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A balanced randomised placebo controlled blinded phase IIa multi-centre study to investigate the efficacy and safety of AUT00063 versus placebo in subjective tinnitus: The QUIET-1 trial

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ABSTRACT

AUT00063 is an experimental new medicine that has been demonstrated to suppress spontaneous hyperactivity by modulating the action of voltage-gated potassium-channels in central auditory cortical neurons of a rodent model. This neurobiological property makes it a good candidate for treating the central component of subjective tinnitus but this has not yet been tested in humans. The main purpose of the QUIET-1 (QUest In Eliminating Tinnitus) trial was to examine the effect of AUT00063 on the severity of tinnitus symptoms in people with subjective tinnitus. The trial was a randomised, placebo-controlled, observer, physician and participant blinded multi-centre superiority trial with two parallel groups and a primary endpoint of functional impact on tinnitus 28 days after the first drug dosing day.

The trial design overcame the scale and logistical challenges of delivering a scientifically robust, statistically powered multi-centre study for subjective tinnitus within the National Health Service in England. The trial was terminated early for futility. Overall, 212 participants consented across 18 sites with 91 participants randomised to groups using age, gender, tinnitus symptom severity and hearing status as minimisation factors. While the pharmacokinetic markers confirm the uptake of AUT00063 in the body, within the expected therapeutic range, with respect to clinical benefit findings indicated that AUT00063 was not effective in alleviating tinnitus symptoms (1.56 point change in Tinnitus Functional Index). In terms of clinical harms, results indicated that a daily dose of 800 mg capsules of AUT00063 taken for 28 days was safe and well tolerated. These findings provide significant advances in the drug development field for hearing sciences, but raise questions about the predictive validity of certain rodent models of noise-induced hearing loss and tinnitus, as least for the mechanism evaluated in the present study. Trial Registration: (EudraCT) 2014-002179-27; NCT02315508.

1. Introduction

Tinnitus is a common medical symptom that can be debilitating. Tinnitus affects between 5% and 43% of the general population depending on the definition of tinnitus, and prevalence increases with age (McCormack et al., 2016). For many people, tinnitus is persistent and troublesome, and has disabling effects such as sleep difficulties, problems concentrating, impaired communication and social interaction, low mood including generalised anxiety and

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Abbreviations

ABR	Auditory Brainstem Response
AE	Adverse Event
ANCOVA	Analysis of Covariance
CI	Confidence Interval
ECG	Electrocardiogram
eCRF	electronic Case Report Form
EEG	Electroencephalography
IDMC	Independent Data Monitoring Committee
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
NRES	National Research Ethics Service
QTc	corrected Q-T interval
SAE	Serious Adverse Event
SD	standard deviation
TFI	Tinnitus Functional Index

depressive symptoms (Hall et al., 2018b).

The most prevalent form of the condition is subjective tinnitus, a condition in which the experience of an auditory sensation is of the individual alone. Risk factors for developing subjective tinnitus include noise exposure, hearing loss, ototoxic medication, and head injury (e.g. Baguley et al., 2013). Because otological conditions, especially high-frequency sensorineural hearing loss, present one of the major risk factors for tinnitus, the condition is often deemed to reflect neural changes in the central auditory system in response to cochlear damage (Eggermont and Roberts, 2015). Indeed, subjective tinnitus is generally considered to have a central nervous system component. While the trigger for tinnitus is thought to occur at the peripheral level, a cascade of neuro-plastic events throughout the central auditory pathway develops and sustains the symptoms. Hence, preventing or treating sensorineural hearing impairment could be more successful in avoiding or delaying tinnitus than current intervention approaches which merely help people to manage their existing tinnitus symptoms (Noreña, 2011).

The clinical rationale for the study is therefore motivated by the absence of a singularly effective treatment for subjective tinnitus which either alleviates the reactions to the tinnitus or which reduces the tinnitus percept (e.g. Baguley et al., 2013). Cognitive behaviour therapy delivered by qualified psychologists (Martinez-Devesa et al., 2010), and sound therapies such as amplification devices and sound generators (Sereda et al., 2018) may have limited benefit for some people. But there are currently no approved pharmaceutical treatments for tinnitus, even though surveys suggest that people would prefer a pill to reduce or eliminate their tinnitus percept (Tyler, 2012). A survey across General Practitioners and Ear, Nose and Throat specialists in six countries (US, Germany, UK, France, Italy and Spain) indicated a broad range of off-label pharmacological prescriptions to treat conditions associated with subjective tinnitus including anti-vertigo products and corticosteriods, with antidepressants and sedatives being particularly common when the condition has lasted $\geq$3 months (Hall et al., 2011). Respondents were generally dissatisfied with current drug treatments; noting their lack of specificity for tinnitus and general ineffectiveness. Given the urgent need for a targeted drug treatment for tinnitus, it is perhaps surprising that there are so few research programmes in the pipeline (Langguth et al., 2019). However, this simply reflects the historical absence of a clear understanding of the disorder. While there have been significant advances in understanding tinnitus mechanisms over the past few decades, there have been no major breakthroughs in the theoretical understanding and availability of preclinical tools necessary to conduct a robust drug development programme for the central auditory system. It is important to note that there is no cumulative evidence from relevant studies to draw upon. Pharmacology trials in hearing are “pretty much white space” (Jarvis, 2014).

Noise exposure damages the cochlea, triggering hearing loss in animals and humans and provoking neural degeneration in peripheral and central auditory structures. Neuronal excitability is one of the known consequences of noise damage over the short term, and this is a potential neural correlate of tinnitus (Eggermont, 2013; Eggermont and Roberts, 2015; Pilati et al., 2012a; Roberts et al., 2010). In general, voltage-gated potassium channels play a major role in determining intrinsic cellular excitability. The Kv3 family of such channels play a role in shaping the action potential and firing properties. In a rat model, noise exposure has been shown to down-regulate high voltage activated potassium currents that are likely responsible for the high-frequency burst firing of neurons characteristic of spontaneous hyperactivity (Pilati et al., 2012b). In particular, Kv3.1 is a candidate neurobiological target for developing drug treatments for tinnitus because it is a high-threshold potassium channel that is expressed in the cell membrane of fast-spiking neurons throughout the central auditory system (Chambers et al., 2017).

AUT00063 is a novel centrally acting drug developed as a potent and selective modulator of Kv3.1 and Kv3.2 voltage-gated potassium channels, shifting their voltage-dependence of activation towards more negative potentials (Anderson et al., 2018). AUT00063 has been demonstrated to suppress spontaneous hyperactivity induced by noise exposure in dorsal cochlear nucleus fusiform cells in a hamster model (Glait et al., 2018), and in multi-unit activity recorded in the inferior colliculus in a mouse model (Anderson et al., 2018). Based on this evidence, the authors set out to investigate whether modulation of Kv3 channels in the auditory brainstem and midbrain could provide symptomatic relief in people with tinnitus associated with noise exposure. AUT00063 is the first drug developed to target the central (‘neural’) component of tinnitus associated with the perception of tinnitus.

The present phase IIa trial followed a first-in-human, phase I double-blind, randomised, single and repeat dose escalating trial in healthy young (18–45 year old) men and older men and women (aged 60–75 years) designed to assess safety, tolerability, and pharmacokinetics of AUT00063 following oral doses. Based on the Phase I trial data, side effects were minor, mostly mild in nature and the most frequent were headache, fatigue, tiredness, abdominal pain and toothache (Autifony Therapeutics Ltd, unpublished results). Most of these side effects resolved spontaneously. Based on the pharmacokinetic data from the volunteers, it was concluded that 800 mg administered daily would result in plasma concentrations within the therapeutic range for tinnitus. The half-life was found to be in the region of three days, and hence steady state concentrations were achieved between 1 and 2 weeks following continuous dosing.

The primary objective of the phase IIa trial was to compare the effect of 28 days repeat dosing with AUT00063 on the functional impact of tinnitus in adults with subjective tinnitus associated with noise and/or age-related sensorineural hearing loss, relative to a placebo. A secondary efficacy objective was to compare AUT00063 and placebo groups after 28 days on tinnitus loudness. As is customary in an early stage clinical trial, safety and tolerability of AUT00063 were also important secondary endpoints. Three exploratory objectives compared repeat administration of AUT00063 and placebo on: i) within-subject changes in the functional impact of tinnitus and tinnitus loudness,

ii) the effect of AUT00063 on a pharmacokinetic marker (i.e.

plasma concentration of AUT00063), iii) the effect of AUT00063 on discrete outcome domains associated with the functional impact of tinnitus (intrusiveness, sense of control, cognitive interference, sleep disturbance, auditory difficulties attributed to tinnitus, relaxation, tinnitus-specific 'quality of life' and emotional distress), and iv) individual patterns of clinically meaningful improvement or worsening of the clinical impact of tinnitus. All objectives (including exploratory objectives) were pre-defined in the study protocol.

