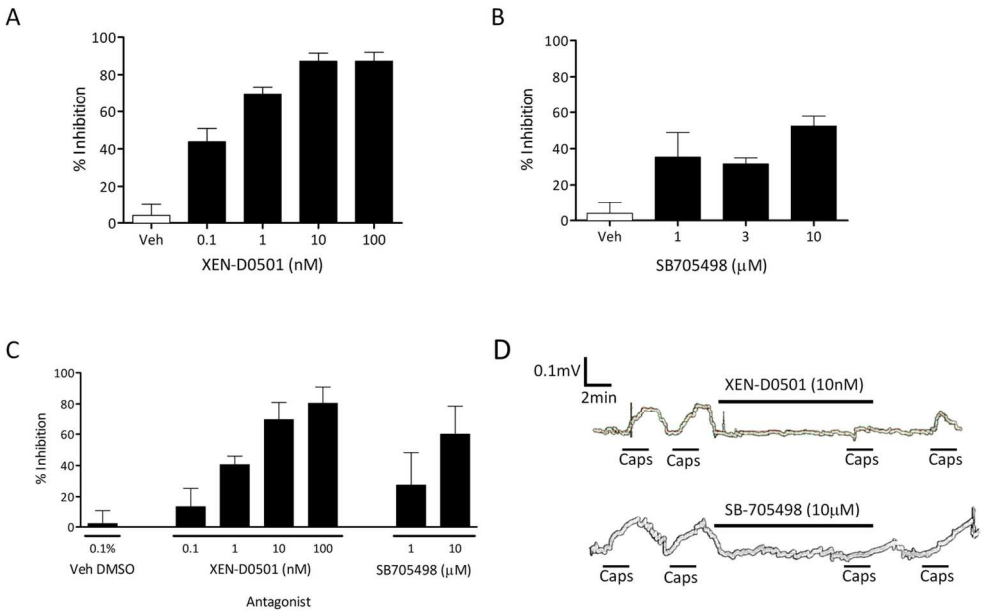


Figure 2. The effect of vehicle (0.1% DMSO v/v) or (A) XEN-D0501 (0.1-100nM.) or (B) SB705498 (1-10μM) on depolarization of the guinea-pig vagus induced by capsaicin (1μM). A similar effect was produced in human vagus (C); typical example traces (D). Data expressed as mean +/-S.E.M, n=4 and n=3 observations per treatment group in guinea-pig and human studies, respectively.

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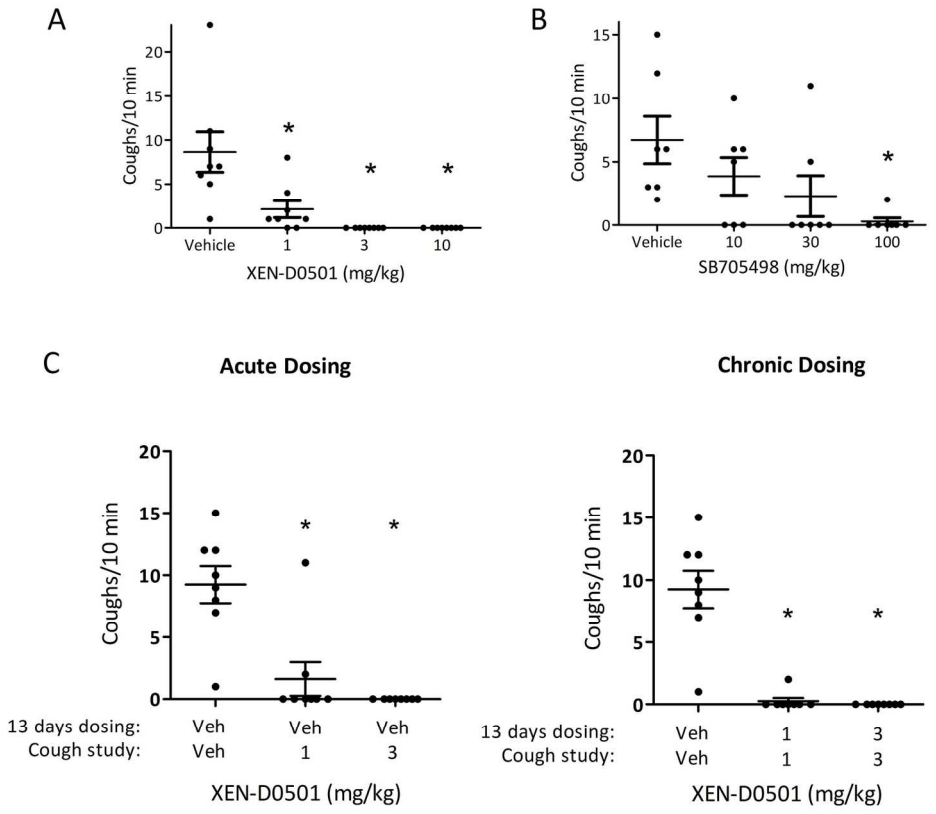


