Autifony

Clinical trial report

Trial title	A randomised, double-blind, placebo-controlled, single and repeated dose escalation study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of AUT00201 in healthy male and female volunteers
Version and date of report	Version 1, 31 August 2021
EudraCT number	2019-002160-26
HMR trial code	18-022
Sponsor trial code	AUT011201
Investigational product	AUT00201
Trial indication	None
Phase of trial	Phase 1
Principal investigator	[redacted] HMR Cumberland Avenue, Park Royal London NW10 7EW, UK [redacted]
Trial sponsor	Autifony Therapeutics Limited Stevenage Bioscience Catalyst Gunnels Wood Road, Stevenage Hertfordshire SG1 2FX, UK [redacted] [redacted] [redacted]
Date first subject screened	10 October 2019
Date of last subject visit	23 December 2020

This trial was conducted in accordance with EU Directive 2001/20/EC, applicable national statutory requirements, and ICH GCP, including the archiving of essential documents. The protocol was approved by the Medicines and Healthcare products Regulatory Agency and an independent recognised research ethics committee before the trial began, and written informed consent was obtained from each subject. This report has been prepared in accordance with ICH E3 and ICH M4E.

1 Signatures

We, the undersigned, confirm that this report is an accurate and comprehensive record of those parts of the trial for which we are responsible:

[redacted] 06-Sep-2021
Date

[redacted] 06-Sep-2021
Date

[redacted] 06-Sep-2021
Date

I, the undersigned, confirm that an audit has been done on this trial report. The results of that audit revealed no significant deviations from the International Council for Harmonisation Guideline for Good Clinical Practice.

[redacted] 07-Sep-2021
Date

2 Synopsis

Sponsor: Autifony Therapeutics Limited	
Name of finished product: AUT00201	**Name of active ingredient:** AUT00201
Title: A randomised, double-blind, placebo-controlled, single and repeated dose escalation study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of AUT00201 in healthy male and female volunteers	
Investigator(s): [redacted]	
Trial centre(s): Hammersmith Medicines Research, Cumberland Avenue, Park Royal, London NW10 7EW	
Publication(s): None at the time of this report.	
Trial period: 10 October 2019–23 December 2020	**Phase of Development:** 1
Date of the report: 31 August 2021	

Objectives:

Primary:

- To assess the tolerability of single oral doses of AUT00201 in healthy men and women
- To assess the tolerability of repeated oral doses of AUT00201 in healthy men

Secondary:

- To assess the safety of single oral doses of AUT00201 in healthy men and women
- To assess the safety of repeated oral doses of AUT00201 in healthy men
- To assess the pharmacokinetic (PK) profile of AUT00201 after single oral doses of AUT00201 in healthy men and women
- To assess the PK profile of AUT00201 after repeated oral doses of AUT00201 in healthy men
- To determine the effect of food on the bioavailability of single oral doses of AUT00201 in healthy men

Exploratory:

- To further assess the PK profile of AUT00201 after single oral doses of AUT00201 in healthy men and women
- To further assess the PK profile of AUT00201 after repeated oral doses of AUT00201 in healthy men
- To explore whether single oral doses of AUT00201 modify brain activity, as measured using pharmaco-electroencephalography (phEEG) in healthy men
- To explore the effects of repeated oral doses of AUT00201 on audiological assessments
- To explore the metabolite profile of single and repeated oral doses of AUT00201

Methods:

This was a randomised, double-blind, placebo-controlled, dose escalation study to assess the safety, tolerability, PK and pharmacodynamics (PD) of AUT00201. The study was in 2 parts, as follows.

Part A investigated single doses of AUT00201, and was divided into 3 sub-parts:

- Part A1 investigated ascending doses in healthy men,
- Part A2 investigated ascending doses in healthy women
- Part A3 was an open-label assessment of the effect of food on the bioavailability of AUT00201 in healthy men

Part B investigated repeated doses of AUT00201 in healthy men.

It was intended that cohorts should receive higher doses as the study progressed. However, the dose would not be escalated in any study part unless the safety and tolerability of previous doses were acceptable, and plasma concentrations of AUT00201 and AUT00208, a synthetic-route intermediate and potential degradant of AUT00201, were predicted to remain below the toxicokinetic exposure limit.