2. Material and methods

2.1. Trial design

As a short-form name, the trial was called QUIET-1 (QUest In Eliminating Tinnitus). This was a randomised, placebo-controlled, multi-centre superiority trial with two parallel groups and a primary endpoint of the functional impact on tinnitus 28 days after the first drug dosing day. This trial was designed to demonstrate superiority of AUT00063 (800 mg once daily) over placebo. Staff collecting outcome data (observer), physicians and participants were blinded to treatment allocation (triple-blinded design). Stratified randomisation used age, gender, tinnitus symptom severity and hearing status as minimisation factors, with a 1:1 treatment allocation. Our reporting of QUIET-1 follows the rigorous standards defined in the Consolidated Standards of Reporting Trials (CONSORT) Statement (Schulz et al., 2010).

The protocol was independently reviewed and approved by the National Research Ethics Service (NRES) Committee Yorkshire and the Humber – Leeds East (Ref: 14/YH/1090) and the Medicines and Healthcare products Regulatory Agency (MHRA) (Eudract number: 2014-002179-27). The trial Sponsor was the Autifony Therapeutics Ltd (Stevenage, UK). The trial was registered on ClinicalTrials.gov on 09 December 2014 (Identifier: NCT02315508) and the results were published on the EU clinical trials register (www.clinicaltrialsregister.eu) on 08 Dec 2016 (EudraCT: 2014-002179-27).

2.2. Participants

A diverse range of methods, continuous monitoring, mitigation strategy and adequate resourcing were essential for achieving the recruitment rate required by the QUIET-1 protocol (Sanchez et al., 2018). Participant recruitment was primarily from direct referral to those National Health Service (NHS) organisations approved for recruitment and by posters displayed in public areas in those local hospitals. Additionally, the trial team placed a series of features in several national newspapers, hearing-related magazines (for the public and hearing healthcare professionals), and hearing charity websites. Some recruiting sites advertised through NHS general practitioner centres or engaged in additional regional advertising in newspapers and magazines and interviews on local radio stations, following ethical review and approval.

Table 1 provides details of inclusion and exclusion criteria for the trial according to the final version of the Clinical Trial Protocol (version 1.5). We sought to identify subjective tinnitus with noticeable tinnitus symptoms that had existed for not less than 6 months, and not more than 18 months at study start and within a defined range of sensorineural hearing loss. These eligibility criteria were based on discussion with Sponsor and audiologist experts. There is no widespread standard for grading the temporal characteristics of tinnitus, but many clinical studies use 6 months as a benchmark for defining the boundary between acute and chronic tinnitus. Tinnitus experienced for >18 months can be considered as a long-standing tinnitus which may involve psychological or other mechanisms responsible for maintaining some of the symptoms and which differ from those mechanisms responsible for its initial generation. A score $\geq$24 and $\leq$ 68 on the TFI was informed by the best available evidence at the time which was an estimate of the interquartile range on the TFI using UK-based data on the target population (Fackrell et al., 2018; ClinicalTrials.gov Identifier: NCT01541969).

Four sets of substantial amendments were submitted and approved by the NRES and MHRA, and all changes relevant to the eligibility criteria are reported. The trial team operated a central telephone number where those interested could find out if they met the basic entry criteria. Individuals answered a scripted pre-screening interview with general questions on tinnitus characteristics, otological comorbidities and general health. This was not part of the formal trial assessment as no personal details were taken. Those passing the screen were simply advised to contact their nearest recruiting site.

Participants were involved in the trial for up to 10 weeks from start to finish and the schedule of clinical research activities is illustrated in Fig. 1. These comprised 4 or 5 visits to the clinic plus a 5–10-min telephone call at the mid-way point. In brief, a 2–3 h initial screening visit included informed consent followed by medical and audiometric assessments required for determining eligibility. This could take place at any time up to 28 days before dosing. The second visit was a 1-1 ½ hour training baseline which was intended to expose participants to the loudness matching procedure and to administer a structured interview for estimating lifetime noise exposure. At the discretion of the trial team, these assessments could be carried out on the same day as screening. The (third) visit repeated some of the medical and audiological assessments and took 4-4 ½ hours. This visit is called 'Day 1' (D1) because it corresponds to the first dose which was taken under clinical supervision. Participants at the Nottingham site undertook additional assessments of pharmacological electroencephalography (pharmacoEEG) and Auditory Brainstem Response (ABR) activity, extending the D1 visit from 4 h up to 11 h including a wait of approximately 6 h in between pre- and post-dose measures. These were optional. The last day of dosing (Day 28, D28) was the study end point (visit 4). This 2–3 h visit entailed repeat medical and audiological assessments. And again, for participants at the Nottingham site optional assessments of pharmacoEEG and ABR activity extended this visit up to 5 h. The main purpose of the final visit (visit 5) at Day 42 (D42) was to enable medical assessments for the safety analysis. This took about 1 ½ hours.

Participants received a financial compensation for loss of working hours and/or inconvenience. In addition, travel and/or meal expenses were reimbursed for each visit.

2.3. Study settings

The trial took place across 18 NHS organisations. Recruitment was open from 23 October 2014 to 08 October 2015, but the opening of sites was staggered over this period and this is fully reported in Table 2. The first 10 sites were opened by January 2015 and the additional 8 sites were opened through April to July 2015 to support slower than expected recruitment. Three of the additional sites opened did not screen any participants as the trial was terminated early for futility.

2.4. Intervention

AUT00063 was formulated for oral administration in Swedish Orange, hard gelatin (size 0) capsules containing white to slightly coloured powder that included 200 mg of AUT00063 as the parent compound along with the following excipients: sodium lauryl

Table 1
Inclusion and exclusion criteria assessed during the telephone pre-screening and screening visit (visit 1) according to the final version of the Clinical Trial Protocol (version 1.5). Four sets of substantial amendments were submitted and approved by the NRES and MHRA and all changes relevant to the eligibility criteria are given in column 2.

Eligibility criteria	History of substantial changes to the Clinical Trial Protocol
Inclusion criteria (Clinical Trial Protocol v1.5, dated 2 July 2015)	
1. English speaking male or female, ≥18 years of age, UK residents, registered with a UK General Practitioner	
2. Stable tinnitus (consistent from day to day), score ≥24 and ≤ 68 on the Tinnitus Functional Index	
3. Duration of noticeable tinnitus symptoms ≥6 months and ≤18 months at enrolment	
4a. Pure Tone Average (for frequencies at 0.5, 1, 2, and 4 kHz) ≤60 dB Hearing Level (HL), across the two ears	v1.3 (13 Nov 2014) amended to include = 20 and = 60 dB HL in the Pure Tone Average calculation. v1.4 (9 Feb 2015) requirement for Pure Tone Average ≥ 20 dB removed
4b. Any single frequency at 0.5, 1, 2, 4, 6 or 8 kHz with an audiometric threshold estimate >20 dB to	v1.4 (9 Feb 2015) individual frequency thresholds >20 dB instead of lower Pure Tone Average ≥ 20 dB
4c. If a hearing aid or sound generator user then confirmed consistent daily device usage over the past six months	
5. Normal life expectancy for age, based on physician's judgement.	
6. Females of child-bearing potential must have a negative pregnancy test at screening and baseline visits, and practice two reliable methods of contraception throughout the study.	v1.1 (14 Aug 2014) amended to ensure that women use two methods of contraception
7. Able to understand and comply with the requirements of the study and signed Informed Consent Form.	
Exclusion criteria (Clinical Trial Protocol v1.5, dated 2 July 2015)	
1. History of hypersensitivity or idiosyncratic reaction to any component of the test medication	
2. Any acute disabling tinnitus	
3. Diabetes mellitus with an HbA1C>8% (64 mmol/mol) to allow the inclusion of patients with well controlled Type 2 diabetes	
4. Previous cardiac rhythm disorders or ECG rhythm abnormalities whether symptomatic or not, and considered to be clinically significant	s
5. Severe hearing impairment such that oral-only communication is unreliable	
6. History of important cardiac, endocrine, pulmonary, neurologic, psychiatric, hepatic, renal, hematologic, immunologic, or other major diseases deemed clinically significant at the time of the study which might be jeopardised by entering the study	
7. Moderate or severe depression or generalised anxiety as indicated by a score of ≥11 out of 21 on the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983)	
8. Alcohol or drug abuse deemed clinically significant, based on physician's judgement.	
9. History of poor cooperation, non-compliance with medical treatment, or unreliability, based on physician's judgement.	
10. Participation in any clinical research study evaluating another investigational drug or therapy within 30 days or at least 5 half-lives (whichever is longer)	
11. Participation in a hearing study, involving an intervention, within 3 months from the last study visit	
12. Use of central nervous system active drugs except analgesics and those specified in the Clinical Trial Protocol	
13. Non-study treatments for the management of tinnitus, severe insomnia, major depressive disorder, severe anxiety, or post-traumatic stress disorder. (Counselling is allowed as long as it was implemented prior to screening).	
14. Central nervous system pathologies (such as Multiple Sclerosis, Parkinson's disease etc).	
15. Tinnitus as a concomitant symptoms of a known otological condition (including but not limited to otitis externa, otitis media, otosclerosis, cholesteatoma, Ménière's disease, or other vestibular problems, acoustic neuroma, or temporo-mandibular joint disorder)	
16. Pulsatile tinnitus (rhythmical sounds that often beat in time with the heartbeat)	
17. Intermittent tinnitus (comes and goes from one day to the next), based on physician's judgement.	
18. Surgical or medical condition that would be expected to significantly affect absorption of medicines	v1.3 (13 Nov 2014) amended to include only those surgeries or medical conditions expected to affect absorption
19. Presence or history of relevant severe adverse reaction to any drug or a history of sensitivity to potassium channel modulators	
20. Blood pressure and heart rate (in seated position) outside the clinically relevant ranges specified	
21. From the electrocardiogram representation of ventricular depolarization and repolarisation, a corrected QT interval <330 ms in males or <340 ms in females or >450 ms for both males and females	v1.1 (14 Aug 2014) amended to include corrected QT interval >450 ms
22.Clinically relevant out-of-range values in ay haematology, urinalysis or clinical chemistry tests	