Figure 3. Inhibition of Capsaicin-induced Cough in a Conscious Guinea-pig model. The effect of vehicle (0.5% methylcellulose, 0.1% Tween80, 5ml/kg, i.p.) or (A) XEN-D0501 (1, 3 or 10 mg/kg, i.p., n=8) or (B) SB705498 (10, 30 or 100 mg/kg, i.p., n=7) administered 1 hour prior to cough recording on capsaicin (60µM in 1% ethanol, 1% Tween 80 in 0.9% sterile saline for 5 mins, coughs counted for 10mins) -induced cough in a conscious guinea-pig model. (C) In repeat dose studies capsaicin-induced cough was assessed after acute and chronic dosing of XEN-D0501 (1 or 3 mg/kg, i.p., bd, n=8) or vehicle. Data expressed as mean +/-S.E.M. Inhibition of cough by the TRPV1 antagonists in vivo was analysed initially utilising a Bartlett's Test in order to determine whether the variances were equal and then either a parametric test (Analysis of Variance followed by a Bonferroni's Multiple Comparison Test-data set in A) or a non-parametric test (Kruskal-Wallis followed by Dunn's Multiple Comparison Test-data sets B & C) to compare responses from the antagonist group with those from the vehicle control group. Data presented are means +/-S.E.M with statistical significance set at a value of $p<0.05$.

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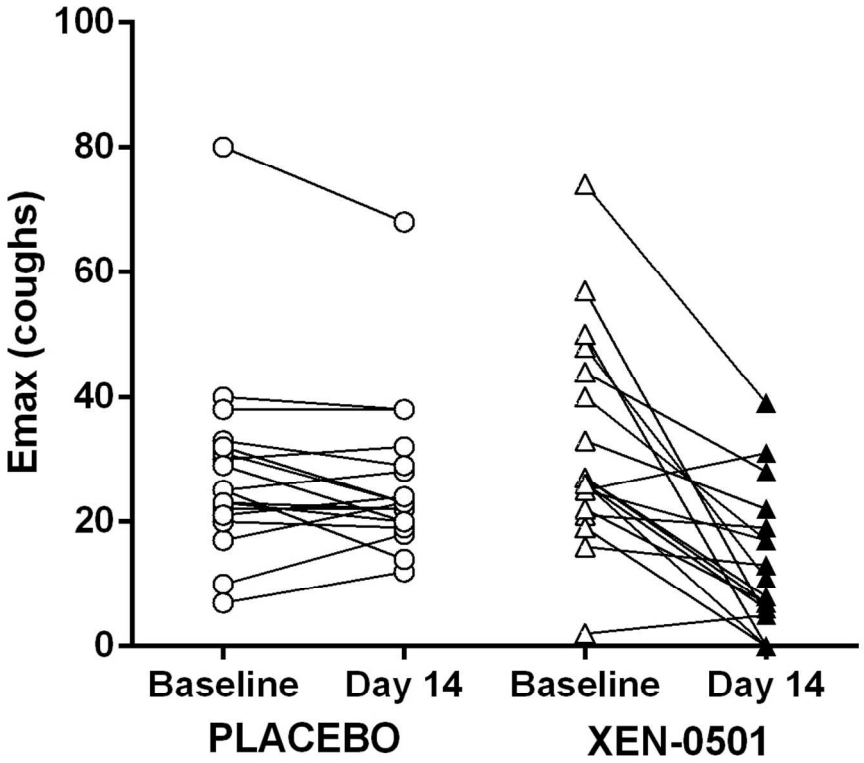


Figure 4. Capsaicin Evoked Cough Responses. Individual patient maximal cough responses to capsaicin (Emax), at baseline and after 2 weeks of treatment with placebo and XEN-D0501. XEN-D0501 significantly reduced E_{max} compared with placebo, $p<0.001$.

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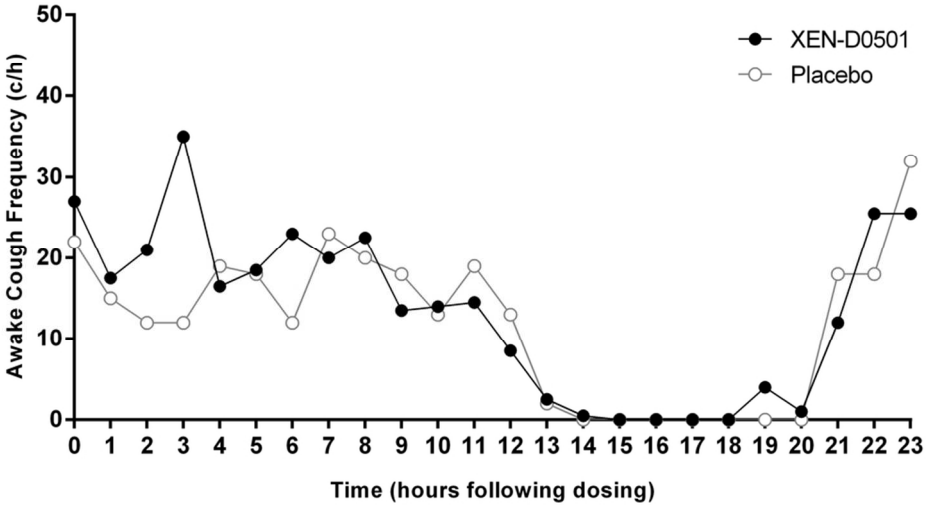


Figure 5. Hourly Cough Frequency. Comparison of cough frequency hour by hour on day 14 of treatment with XEN-0501 compared with placebo; data shown are medians.

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