Part A2 and A3 could not proceed until the Safety Review Group (SRG) had reviewed Part A1 safety, tolerability and PK data (up to 24 h after dosing) from all dose levels (for Part A2) and from at least 3 dose levels (for Part A3). The first dose selected in Part A2, and any dose selected in Part A3, could be no higher than 50% of the highest dose previously tested in Part A1 that caused no safety concerns, and doses were increased such that they did not exceed 3 times the highest dose level that was found to be safe and well tolerated. A dose level could be repeated or decreased, as required, based on emerging results.

An individual daily dose could be tested in Part B only if that (or a higher) individual dose had already shown acceptable tolerability in Part A.

All unplanned dose levels were given in the fed or fasted state based on emerging PK data from Part A3 (food effect).

Part A

There was a wash-out of at least 10 days between treatments in Part A.

Part A1

24 subjects were enrolled in 3 groups of 8 men each (Groups 1–3). Each subject attended up to 4 study sessions (Sessions 1–4), in which they received either a single dose or 2 single doses (one in the morning and one in the evening) of AUT00201 or placebo, by mouth as capsules. Each subject received up to 2 doses of placebo and up to 5 doses of AUT00201 during the trial. Groups were treated sequentially. The first dose received by each group was equivalent to, or lower than, the highest dose received by the previous group (if applicable).

Subjects were randomised to one of 4 treatment sequences such that, in each session, 6 subjects received AUT00201 and 2 received placebo (3:1 ratio).

Because AUT00201 had never been given to humans before, each new ascending dose was staggered: 3 sentinel subjects were dosed first and the remaining 5 subjects at least 23 h later.

The planned starting dose for Group 1, Session 1 was 3 mg. Subsequent doses were decided by the SRG and are given in Table S1.

Table S1: Part A1 doses of AUT00201

Group	Session 1	Session 2	Session 3	Session 4
1	3 mg	6 mg	12 mg	20 mg
2	20 mg	60 mg	160 mg	300 mg
3	160 mg	320 mg	2 x 160 mg	2 x 200 mg

Doses were given in the fasted state in Groups 1 and 2 and in the fed state in Group 3.

Part A2

8 women were enrolled in a single group (Group 1). Up to 4 study sessions were planned (Sessions 1–4). In each session, subjects would receive either a single dose or 2 single doses (one in the morning and one in the evening) of AUT00201 or placebo, by mouth as capsules. Therefore, each subject would receive up to 2 doses of placebo and up to 6 doses of AUT00201 during the trial. In the event, subjects only participated in 2 study sessions, owing to the trial objective being met, with no further dosing required.

In each session subjects were randomised to AUT00201 or placebo in a 3:1 ratio. Part A2 doses of AUT00201 are given in Table S2.

Table S2: Part A2 doses of AUT00201

Group	Session 1	Session 2	Session 3	Session 4
1	160 mg	2 x 140 mg	–	–

Doses were given in the fasted state in Session 1, and in the fed state in Session 2. Subjects did not participate in Sessions 3 and 4 because the sponsor considered the objective for this sub-part to have been met after Sessions 1 and 2.

Part A3

Up to 2 groups of 8 men were planned (Groups 1 and 2). Group 2 was optional and was not deemed necessary.

Each subject in Group 1 received a single oral dose of 80 mg AUT00201 on 2 occasions, in the fed and fasted state, respectively. There were at least 10 days between treatments.

Subjects were randomised to one of 2 treatment sequences, such that, in each session, 4 subjects each received AUT00201 in the fed and fasted states in a 1:1 ratio (Table S3).

Table S3: Part A3 doses of AUT00201

Group		Session 1	Session 2
1	n=4	80 mg fed	80 mg fasted
	n=4	80 mg fasted	80 mg fed

Part B

Up to 4 groups of 8 subjects (Groups 1–4) were planned; 2 groups were enrolled. Each group received AUT00201 or placebo up to twice daily by mouth, as capsules, for 14 days. In each group, 6 subjects received active treatment and 2 received placebo (3:1 ratio).

The dose, dosing regimen (once or twice daily), and whether the dose was taken in the fed or fasted state, was determined based on review of the available safety, tolerability and PK results from Part A and (if applicable) previous groups in Part B.