sulphate, colloidal silicon dioxide, mannitol and microcrystalline cellulose. Matching placebo capsules were also manufactured with identical visual characteristics to ensure a blinded placebo-controlled study. Capsules were packed in an opaque white, high density polyethylene bottle with child-resistant closures that were induction sealed. Participants were instructed to take, with food, 4 × 200 mg capsules of AUT00063 once daily to achieve the 800 mg dose. At the end of the D1 visit, participants were given 27 days of study medication (plus 4 extra capsules).

2.5. Outcomes

The primary endpoint was the difference between groups in the mean change from D1 to D28 of tinnitus symptom severity, as measured by the Tinnitus Functional Index (TFI) global score (Meikle et al., 2012). To reduce risk of bias due to participants inflating the self-reported severity of their tinnitus symptoms in order to be enrolled onto a trial, the pre-intervention baseline (D1) measure of the TFI was pre-determined after enrolment into the trial. Exploratory analyses of the TFI investigated subscale scores and binary outcomes according to whether individual treatments were successful (change in TFI ≥ −13 points) or not (≥+10 points). TFI was selected because it assesses a range of tinnitus symptoms experienced over the preceding week and had some prior clinical evidence on which to estimate sample size to detect treatment-related change (Fackrell et al., 2016; Meikle et al., 2012). Twenty-

	Screening (up to 28 days before Day 1)	Training baseline (up to 21 days before Day 1)[1]	Dosing day (Day 1)	Check up (Day 14, ±2 days)	End point (Day 28, -2 days or +1day)	Close out (Day 42, ±3 days)
Out-patient visit	✓	✓	✓		✓	✓
Phone call				✓		
Informed consent and Medical history	✓					
Demographics	✓					
Hospital Anxiety and Depression Scale	✓					
Physical examination	✓					✓[2]
Height/weight	✓					
12-lead electrocardiogram	✓					✓
Vital signs	✓		✓[3]		✓	✓
Urinalysis	✓		✓[3,4]		✓	✓
Blood sample for Serum biochemistry and Haematology	✓[5]		✓[3]		✓	✓
Previous and concomitant medication	✓	✓	✓[3]	✓	✓	✓
Adverse events	✓	✓	✓[3]	✓	✓	✓
Audiometric assessment (PTA)	✓		✓[3]		✓	
Tinnitus Functional Index	✓		✓[3]		✓	✓
Check eligibility	✓		✓[3]			
Noise exposure questionnaire		✓				
Loudness matching		✓	✓[3]		✓	
Randomisation			✓			
Leeds Sleep Evaluation Questionnaire			✓[3]		✓	
Auditory Brainstem Response (ABR)[7]			✓[3]		✓	
pharmacoEEG (phEEG)[7]			✓[3,8]		✓[6,10]	
Distribute drug			✓			
Diary card[9]				✓		
Blood for pharmacokinetic analysis					✓[6]	
Clinical Global Impression (Improvement) Scale					✓	

Fig. 1. Schedule of the clinical research activities to be undertaken by each participant during the QUIET-1 trial, with details of the individual assessments conducted at each visit. Superscript numbers denote the following: [1]training baseline assessments may be carried out on the same day as screening; [2]brief physical examination; [3]pre-dose assessment; [4]includes urine pregnancy test for women of child-bearing age; [5]includes serum pregnancy test for women of child-bearing age; [6]to be taken before last dose; [7]at Nottingham site only; [8]up to 8 h post-dose assessment depending on participant's availability; [9]distributed before discharge on Day 1; and [10]optional assessment according to participant's availability.

five items span eight subscales (intrusiveness, sense of control, cognitive interference, sleep disturbance, auditory difficulties, difficulties relaxing, quality of life, and emotional distress). Each item is scored on an 11-point Likert scale, giving a global score from 0 to 100, and with higher scores indicating greater symptom severity. For a UK clinical population of adults with tinnitus, the TFI has been confirmed to have high construct validity (correlation with the Tinnitus Handicap Inventory, $r = 0.80$), high internal consistency ($\alpha \geq 0.95$), and high test-retest reliability (intraclass correlation = 0.87) (Fackrell et al., 2018).

Tinnitus loudness was the secondary clinical efficacy endpoint (D1 to D28), measured by adjusting the level of a tone until its loudness was "about the same loudness" as the participant's tinnitus. Estimates of tinnitus loudness largely depend on the type of measurement used and at the time of study design it was unclear which available procedures had acceptable reliability and validity. Choice of loudness-matching technique was informed by the procedure developed at the Oregon Tinnitus Clinic (Vernon and Meikle, 1981). The modified procedure required testing, in 2 dB-steps, only at 1 kHz in the non-tinnitus ear (or ear where the tinnitus was least

Table 2
Recruiting NHS sites across England.

Site code	Date opened to enrolment	Patients assessed for eligibility at screening visit (n = 212)	Randomised to AUT00063 (n = 44)	Randomised to placebo (n = 47)
03	10/10/2014	22	7	4
09	10/10/2014	12	5	1
08	17/10/2014	14	2	6
02	23/10/2014	32	7	7
04	27/10/2014	27	9	6
10	03/11/2014	13	3	3
06	06/11/2014	4	0	1
13	21/01/2015	9	0	4
12	11/02/2015	7	1	1
01	17/02/2015	28	4	3
17	23/04/2015	6	1	2
18	14/05/2015	14	3	3
14	22/06/2015	2	0	0
15	22/06/2015	10	1	3
19	28/07/2015	12	1	3
11	28/07/2015	0	0	0
05	25/08/2015	0	0	0
20	03/09/2015	0	0	0

dominant). This frequency reduces the influence of loudness recruitment since 1 kHz is a frequency generally within the normal hearing range and also well below the typical pitch match frequency. To familiarise participants with the procedure enhancing stability of the baseline measure, loudness matching was completed during the training baseline visit. This data was not used in the analysis.

To promote data quality, a Study Procedures Manual for audiology assessments was created and all relevant trial staff across the sites were trained in study-specific procedures (TFI, loudness matching, and estimating lifetime noise exposure) by a member of the Nottingham team. Noise exposure was assessed using a structured interview that consider all activities across the lifespan that the respondent had experienced as noisy (defined based on sound level estimated from vocal effort) (Lutman et al., 2008). A list of Frequently Asked Questions encouraged consistency across the trial sites by sharing responses to audiology-related queries, especially on the noise exposure procedure. Pure Tone Average and TFI global scores could differ across Screening and D1 visits due to test-retest variability. Participants, who were eligible for inclusion at Screening but not at D1, were recommended for randomisation, but any uncertainties by the trial team or unexpectedly large differences were referred to the Sponsor.

The other secondary clinical efficacy endpoints (D1 to D28) concerned the safety and tolerability profile of AUT00063. These questions were assessed by medically qualified members of the study team in terms of effects on vital signs (i.e. systolic blood pressure, diastolic blood pressure, heart rate andtemperature), physical examination (i.e. lymph nodes, gastrointestinal, cranial nerves, peripheral nervous system, heart, chest, ears and general appearance), and laboratory exams (i.e. serum biochemistry, serum haematology, and urinalysis). Electrocardiogram (ECG) (including corrected QT interval (QTc) which is an index of cardiac repolarisation) was collected at the screening visit and Day 42. Data on Adverse Events (AEs) and Serious Adverse Events (SAEs) were also captured throughout the trial. An AE was defined as any unfavourable and unintended sign, symptom or disease temporarily associated with the intervention, whether or not it was considered related to the medicinal product. It was rated as mild, moderate or severe by the sitesite Investigators. An SAE was life-threatening or resulted in death, resulting in hospitalisation or prolongation of existing hospitalisation, resulted in persistent or significant disability or incapacity, was a congenital anomaly or birth defect or was otherwise considered medically important. Treatment-emergent AEs were those that started or increased in severity on or after the first dose of study medication.

Exploratory outcomes were the blood plasma concentration of AUT00063 as a marker for drug exposure, overall patient-reported experience of treatment-related change and neural activity. Blood plasma concentration of AUT00063 was measured in ng/ml at a pre-dose time point on D28. Samples were stored and dispatched to a commercial company (Aptuit) for analysis. On D28, participants were also asked to reflect on whether they had experienced any treatment-related change using the Clinical Global Impression (Improvement) scale. This is a single 7-point Likert scale with categories from 'much worse' to 'much better', with 'no change' at the mid-point. Finally, neural activity was assessed by pre- and post-dosing by pharmacoEEG and Auditory Brainstem Response recording. Only seven participants completed the electrophysiology assessments at one recruiting site, and so the data are inconclusive (not reported).

2.6. Sample size

The analysis of the primary outcome was planned for an ANCOVA of the global TFI change from D1 to D28, controlling for randomisation strata and baseline covariates to estimate the mean difference between the AUT00063 and placebo groups. Sample size estimation was based on unpublished data from a large UK sample of people with tinnitus (n = 285 enrolled from the general population into a trial, ClinicalTrials.gov Identifier: NCT01541969 and n = 151 recruited via audiology clinics). The mean global TFI score for this group was 44.9 points (SD 21.2). The minimum clinically important benefit was taken to correspond to a reduction in the group mean TFI of 13 points (Meikle et al., 2012).

Based on a two-group *t*-test, a sample size calculation determined that 57 participants would be required per treatment arm to detect a statistically significant difference between AUT00063 and placebo groups (nQuery Advisor 5.0, Statistical Solutions Ltd). This calculation was based on the above information as well as a 1:1 treatment allocation and a two-sided hypothesis with 90% power at an alpha level of 0.05. Accounting for a planned interim futility analysis (see section 2.7) indicated that 59 participants per arm were required. The permitted sample size was increased to 76 per group (152 in total) in order to mitigate a number of operational risks. These were that: i) the study sample may not adequately match the reference population in terms of the assumed distribution of TFI scores and heterogeneity in other relevant

characteristics, ii) drop out should be accounted but was unknown, and iii) any subsequent upward adjustment of the sample size (potentially arising from recommendations of the planned interim analysis) would have severe project management implications.

2.7. Interim analysis and stopping rules

One interim analysis of the primary endpoint and safety data was planned for when approximately 50% of the participants had completed D28. Statistical significance and futility boundaries were estimated for the interim and final analysis based on 50,000 simulations from the PASS® software (NCSS, Kaysville, Utah) by simulating a group sequential test for two means assuming normality testing. At the interim analysis, the two-sided significance boundary for clinical efficacy was 0.00312 and for futility of detecting $\mu_{AUT00063} > \mu_{placebo}$, the one-sided O'Brien-Fleming boundary was 0.39,141. Hence, at the final analysis, the two-sided significance boundary for clinical efficacy would be 0.04761. The Independent Data Monitoring Committee (IDMC) was advised to consider making recommendations for early termination only where there was a clear demonstration of futility.

2.8. Randomisation and blinding

Participants were randomised using a computer-generated allocation sequence that was managed within a telephone-based central randomisation service (http://www.sharpservices.com/). Participants were randomly assigned to either AUT00063 or placebo groups with a 1:1 allocation. Minimisation techniques were used to balance allocation to the two arms according to four stratification variables assessed at the screening visit: (i) age (<65 years, ≥65 years), (ii) gender (male, female), (iii) average hearing loss (mild = ≤40 dB and moderate = ≥41 dB), and (iv) tinnitus symptom severity as measured by the global TFI (slight-moderate = 24–35.4, and moderate-severe = 35.5–68). For the minimisation procedure only, average hearing loss was defined by the Pure Tone Average of air-conduction thresholds at six frequencies (0.5, 1, 2, 4, 6 and 8 kHz), averaged across the two ears. This measure captured threshold sensitivity at both low and high frequencies considered relevant to the underlying pathophysiology targeted by AUT00063.

The Sponsor, the Contract Research Organisation responsible for monitoring the study, pharmaceutical services staff and local study site personnel were all blinded to treatment allocation, with audit trails to confirm no unblinding of participants. Thus, randomisation was conducted without any influence of the Sponsor or local study site personnel. According to the Clinical Trial Protocol, several individuals were unblinded: i) the clinical project controller for the telephone-based central randomisation service, ii) two Contract Research Organisation statisticians working on processing and analysing the primary outcome, safety and harms data for the planned interim analysis presented to the IDMC, and iii) two Contract Research Organisation biostatisticians who prepared all the Tables and Listings after termination of the trial and database lock. To safeguard the integrity of the trial, the statistical teamteam preparing thethe interim analysis were independent from the Data Management team.

2.9. Data monitoring and fidelity of the trial procedures

The majority of the participant data was entered into an electronic Case Report Form (eCRF) database maintained by a Contract Research Organisation. ECG tracings and reports were separately managed through a clinical study portal and database subcontracted to ERT (www.ert.com/), while EEG recordings for the Nottingham site were managed by Biotrial (www.biotrial.com/). ERT and Biotrial are specialists in conducting clinical trials to regulatory standards. Data monitors in the Contract Research Organisation, ERT and Biotrial data management teams remotely monitored data and sent regular queries to local trial sites when data entry discrepancies were suspected. Site monitoring visits were also performed generally every three weeks or according to recruitment rate against a Monitoring Plan by Clinical Research Associates acting on behalf of the Contract Research Organisation. The purpose of these visits was to verify site data by cross-checking all source data against the eCRF. The Principal Investigator at each site reviewed and approved every page of the eCRF.

There was continual oversight of participant safety and of the safety data. A medical monitor reviewed all laboratory test results and safety data bi-weekly, sending data queries to local trial sites. In addition, the medical monitor addressed queries about eligibility and dealing with adverse events and adverse reactions. The Sponsor's Chief Medical Officer was in monthly contact to ensure any potential safety data trends were identified and that all adverse events were rigorously checked. An annual safety assessment and six-monthly line listing was sent to all local recruiting sites after submission to the relevant regulatory bodies (MHRA, NRES). The integrity of the blood samples was monitored by recording detailing dosage levels and timings, storage temperature, and details of the transit of the samples from each trial site to BIOLIM, the subcontracted company conducting the analysis (http://www.biolim.pl). Aptuit (a pharmaceutical services company, www.aptuit.com) cross-referenced these data against the telephone-based central randomisation service data.

A full audit to verify the compliance of the Trial Master File was conducted after the planned interim analysis and decision to terminate the study early by the Contract Research Organisation. This process was assessed by the Sponsor.

2.10. Statistical methods

Safety and tolerability of AUT00063 were investigated using the 'Safety Analysis Set' which included all enrolled participants who had received at least one dose of study medication.

Clinical efficacy analysis using analysis of covariance (ANCOVA) was planned for a 'Per Protocol Set' which should include those participants meeting all inclusion criteria, completing at least one post-baseline measurement for the primary efficacy variable, and with no major protocol violations. Because the trial was terminated early and there was no Blinded Data Review Meeting, we were unable to conduct a 'Per Protocol' analysis as intended. Instead, the primary efficacy analysis was conducted using the 'Full Analysis Set' which included those participants who received at least one dose of study medication, *and* completed at least one post-baseline measurement for the primary efficacy variable. All analyses were conducted by a trial statistician who was blinded to the group allocation and followed the pre-defined Statistical Analysis Plan. Details of the characteristics of the Full Analysis Set, as assessed at the screening visit, are given in Table 3. Missing data were not imputed and so complete case analyses are reported. Notably, eight participants had missing noise exposure estimates (the covariate of interest) and so a total of 68 datasets were available for this analysis. ANCOVA findings were also confirmed for a 'Completers Population' which was defined as the subset of those participants who completed the full 28 days of study treatment (n = 63).