Part B doses and dosing regimens are given in Table S4.

Table S4: Part B doses of AUT00201

Group	Dose regimen
1	twice-daily 100 mg fed
2	twice-daily 40 mg fed

Subjects were screened within the 28 days before their first dose of trial medication.

Part A: In each study session, subjects remained resident on the ward from the day before their dose (Day–1) until 48 h after dosing (Day 3).

In Parts A1 (Groups 1 and 2 only) and A3, they attended 3 outpatient visits in each session (Days 4, 5, and 7). In Part A1 (Group 3) and Part A2, they attended 2 outpatient visits in each session (Days 4 and 5). All subjects returned for a follow-up visit 12–16 days after their final dose (Day 13–17).

The duration of residence could be altered, and outpatient visits could be added, based on emerging data.

Part B: Subjects remained resident on the ward from the day before their first dose (Day –1) until 48 h after their final dose (Day 16). They attended 2 outpatient visits (Days 17 and 18) and returned for a follow-up visit 12–16 days after their final dose (Day 26–30).

Number of subjects:

Planned: Up to 80 healthy volunteers (up to 40 men and 8 women in Part A, and up to 32 men in Part B), excluding replacements.
Enrolled: 57 healthy volunteers (33 men and 8 women in Part A, and 16 men in Part B), including 1 replacement.
Completed: 55 healthy volunteers (32 men and 8 women in Part A, and 15 men in Part B).

Diagnosis and main criteria for inclusion:

Healthy men aged 18–45 years (Parts A1 and A3, and Part B), or healthy women aged 18−65 years (Part A2), with a body mass index of 18.0–31.0 kg/m2; deemed healthy on the basis of clinical history, physical examination, electrocardiogram (ECG), electroencephalogram (EEG; Part A1 only), vital signs, and laboratory tests of blood and urine; willing to comply with the contraception requirements and give fully informed written consent to participate in the trial.

Test and reference products, dose, mode of administration and batch numbers:

Each subject received oral doses of AUT00201 or placebo. AUT00201 was administered as capsules containing 3, 20 or 100 mg active substance. All treatments were administered orally, in the fed or fasted state, with about 240 mL of water.

Matching placebo capsules were supplied, identical to the AUT00201 capsules minus the active ingredient and [redacted] excipients.

The drug product was presented as Swedish orange coloured, hard gelatine capsules (size 0 or size 3), with no identifying markings, in strengths of 3, 20 and 100 mg, for use in the trial. AUT00201 is a white to slightly coloured solid powder. [redacted] Capsules were packed in opaque white, high density polyethylene bottles with tamper-evident, child-resistant closures.

The drug product was manufactured in accordance with Good Manufacturing Practice at [redacted] The shelf life of AUT00201 was about 1 year, when stored in the clinical trial packaging, below 30°C. There were several extensions to the shelf life, as described below.

Subjects received doses as described in Table S1 (Part A1), Table S2 (Part A2), and Table S4 (Part B); subjects in Part A3 received 80 mg AUT00201 in both the fed and fasted states.

[redacted]

Duration of treatment:

In Parts A1 and A2, subjects received up to 5 doses of AUT00201.

In Part A3, subjects received 2 single doses: once each in the fed and fasted states.

In Part B, subjects received twice-daily repeated doses for 14 days.

Criteria for evaluation and endpoints:

Tolerability: AEs.

Safety: Laboratory assessments (routine haematology, biochemistry, and urinalysis), physical examination, 12-lead ECG, 5-lead telemetry, 3-lead Holter monitoring, vital signs (supine and standing), the Columbia Suicide Severity Rating Scale (C-SSRS) and results of EEGs (Part A1 only).

Pharmacokinetic: Blood samples for assay of AUT00201 were taken before and at frequent time points up to 144 h after each dose in Part A, and before each daily repeated morning dose and frequently up to 96 h after the last dose in Part B. Urine was collected continuously for assay of AUT00201 for 48 h after single doses in Part A and 48 h after the first and last doses in Part B. Plasma samples were also assayed for AUT00208.

The following PK parameters were derived.

Single doses: C_{max}, C_{max}/D, t_{max}, t_{last}, AUC_{24}, AUC_{24}/D, AUC_{48}, AUC_{last}, AUC_{last}/D, AUC_{inf}, AUC_{inf}/D, AUC_{extr}, $t_{½}$, λ_z, CL/F, V_z/F, MRT_{last} and MRT_{inf} of AUT00201 in plasma. $AUC_{0–12}$, $AUC_{12–24}$, $C_{max,0–12}$, $C_{max,12–24}$, $t_{max,0–12}$, and $t_{max,12–24}$ in case of twice-daily dosing, with the second dose given 12 h after the first dose. Dose proportionality was assessed.

Ae_t, Ae/D and CL_R of AUT00201 in urine.

Repeated doses: PK parameters of AUT00201 in plasma.

- On Days 1 and 14: AUC_{tau}(AM), AUC_{tau}(PM), AUC_{24}, C_{max}(AM), C_{max}(PM), t_{max}(AM) and t_{max}(PM).
- On Day 14: AUC_{inf}, AUC_{48}, $R_{ac}(C_{max})$, R_{ac}(AUC), $t_{½}$, λ_z, CL_{ss}/F, V_Z/F, MRT_{inf}, and MRT_{tau}.

On Days 2–14: C_{trough}, to assess attainment of steady state.
PK parameters of AUT00201 in urine.

- On Days 1 and 14: Ae_{12}, Ae_{12}/D, Ae_{24}, Ae_{24}/D, and Ae_{48}, Ae_{48}/D; and $CL_{R,12}$ and $CL_{R,24}$.
- On Day 14: $CL_{R,48}$

Pharmacodynamic:

Part A: phEEG was assessed in Part A1.

Part B: Audiological assessments were done at baseline and about 4 h postdose on Days 1, 7 and 14. Leeds Sleep Evaluation Questionnaire (LSEQ) was done at predose on Day 1, Day 7 and Day 14, and at 24 h after the last dose.

Statistical methods:

The trial was an exploratory one, and there were no null hypotheses to be tested.

Safety and tolerability data: Safety and tolerability data were not subjected to formal analysis. All data were summarised using descriptive statistics (see below).

Pharmacokinetic data: PK parameters were summarised using descriptive statistics (including mean, standard deviation (or standard error), median, minimum, and maximum; 95% confidence intervals [CI] were presented where appropriate for data interpretation).

Food effect

To assess the effect of food on AUT00201 PK in Part A3, C_{max}, AUC_∞ and AUC_{24} were logarithmically transformed and subjected to analysis of variance (ANOVA), with session and treatment as fixed effects and subject as a random effect. The estimated least-squares (LS) means and residual variation from the model were used to construct the 90% CIs of the ratio (fed:fasted) of geometric means of each parameter. Effect of food was concluded if the 90% CIs were not contained within the 80–125% acceptance interval.

Dose proportionality

In Parts A1, A2 and B, dose proportionality of the PK of AUT00201 in plasma was assessed using the power model. For the dose-dependent parameters AUC_{inf}, AUC_{tau} and C_{max}, dose proportionality was concluded if the 95% confidence interval of the slope (log PK parameter versus log dose) included the value 1.0.

Pharmacodynamic data: Audiology data were summarised using descriptive statistics (as described above) and analysed using parametric or inferential statistics.

Inferential statistics were performed with the absolute mean change from baseline in otoacoustic emission (OAE) signal-to-noise ratio (SNR) and Portable Automated Rapid Testing (PART)-Spatial Release Test data to Days 1, 7, and 14 were analysed using an analysis of covariance (ANCOVA) model with treatment as a fixed effect and the baseline assessment as a continuous covariate, for each area of the test and frequency. The covariate adjusted LS means (with standard errors) change from baseline, and treatment differences (AUT00201 – placebo, with associated 2-sided 95% CI and p value), were presented.

Results:

Safety and tolerability: There were no deaths, serious AEs (SAEs), or otherwise significant AEs. No subject was withdrawn from the study owing to a treatment-emergent AE (TEAE). All TEAEs were of mild or moderate intensity.