The planned ANCOVA accounted for the four minimisation variables (i.e. age, gender, hearing status and tinnitus symptom severity) to allow systematic and random variability to be separately estimated (Taves, 1974). To improve the precision of the estimate of clinical efficacy of the drug treatment, analysis also

Table 3
Demographics and characteristics of the Full Analysis Set assessed at the screening visit. A noise exposure estimate of 1.0 denotes an equivalent lifetime exposure to occupational, recreational and firearm noise (8 h per day, 5 days per week, 48 weeks per year over a 50-year working lifetime) corresponding to 81–90 dB (A). For more details of the calculation and interpretation of lifetime noise exposure, see Lutman et al. (2008).

	AUT00063 (n = 36)		Placebo (n = 40)	
	n	Mean (SD)	n	Mean (SD)
Age (years)	36	52.78 (11.27)	40	55.23 (10.56)
Gender	36	9 female; 27 male	40	8 female; 32 male
Height (cm)	36	176.17 (7.45)	40	174.83 (9.34)
Weight (kg)	36	86.58 (20.37)	40	85.03 (18.31)
Body Mass Index (kg/m^2)	36	27.72 (5.68)	40	27.88 (5.87)
Hearing loss (defined by the pure tone average 0.5–4 kHz across both ears, dB HL)	36	19.74 (11.64)	40	21.28 (11.42)
Noise exposure estimate (0–4)	35	1.0 (0.92)	33	0.9 (0.91)
Duration of noticeable tinnitus symptoms (years)	36	1.0 (0.37)	40	1.1 (0.32)
Tinnitus Functional Index (0–100)	36	42.01 (12.45)	40	45.44 (11.86)
Depressive subscale of Hospital Anxiety and Depression Scale (0–21)	36	3.36 (2.59)	40	2.88 (2.42)
Anxiety subscale of Hospital Anxiety and Depression Scale (0–21)	36	4.97 (3.27)	40	4.40 (2.59)

included three additional covariates which might also be expected to influence the primary outcome; the baseline (D1) TFI global score, duration of noticeable tinnitus symptoms, and noise exposure estimate. These covariates were chosen based on informed expert opinion of the study team and the Scientific Advisory Board. Noise exposure was an indicator for the degree of noise-related cochlear damage, which relates to the underlying pathophysiology targeted by AUT00063.

3. Results

The number of randomised participants was not sufficient to achieve the statistical power determined by the sample size calculation. This was a consequence of the premature termination of the study resulting from futility in the planned unblinded interim analysis. The interim analysis was conducted by the IDMC, who reviewed unblinded safety and primary efficacy outcome (TFI) data from 58 participants. The recommendation was to discontinue the QUIET-1 trial based on efficacy data, as the p-value of the primary analysis had exceeded the futility boundary stated in the IDMC charter. No safety issues were identified. On the basis of this recommendation and following an internal review of the data by the Sponsor, the Sponsor decided to terminate enrolment into the QUIET-1 trial early; 91 participants had been enrolled.

3.1. Participant flow

In total, 212 participants were consented across the 15 of the centres opened for recruitment. Two additional participants were assessed for eligibility, but without written informed consent, and so their data was not included. These two participants also failed to meet the inclusion criteria at the eligibility assessment. In total therefore, 123 participants were excluded at the eligibility assessment (visit 1). Fig. 2 provides primary reasons for exclusion. Where several reasons were given for an individual, the major reason was recorded as the first one given in the sequential order of the study protocol (seven inclusion and 22 exclusion criteria). “Other reasons” included two participants who were referred to their General Practitioner for further assessment before being eligible for rescreening, three participants who needed rescreening, and one who was unwilling to comply with the Clinical Trial Protocol regarding alcohol intake.

Of the 91 participants randomised, all received at least one dose of study medication and so these comprised the Safety Analysis Set. Fifteen participants did not complete the study to D28 and so the Full Analysis Set comprised 76 participants. Of these, five (AUT00063 = 4; Placebo = 1) interrupted their treatment schedule and did not complete the full 28-day prescription. These were excluded from the Completers Population.

3.2. Characteristics of the Full Analysis Set (n = 76)

Overall, the mean (median) age was 54.07 (55) years and ranged from 27 to 76. There was a substantial preponderance of men: n = 61 (80.3%) versus n = 15 (19.7%) women. The majority of participants (n = 65, 85.5%) were aged less than 65 years. All participants were of Caucasian ethnic origin, with the exception of a single Asian male allocated to the placebo group. There were no notable differences between the treatment groups for these baseline demographic variables or for height, weight and Body Mass Index. Data for the key demographic and clinical characteristics of the 76 participants in the Full Analysis Set are reported in Table 3. Eight participants did not have noise exposure estimates. Three of these reported having experienced an explosion and so exposure could not be estimated reliably, following Lutman et al. (2008). The remaining five had missing data (see section 3.4 for more details).

The characteristics of the Safety Analysis Set (n = 91) were comparable to the Full Analysis Set. Mean (median) age was 54.16 (55) years, with 85.7% aged less than 65 years. Again, there were almost four times more men (n = 71, 78.0%) than women (n = 20, 22.0%). Baseline tinnitus symptom severity was 42.3 (12.5) in AUT00063 and 46.6 (11.6) in placebo.

3.3. Fidelity of implementing the intervention

Two deviations were noted by the Sponsor during the conduct of the study.

Initially the Clinical Trial Protocol allowed for D28 (endpoint) visit to be conducted on Days 26, 27 or 28, while the amendment in version 1.5 dated 02 July 2015 extended this to allow an endpoint visit on Day 29. At several sites, when the endpoint visit was on Day 29, some participants took the four extra capsules which constituted a 29th day of dosing. This was not deemed critical to the scientific interpretation of clinical efficacy or to participant safety; participants were included in the Completers Population analysis. The second deviation pertained to those five participants who interrupted their treatment schedule and did not complete the full 28-day prescription. To verify that this did not bias the main study finding, these were excluded from an analysis of the Completers Population.