Table S5: Summary of treatment-emergent adverse events

Subjects with:	Part A (N=41)	Part B (N=16)	All Subjects (N=57)
	n (%) [number of TEAEs]		
TEAEs	18 (43.9) [35]	10 (62.5) [27]	28 (49.1) [62]
TEAEs related to treatment	6 (14.6) [8]	6 (37.5) [10]	12 (21.1) [18]
TEAEs leading to withdrawal	0	0	0
Treatment-emergent serious AEs	0	0	0
Mild as highest TEAE severity	7 (17.1)	9 (56.3)	16 (28.1)
Moderate as highest TEAE severity	11 (26.8)	1 (6.3)	12 (21.1)
Severe TEAEs	0	0	0
Fatal TEAEs	0	0	0

N: total number of subjects; n: number of subjects with a TEAE; TEAE: treatment-emergent adverse event. Subjects with > 1 TEAE in any treatment session are counted only once per system organ class and preferred term.

Part A

In Part A, male and female subjects received single doses of AUT00201. 18 (43.9%) subjects had at least 1 TEAE during the study across all treatment sessions (35 TEAEs in total). 6 (14.6%) subjects had at least 1 TEAE considered by the investigator to be possibly drug related across all treatment sessions (8 TEAEs in total).

Part A1 (male subjects)

After dosing with AUT00201 in the fasted state, TEAEs were recorded in: 1 (16.7%; 2 TEAEs) subject after 3 mg; 3 (25.0%, 5 TEAEs) subjects after 20 mg; 1 (16.7%; 3 TEAEs) subject after 60 mg; 4 (66.7%; 4 TEAEs) subjects after 160 mg; and 1 (16.7%; 1 TEAE) subject after 300 mg. No subjects had TEAEs after 6 or 12 mg AUT00201 in the fasted state.

After dosing with AUT00201 in the fed state, TEAEs were recorded in 1 (16.7%; 1 TEAE) subject after a single dose of 160 mg, and 1 (16.7%; 3 TEAEs) subject after 2 doses of 160 mg taken 12 h apart (2 × 160 mg). No subjects had TEAEs after 320 mg or 2 × 200 mg AUT00201 in the fed state.

4 (16.7%) subjects had 5 TEAEs after placebo in either the fed or fasted state.

There was little evidence that dose level, or dosing regimen (single dose or two doses 12 h apart), affected the incidence of TEAEs: possibly drug-related TEAEs were recorded in 2 (16.7%; 2 TEAEs) subjects after 20 mg and 1 (16.7%; 1 TEAE) subject each after 160 mg and 300 mg AUT00201 in the fasted state; and in 1 (16.7%; 1 TEAE) subject after 2 × 160 mg AUT00201 in the fed state. No subject had possibly drug-related TEAEs after 3, 6, 12 or 60 mg AUT00201 in the fasted state, or after 160, 320, or 2 × 200 mg AUT00201 in the fed state. 1 (4.2%) subject had 2 possibly drug-related TEAEs after placebo.

Part A2 (female subjects)

TEAEs were recorded in 1 (16.7%; 1 TEAE) subject after 160 mg AUT00201 in the fasted state, and in 2 (33.3%; 2 TEAEs) subjects after 2 doses of 140 mg AUT00201 in the fed state, taken 12 h apart. 2 (50.0%) subjects had 3 TEAEs after placebo in the fasted or fed state.

A single possibly drug-related TEAE was recorded in 1 (25.0%) subject after placebo; no subject in Part A2 had a possibly drug-related TEAEs after dosing with AUT00201.

Part A3 (fed/fasted crossover, male subjects)

Dosing in the fed state did not affect the incidence of TEAEs: after 80 mg AUT00201 given to the same subjects in the fed and fasted states, TEAEs were recorded in 2 (25.0%) subjects after each treatment (3 and 2 TEAEs after dosing in the fed and fasted state, respectively). No possibly drug-related TEAEs were reported after either treatment.

All of Part A

Headache was the most common TEAE overall, reported by 6 (14.6%) subjects across all treatment sessions (8 TEAEs). Headache was considered by the investigator to be possibly drug related in 2 (4.9%) subjects across all treatment sessions (3 TEAEs), but there was no clear association with AUT00201 dose. Other TEAEs that were considered possibly drug-related were only recorded in single subjects, and included dizziness, abdominal discomfort, diarrhoea, dry mouth, and muscle twitching. No relationship between AUT00201 dose and the prevalence of those TEAEs was apparent.