3.4. Fidelity of the research assessments

A full ‘Per Protocol’ evaluation was not conducted, but three

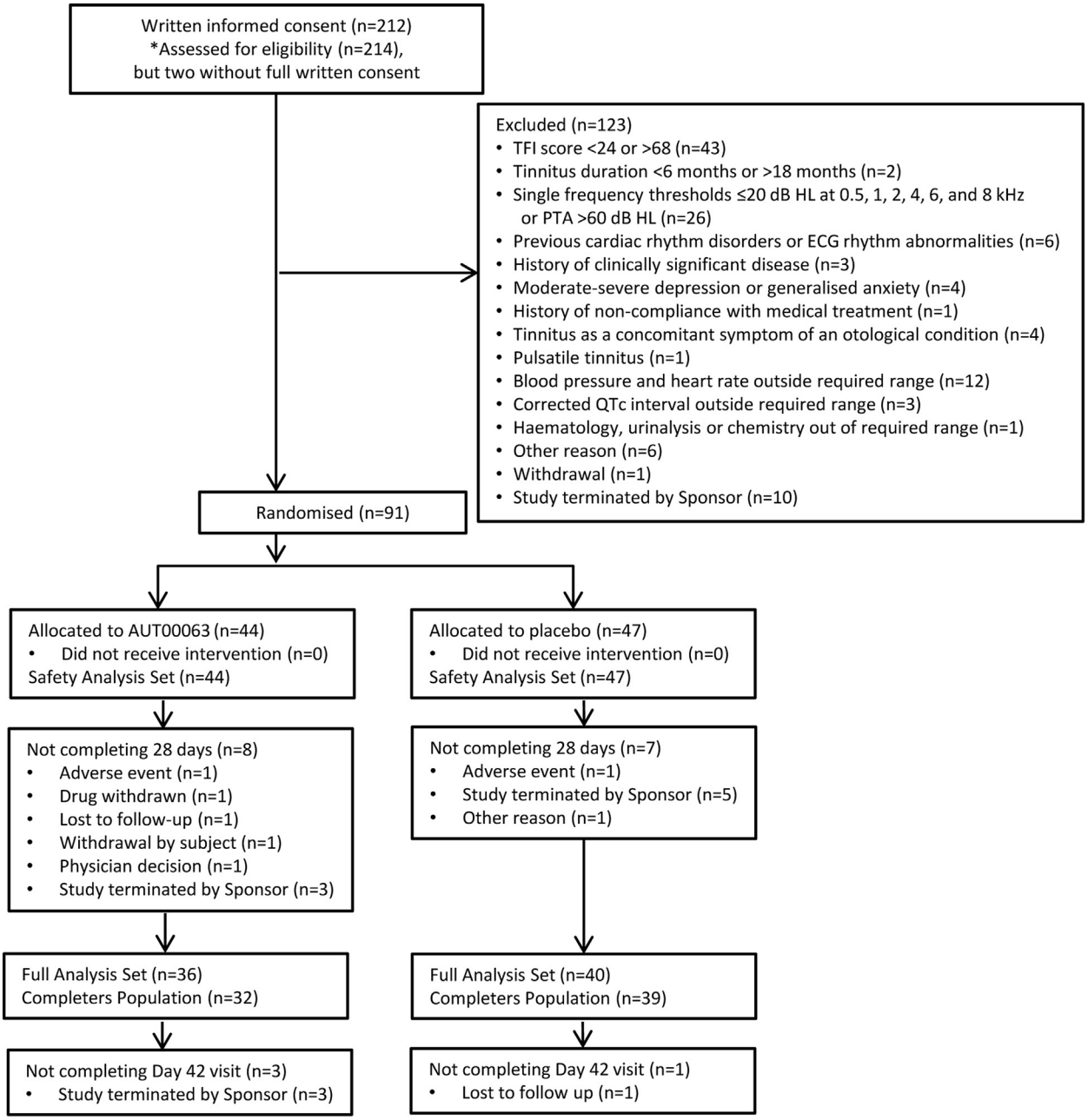

Fig. 2. Flow of participants in the QUIET-1 clinical trial. The study was terminated by the Sponsor after the interim analysis for futility which accounts for some of the participants who did not complete all scheduled visits Two participants were randomised but subsequently withdrawn at Sponsor request for administrative reasons.

deviations to the Clinical Trial Protocol were noted by the Sponsor during the conduct of the study. None of these were deemed critical to the scientific interpretation of clinical efficacy or to participant safety. First, according to the Clinical Trial Protocol the audiological assessment at the screening visit requiredcalculating a four-frequency pure tone average for determining eligibility (see Table 1 Inclusion criterion 4a), and a six-frequency pure tone average for determining stratification using the telephone-based central randomisation service (see section 2.8). Sometimes this information from the D1 visit was used instead. Similarly, the TFI global score at D1 was sometimes used for stratification. Descriptive data for the baseline D1 visit were carefully investigated to examine how well balanced was the hearing loss and tinnitus symptom severity across the two groups. Moreover, a post-hoc analysis confirmed that these deviations did not impact on participant eligibility (not reported). Second, one participant allocated to AUT00063 had depression and anxiety scores outside the eligible range (scores 11 and 15, respectively), while one participant allocated to placebo should have been excluded for high anxiety (score = 11). Both contributed to the Completers Population. Third, for the noise exposure estimate, data for five participants were coded as N/A in the electronic case report form (one on AUT00063 and four on placebo). For these participants, it was unclear whether noise exposure did not reach eligibility for recording (i.e. ≤80 dB(A)), or whether the structured interview was not completed, and so the noise exposure estimate was counted as a missing data entry (and data were excluded from the ANCOVAs for the Full Analysis Set and the Completers Population).

3.5. Planned primary and secondary efficacy outcomes

Treatment-related change in the global TFI score showed no statistically significant difference between AUT00063 800 mg once-daily and placebo groups in the Full Analysis Set (Table 4). The adjusted mean difference in TFI was 1.56, $p = 0.678$ and the 95% confidence interval around the mean difference was −5.90 to 9.01. The ANCOVA for the Completers Population ($n = 71$) gave an almost identical null result (Table 4).

There was no effect of AUT00063 on the tinnitus loudness match in the Full Analysis Set. The adjusted mean loudness match difference between AUT00063 and placebo groups was −1.22 dB SL, $p = 0.530$ and the 95% confidence interval around this mean difference was −5.10 to 2.65. Worthy of note, tinnitus symptoms measured by TFI and loudness matching did not change over the four weeks of treatment for either treatment group (see Table 5). This lack of a 'placebo' effect is considered in the Discussion.

3.6. Adverse events (AEs)

Safety and tolerability data from the Safety Analysis Set indicated that AUT00063 at 800 mg once-daily for 28 days was acceptable. Overall there were 177 recorded treatment-emergent AEs (AUT00063 = 115; placebo = 62), reported across 32 of the 44 participants receiving AUT00063 and 23 of the 47 participants receiving placebo (Table 6). The majority of AEs were of mild severity (mild = 125; moderate = 29; severe = 21; uncoded = 2) and symptoms recovered or resolved without any need to change the dose. Headaches and dizziness are thought to be related to treatment with AUT00063 and are an expected side effect when not overly severe. Headaches were reported in similar numbers across the AUT00063 and placebo. Treatment with AUT00063 was associated with dizziness in seven participants receiving AUT00063 (15.9%), none on placebo. Incidence of gastrointestinal disorders and of infections was higher in participants treated with AUT00063 than in those treated with placebo. Gastrointestinal disorders were reported by 10 participants (22.7%) on AUT00063 and five (10.6%) on placebo, while infections were reported by nine participants (20.5%) on AUT00063 and two (4.3%) on placebo. Due to the nature and variety of the gastrointestinal disorders and infections, it is not thought that these treatment-emergent AEs were attributable to treatment with AUT00063.

The reporting frequency of SAEs was similar between AUT00063 and placebo, with one SAE being reported by one participant in each treatment group: placebo (2.1%, pyrexia of unknown origin, related); AUT00063 (2.3%, anxiety, not related). There were no deaths or other significant AEs reported during the study. The overall safety-related withdrawal rate was approximately 4%, and was similar between active and placebo groups. Analysis of vital signs, ECG and laboratory parameters did not reveal any clinically significant pattern in participants treated with AUT00063. For example, no abnormal heart rate was recorded on physical examination and changes in heart rate from screening were negligible at D1 and D28, in AUT00063 and placebo groups.

3.7. Planned exploratory outcomes

Findings from the exploratory analyses were as follows:

i) Pharmacokinetic analysis of the plasma concentration of AUT00063 for the Safety Analysis Set assessed at D28 ($n = 38$) revealed good exposure levels following treatment with AUT00063, with mean (median) plasma levels of AUT00063 of 4818.82 (4704.50) ng/ml; range 119.90–8011.30.
ii) For the eight TFI subscale scores, descriptive data for the Full Analysis Set are summarised in Table 7. Small reductions in scores were observed across most subscales from dosing day (D1) to D28, but for both treatment groups. Large standard deviations limit interpretation (c.f. Fackrell et al., 2016, 2018).
iii) The Full Analysis Set was subjected to exploratory analyses of success rate using two logistic regressions with the following predictors: treatment (AUT00063 vs placebo), gender (female vs male), age (years), tinnitus duration (years), functional impact of tinnitus (global TFI score), and degree of sensorineural hearing loss (pure tone average dB HL). Results are reported in Table 8. The first explored whether one might predict the binary outcome 'improver' from 'non-improver'. Five of the 36 participants in the AUT00063 group (13.9%) and eight of the participants in the placebo group (20.0%) suggested an 'improvement' defined by an individual treatment-related change in TFI ≥ -13 points. Remaining participants were classed as 'non-improvers'. Only tinnitus symptom severity was a predictor of improvement ($p = 0.0268$).

It was possible that there was a small subgroup of people with subjective tinnitus who did worse on AUT00063. Three participants (3/76 = 3.9%) treated with AUT00063 rated their tinnitus as "much worse" using a measure of the Clinical Global Impression (Improvement) scale at D28, compared to no participants on placebo. These participants also had a corresponding TFI change of >10 points (i.e. 10.8, 11.2, and 22 points respectively). The second logistic regression therefore explored whether one might predict the binary outcome 'worsener' from 'non-worsener' (Table 8). Eight of the 36 participants in the AUT00063 group (22.2%) and five of the participants in the placebo group (12.5%) suggested a 'worsening' defined by an individual treatment-related change in global TFI score ($\geq +10$ points). Remaining participants were classed as 'non-worseners'. Results showed no statistically significant effect, indicating an absence of any specific predictive characteristics for treatment-related change.

The Clinical Global Impression (Improvement) scale ratings were interrogated more broadly across the Full Analysis Set. A total of eight participants (8/76 = 10.5%) rated their tinnitus as 'slightly improved'; one participant (1/36 = 2.8%) in the AUT00063 group and seven (7/40 = 17.5%) in the placebo group. All other ratings

Table 4
Analyses examining the overall effect of AUT00063 compared to placebo (D28-D1) for the Full Analysis Set and the Completers Population. A positive adjusted mean difference indicates a smaller decrease in tinnitus symptom severity in the AUT00063 group compared to placebo. Vice-versa for a negative mean difference.

Comparison	Adjusted mean difference Estimate (95% CI)	P-value (two-sided)
Full Analysis Set		
AUT00063 versus placebo, TFI global score	1.56 (−5.90 to 9.01)	0.678
AUT00063 versus placebo, tinnitus loudness	−1.223 (−5.10 to 2.65)	0.530
Completers Population		
AUT00063 versus placebo, TFI global score	1.92 (−5.53 to 9.36)	0.608

Table 5
Analyses examining the change from dosing day (D1) to study end point (D28) on the primary and secondary efficacy endpoints for the Full Analysis Set. CI = Confidence Interval. ND = Not Done. A negative mean difference indicates a decrease in tinnitus symptom severity.