Part B

In Part B, male subjects received 14 days' repeated AUT00201 twice daily (BID). 10 (62.5%) subjects had at least 1 TEAE during the study (27 TEAEs in total). TEAEs were recorded in 4 (66.7%; 5 TEAEs) subjects after 40 mg and 3 (50.0%; 9 TEAEs) subjects after 100 mg AUT00201 BID; 3 (75.0%) subjects had a total of 13 TEAEs after placebo BID.

6 (37.5 %) subjects had at least 1 TEAE considered by the investigator to be possibly drug related (10 TEAEs in total). Unlike in Part A, there was evidence in Part B that dose level might have affected the incidence of TEAEs: possibly drug-related TEAEs were recorded in

2 (33.3%; 2 TEAEs) subjects after 40 mg and 3 (50.0%; 5 TEAEs) subjects after 100 mg AUT00201 BID; 1 (25.0%) subject had 3 TEAEs after placebo BID. However, owing to the small group sizes (N=6 on each active treatment, N=4 on placebo), that trend was not strong enough to be conclusive.

In Part B, TEAEs that were considered possibly drug-related were recorded only in single subjects, and included: gastrointestinal disorders (abdominal discomfort, abdominal distension, lip dry, and mucous stools); nervous system disorders (dizziness, dizziness postural, and insomnia); general disorders and administration site conditions (fatigue); musculoskeletal and connective tissue disorders (muscle spasms); and skin and subcutaneous tissue disorders (rash). As in Part A, no clear relationship between AUT00201 dose and the prevalence of those TEAEs was apparent. However, TEAEs of dizziness experienced by a subject (2006) who received 100 mg AUT00201 BID may have been associated with vital signs of potential clinical importance (PCI) (postural decreases in systolic blood pressure and increases in heart rate). Vital signs are discussed further below.

Parts A and B

There were no clinically significant changes to vital signs. However, vital signs of PCI were common throughout the study: ≤ 66.7% of subjects per treatment after 1 or 2 single doses in Parts A1 and A2; 87.5 and 100.0% of subjects after single doses in the fasted and fed state, respectively, in Part A3; and 83.3−100.0% of subjects after repeated doses in Part B, had vital signs of PCI. While the incidence of vital signs of PCI tended to increase with AUT00201 dose in Part A, there was no increase with dose in Part B. However, it is worth noting that vital signs were recorded over a much longer period in Part B (16 days) compared with Part A (4 days per treatment).

Overall, AUT00201 was well tolerated. There were no clinically significant findings or changes in clinical laboratory variables, the results of physical examinations, or ECGs, during the study. There were no positive results on C-SSRS. While 2 subjects had EEG abnormalities after dosing in Part A1, those were short-lived and showed no clear association with AUT00201 administration.

Pharmacokinetics

Part A

Selected PK parameters of AUT00201 are summarised in Table S6 and Table S7 (Part A1), and Table S8 (Parts A2 and A3).

AUT00201 was rapidly absorbed in Part A. After single or two 12-hourly (BID) doses of 3−320 mg in male subjects in either the fasted or fed state (Part A1), most t_{max} were 0.5−4.0 h, but 2 subjects (after 300 mg fasted and 160 mg BID) had t_{max} of 12 h in one of their study sessions.

C_{max} increased less than dose-proportionally after fasted doses of greater than 6 mg, while the relationship between AUC and dose was more variable. Statistical analysis confirmed with > 90% confidence that C_{max} increased less (slope 0.81, 90% CI 0.74, 0.88), and AUC more (AUC_{inf}: slope 1.13, 90% CI 1.09, 1.16; AUC_{24}: slope 1.06, 90% CI 1.02, 1.10), than

dose-proportionally across the 3−300 mg dose range. The C_{max} data suggest that AUT00201 may be more slowly absorbed after higher doses, but the AUC results suggest that overall bioavailability is greater after higher doses.

In Part A1, plasma concentrations were generally higher after doses in the fed state than in the fasted state. Geometric mean AUC was approximately dose proportional in fasting male subjects receiving 160 mg (AUC_{inf} 3405 ng·h/mL) and 300 mg (AUC_{inf} 7051 ng·h/mL; a 2.1-fold increase with a 1.9-fold increase in dose). In contrast, in the fed state the increase in AUC was much greater than dose proportional: geometric mean AUC_{inf} was 2988 ng·h/mL after 160 mg, and 9129 ng·h/mL after 320 mg (a 3.1-fold increase with a 2.0-fold increase in dose). Prior feeding, which increased the bioavailability of AUT00201, may also have contributed to lower variability in plasma concentration during 1−3 h postdose. The effect of food was further investigated in Part A3.