Comparison	D1 mean (SD)	D28 mean (SD)	n	Adjusted mean Estimate (95% CI)	P-value (two-sided)	D28 mean (SD)
Full Analysis Set						
AUT00063, TFI global score	41.82 (14.03)	40.25 (17.02)	36	−2.80 (−10.84 to 5.24)	0.489	40.63 (19.69)
Placebo, TFI global score	45.66 (14.10)	39.87 (17.39)	40	−4.35 (−12.67 to 3.96)	0.299	40.04 (18.19)
AUT00063, tinnitus loudness (dB SL)	17.37 (9.83)	15.40 (10.43)	34	1.51 (−3.10 to 6.13)	0.514	ND
Placebo, tinnitus loudness (dB SL)	16.03 (10.50)	16.18 (9.63)	39	2.74 (−2.40 to 7.87)	0.290	ND
Completers Population						
AUT00063, TFI global score	42.66 (14.330)	42.82 (15.279)	32	−0.09 (−8.10 to 7.91)	0.981	43.35 (18.112)
Placebo, TFI global score	45.22 (13.999)	38.89 (16.468)	39	−2.01 (−10.98 to 6.96)	0.655	39.52 (18.147)

Table 6
Summary of treatment emergent AEs that occurred in ≥5% of the Safety Population. AEs are coded using the Coding Dictionary MedDRA Version 17.0 (or higher). Counting is on a per-participant basis. If a participant reported the same event repeatedly then the event was counted only once.

System Organ Class Preferred Term	AUT00063 (N = 44) n (%)	Placebo (N = 47) n (%)	Overall (N = 91) n (%)
Number of participants with at least one treatment emergent AE	32 (72.7)	23 (48.9)	55 (60.4)
Nervous System Disorders	20 (45.5)	10 (21.3)	30 (33.0)
Headache	13 (29.5)	9 (19.1)	22 (24.2)
Dizziness	7 (15.9)	none reported	7 (7.7)
Gastrointestinal Disorders	10 (22.7)	5 (10.6)	15 (16.5)
Nausea	4 (9.1)	1 (2.1)	5 (5.5)
Infections and Infestations	9 (20.5)	2 (4.3)	11 (12.1)
Nasopharyngitis	4 (9.1)	none reported	4 (4.4)
Ear and Labyrinth Disorders	5 (11.4)	4 (8.5)	9 (9.9)
Ear Pain	3 (6.8)	1 (2.1)	4 (4.4)
Respiratory, Thoracic And Mediastinal Disorders	7 (15.9)	1 (2.1)	8 (8.8)
Oropharyngeal Pain	3 (6.8)	1 (2.1)	4 (4.4)

Table 7
Summary of the changes from dosing day (D1) to study end point (D28) on the TFI subscale scores for the Full Analysis Set. SD = standard deviation. A negative mean difference indicates a decrease in symptom severity.

TFI subscale	n	AUT00063, mean (SD)	n	Placebo, mean (SD)
Intrusiveness	35	−2.33 (17.238)	40	−3.58 (13.989)
Sense of control	36	−2.69 (22.191)	40	−9.43 (23.693)
Cognitive interference	36	−2.36 (17.313)	40	−8.70 (17.611)
Sleep disturbance	36	−4.99 (22.073)	40	−3.50 (13.495)
Auditory difficulties attributed to tinnitus	36	−2.00 (20.656)	40	−3.20 (15.914)
Relaxation	36	−3.51 (23.977)	40	−8.75 (20.233)
Tinnitus-specific 'quality of life'	36	1.66 (14.983)	40	−3.83 (16.384)
Emotional distress	36	2.83 (21.194)	40	−5.75 (16.282)

Table 8
Summary of the logistic regression analyses for the Full Analysis Set to predict improvers/non-improvers and worseners/nonworseners. * denotes significance at $p < 0.05$.

Predictor variable	Odds ratio Estimate (95% CI)	P-value
Improvers vs non-improvers		
Treatment (AUT00063 vs Placebo)	0.586 (0.151–2.277)	0.1007
Gender (female vs male)	1.092 (0.223–5.341)	0.9132
Age (years)	1.069 (0.987–1.158)	0.1007
Tinnitus duration (years)	0.214 (0.028–1.647)	0.1386
Functional impact of tinnitus (global TFI score at D1)	1.068 (1.008–1.132)	0.0268*
Degree of sensorineural hearing loss (pure tone average dB HL)	0.992 (0.928–1.061)	0.8252
Worseners vs non-worseners		
Treatment (AUT00063 vs Placebo)	2.078 (0.589–7.330)	0.2555
Gender (female vs male)	1.207 (0.280–5.215)	0.8007
Age (years)	1.007 (0.941–1.078)	0.8348
Tinnitus duration (years)	1.607 (0.255–10.118)	0.6132
Functional impact of tinnitus (global TFI score at D1)	1.018 (0.966–1.072)	0.5011
Degree of sensorineural hearing loss (pure tone average dB HL)	0.968 (0.903–1.037)	0.3524

were similar between AUT00063 and placebo groups, consistent with the null findings on the planned statistical analyses.

4. Discussion

4.1. Main observations

QUIET-1 was a carefully designed multi-centre phase IIa clinical trial that followed a pre-specified Clinical Trial Protocol and Statistical Analysis Plan, and was statistically powered to detect a difference between experimental and placebo groups. The findings produced a clear outcome. While the pharmacokinetic analysis confirmed systemic exposure to AUT00063 within the predicted therapeutic range, AUT00063 was not effective in alleviating the symptoms of subjective tinnitus at this dose level. The trial was stopped early for futility, a decision which was based on both ethical and resource perspectives. The results indicated that the daily dose of 800 mg capsules of AUT00063 taken for 28 days was safe and well tolerated, with the most common expected side effect (dizziness) being mild in nature, with these symptoms resolving without the need to stop or change dosing. We acknowledge that a 28-day study in 91 participants is not sufficient to exclude long-term safety concerns or rare adverse reactions.

4.2. Reflections on bridging the gap between animal and human research

First, systemic drug exposures to AUT00063 achieved in subjects in the QUIET-1 trial were comparable to those achieved in rodent models. Specifically, the plasma levels of AUT00063 in QUIET-1 participants were similar to those achieved in rodents following a 30 mg/kg i.p. dose which was effective at reducing spontaneous neuronal firing in noise-exposed hamsters (Glait et al., 2018). These data support the conclusion that appropriate drug concentrations were likely to have been achieved in the central nervous system. However, whether or not these concentrations were sufficient to achieve the required engagement with the biological target could not be ascertained from the current data. A potential biomarker for target engagement (pharmacoEEG) was included in the trial protocol, but these measures were only achieved for seven participants, and thus were not informative.

Several important aspects of preclinical data did not translate well from animal to human. Using the available preclinical data from rodent models, the prediction about the human therapeutic benefits of AUT00063 for tinnitus had been inferred from data gathered on a surrogate neurobiological marker (i.e. reduction in spontaneous hyperactivity in the brainstem and midbrain) (Anderson et al., 2018; Glait et al., 2018). It was always realised that this prediction had a number of important caveats, since the exact neural substrate of subjective tinnitus is unknown. One caveat concerns the principle that spontaneous hyperactivity plays a determining role in tinnitus. Other neural mechanisms are likely to be implicated. For example, Roberts et al. (2010) concluded that neural synchrony in spontaneous firing may be the more prominent neural correlate of tinnitus because it is more likely to impact postsynaptic targets and recruit cortical and downstream neurons into a tinnitus percept. Changes in spontaneous neural synchrony also precede hyperactivity in an acute preparation of noise-induced tinnitus, following more closely the perceptual experience of tinnitus (Eggermont, 2013). In 2013, Eggermont reviewed a series of animal studies that had collected data using electrophysiological recordings and behavioural tests in the same animals (Eggermont, 2013). Findings indicated a discrepancy between putative cortical electrophysiological correlates of tinnitus and presumed behavioural ones, leading Eggermont to conclude that cortical measures of spontaneous hyperactivity and neural synchrony seem to be correlates of hearing damage through noise exposure, rather than of tinnitus per se. While most of the discrepancies would disappear if neural activity in subcortical structures were taken to be the main determinant of performance on behavioural tests, but then this would call into question the role of cortical processing in the perception of tinnitus. The search for neural signature of tinnitus requires animal models that show reliable and robust behavioural evidence of subjective tinnitus, under conditions similar to those that cause the condition in humans. The lack of a standard behavioural model is yet another challenge to be solved in order to make substantive advances in drug development for tinnitus. In the meantime, more direct measures of auditory subcortical activity in people with tinnitus could allow better translation of rodent study results in humans (Glait et al., 2018), but again these techniques too are not without challenge (c.f. Pierzycki et al., 2016). A final caveat concerns the equivalence of single (acute) and repeated (chronic) dosing. As Glait et al. (2018) point out; the rodent model used an acute dosing of AUT00063, whereas the QUIET-1 trial design used a chronic dosing in humans. The persistence of the neurobiological effects of AUT00063 is not known since that experimental work has not yet been done. These uncertainties suggest that the confidence in the predictive validity of certain animal models of noise-induced hearing loss and tinnitus were perhaps somewhat optimistic.

We conclude that, despite a compelling body of in vitro and in vivo rodent data from models of noise-induced hearing loss and tinnitus supporting the neurobiological modulatory effects of AUT00063 on central auditory pathologies, the drug did not influence the subjective patient experience of tinnitus. The findings from QUIET-1 underlie the general scientific challenge and commercial risk in transitioning from preclinical drug development in the animal model to clinical application in patients. Such challenge and risk are certainly not restricted to hearing-related conditions and are widely acknowledged across any biological complex system (e.g. Demetrius, 2005; Shanks et al., 2009).

4.3. Learnings about the design of phase IIa pharmaceutical trials in tinnitus

When the QUIET-1 trial was designed there had been few tinnitus trials with novel drugs on which to select an appropriate study population, optimal outcome measures and sample size that would assure an informative outcome from the trial and meet regulatory standards. Several general literature reviews on tinnitus trial design (e.g. Tyler et al., 2007; Landgrebe et al., 2012), and some recent examples of phase II trials (e.g. Suckfüll et al., 2011) were available, but there was little consensus regarding optimal design. A review of the strategies for recruiting participants into hearing-related clinical trials have been published elsewhere (Sanchez et al., 2018), and so here we reflect on the specific eligibility criteria and the choice of outcome measures in the QUIET-1 trial.

The QUIET-1 trial was the first study to evaluate AUT00063 in a population of patients with tinnitus, and as such, was intended to search for evidence of efficacy. In the phase IIa stage of the drug development pipeline, trials are designed to establish an answer to the question about whether the preclinical theory can be translated into human patients, and so they investigate whether the drug has any potential therapeutic benefit in a relatively homogeneous population that might be most sensitive to the drug's mechanism of action. A phase IIa study design is not intended to generate findings that could be generalised to a broader tinnitus population. If the drug fails in this stage, then it is very unlikely to succeed when the variability of the patient group is broadened at the phase III stage. In light of the context of its phase of development, the QUIET-1 trial had more restrictive eligibility criteria than the usual clinical

presentation. Eligibility criteria limited the duration of tinnitus, degree of tinnitus symptom severity, and degree of sensorineural hearing loss in order to promote greater homogeneity. These were all considered important baseline characteristics which could influence sensitivity to drug treatment. These criteria were set to balance the different needs of the trial against feasibility of assessment and likelihood of recruiting the target number of patients.

The duration of tinnitus symptoms required for inclusion in the QUIET-1 study was set to >6 months and <18 months. Although somewhat arbitrary, these durations were chosen to exclude participants with relatively acute tinnitus that might spontaneously remit, and those with a long-standing tinnitus that may involve different mechanisms to those responsible for the generation of tinnitus and may be more resistant to change (Landgrebe et al., 2012).

Regulatory approval for a new drug requires evidence that it provides an *important clinical benefit*. And so although AUT00063 targeted the central component of tinnitus associated with the perception of tinnitus, the trial examined its indirect effects on reactions to tinnitus as the primary outcome measure. The Tinnitus Functional Index (Meikle et al., 2012) was chosen based on reports of its sensitivity to treatment-related change and validity with respect to other diagnostic measures of tinnitus (this is discussed further below). Participants in the QUIET-1 trial were included with a global TFI score $\geq$24 and $\leq$ 68. This range of scores was chosen to reflect those who might be most likely to respond to treatment based on raw data collected from 151 patients recruited from National Health Service audiology clinics into a non-randomised observational study (Fackrell et al., 2018) and 285 members of the general public participating in a randomised controlled trial of a novel tinnitus device (ClinicalTrials.gov Identifier: NCT01541969) (see Supplementary Material for the descriptive statistics used to define the cut-offs). The assumption was that participants with scores up to 24 would have little room to show improvement, whereas those with TFI scores of 68 and above might require additional clinical intervention that could interfere with the trial. These choices seem reasonable given our later work showing that global TFI scores up to 28 denote a *small perceived problem*, while scores of 66 or more denote a *very big problem* in the same UK population (Fackrell et al., 2018).

Finally, the inclusion criteria for hearing thresholds were set to ensure the practicality of patient participation in the trial. An important secondary efficacy endpoint for the study was Tinnitus Loudness Matching which required the participant to listen to sounds and match their loudness to their subjective tinnitus percept. Consequently, thresholds (see Table 1) were selected to exclude participants with moderate to severe hearing loss, which might interfere with their ability to participate in the trial.

Selecting outcomes which are relevant, appropriate and of importance to patients in real-world clinical settings is a critical design component. Indeed, it has been claimed that relatively few trials make a meaningful contribution to patient care, often as a result of the way that the trial outcomes are chosen, collected and reported (Heneghan et al., 2017). For trials evaluating novel drug products, the outcome must also satisfy the standards required by the regulatory authorities. In current tinnitus practice, this means choosing an outcome which demonstrates an improvement in everyday functioning or quality of life *and* one that demonstrates a reduction in the tinnitus percept.

With regards to everyday functioning or quality of life, a review in 2010 (Kamalski et al., 2010) highlighted limitations in the evidence to support the suitability of four of the most common multi-item questionnaires in measuring changes in everyday tinnitus symptom severity (Tinnitus Handicap Inventory, Tinnitus Handicap Questionnaire, Tinnitus Reaction Questionnaire and Tinnitus Questionnaire). Prior to the QUIET-1 trial, the TFI had been developed as a new scale to be responsive to treatment related change (Meikle et al., 2012). We therefore used the best available evidence for what constitutes a clinically meaningful change in tinnitus symptom severity and for the mean and standard deviation of scores in a UK sample to select the TFI as the primary endpoint and to calculate a powered sample size. Since then new evidence specific to the UK population has been published that would lead us to revisit those original considerations. This evidence revises estimates about the structural validity of the TFI and its responsiveness (Fackrell et al., 2016, 2018). Moreover, a global consensus across different stakeholder groups involving patients, healthcare professionals, clinical researchers and representatives from the pharmaceutical industry has recently made a recommendation for tinnitus intrusiveness to be the common standard that reflects the impact of tinnitus and for this construct to be assessed in every pharmaceutical trial of subjective tinnitus (Hall et al., 2018a). In this study, tinnitus intrusiveness was defined as *Noticing the sound of tinnitus is there and it is invading your life or your personal space*. Further research is warranted to determine whether there are any available instruments that adequately measure this particular definition of tinnitus intrusiveness.

Tinnitus loudness is the most commonly assessed perceptual attribute of tinnitus yet there is no gold standard for estimating it (Hall et al., 2016). In the QUIET-1 trial, we chose a loudness matching procedure based on one that had been developed by expert healthcare practitioners (Vernon and Meikle, 1981). Nevertheless, the data gathered during the QUIET-1 trial have enabled us to conclude that this tinnitus loudness matching test performs sub-optimally on psychometric criteria for reliability and validity compared to a simple self-rating tool using a Numerical Rating Scale (Hall et al., 2017). But single questions about loudness are not without their own limitations as they seem to be interpreted by patients as synonymous with functional impacts.

In conclusion, despite attempts to design a trial in patients with subjective tinnitus who might be most sensitive to drug treatment, the study failed to detect superiority of AUT00063 and met criteria for futility at the interim analysis. While we are not saying that futility was attributable to the choice of outcome measures, it is likely that the primary and secondary tinnitus outcomes were not the most sensitive to detecting tinnitus-related changes due to the drug treatment (see also Glait et al., 2018). Again, further research is warranted to identify instruments that are most sensitive to assessing change associated with taking a drug for tinnitus. This may be a specific instrument for assessing a narrowly defined construct that is currently measured by a single question or a subscale or a larger questionnaire.

Declaration of conflicting interests

CH Large is a director and shareholder of Autifony Therapeutics Ltd. At the time of conducting the QUIET-1 trial, Jeannette Watson, Alice Sharman, John Hutchison and Peter Harris were employees of Autifony Therapeutics Ltd.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heares.2019.03.018.

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