Two doses at 12-h intervals (BID) in male subjects on Day 1 yielded peak plasma concentrations similar to those after single doses: geometric mean $C_{max,0–12}$ and $C_{max,12–24}$ were 492 and 377 ng/mL after morning and evening doses of 160 mg in the fed state, compared with C_{max} of 380 ng/mL after a single morning dose in the same conditions. However, 12-hourly 160 mg doses yielded lower AUC than did the equivalent single dose: geometric AUC_{inf} was 7885 ng·h/mL after 160 mg BID and 9129 ng·h/mL after 320 mg. (PK analysis continues overleaf)

Table S6: Summary of AUT00201 plasma pharmacokinetic parameters in Part A1 after single doses: PK parameter population; N=6 per group; N=12 after 20 mg

AUT00201 parameter		Part A1								
		3 mg Male N=6	6 mg Male N=6	12 mg Male N=6	20 mg Male N=12	60 mg Male N=6	160 mg Male N=6	300 mg Male N=6	160 mg (fed) Male N=6	320 mg (fed) Male N=6
C_{max}/D (ng/mL/mg)	geo mean[a]	3.80	3.83	2.82	2.40	2.09	1.96	1.47	2.38	3.79
	95% CI	(2.25, 6.45)	(2.54, 5.80)	(1.32, 6.04)	(1.75, 3.31)	(1.35, 3.25)	(1.37, 2.82)	(0.89, 2.43)	(1.68, 3.37)	(2.88, 4.99)
	Range	2.07–8.17	2.70–6.95	1.43–7.20	1.19–5.80	1.04–3.15	1.47–3.84	0.847–3.22	1.34–3.24	2.34–5.06
t_{max} (h)	Median	1.00	1.50	1.00	2.00	2.50	2.46	3.46	3.00	2.00
	Range	0.5–2.0	1.0–2.0	0.5–2.0	0.5–3.0	1.0–3.9	0.5–3.9	0.5–12.0	1.0–6.0	1.0–4.0
$t_{½}$ (h)	mean[b]	3.52	7.50	5.66	7.17	7.93	9.05	8.43	6.72	8.34
	SD (%CVb)	1.85 (52.5)	2.79 (37.2)	1.51 (26.7)	1.39 (19.3)	1.64 (20.7)	2.35 (25.9)	1.2 (14.2)	1.48 (22.0)	2.07 (24.8)
	Range	1.15–5.78	3.84–10.9	2.87–7.07	4.11–8.48	5.89–10.0	6.79–13.3	6.99–10.6	4.82–8.84	6.01–11.0
AUC_{inf}/D (ng·h/mL/mg)	geo mean[a]	15.3	19.6	13.1	16.3	17.5	21.3	23.5	18.7	28.5
	95% CI	(7.89, 29.5)	(11.2, 34.3)	(8.24, 20.7)	(11.8, 22.4)	(12.8, 24.0)	(16.9, 26.9)	(18.7, 29.5)	(11.9, 29.2)	(19.7, 41.4)
	Range	6.86–42.3	12.5–53.8	7.41–23.1	8.56–49.6	12.4–27.1	15.6–26.7	18.1–31.8	12.2–40.0	16.7–48.2

N: number of subjects receiving treatment; SD: standard deviation; %CVb: between-subject coefficient of variation; CI: confidence interval; AUC_{inf}: area under the concentration time curve (AUC) from time 0 to infinity; AUC_{inf}/D: dose-normalised $AUC_{inf;}$ C_{max}: maximum plasma concentration; C_{max}/D: dose-normalised $C_{max;}$ t_{max}: time at which C_{max} is observed; $t_{½}$: terminal elimination half-life; a: geometric mean; b: arithmetic mean.

Table S7: Summary of AUT00201 plasma pharmacokinetic parameters in Part A1, after 2 single doses in the fed state: PK parameter population; N=6 per group

AUT00201 parameter		Part A1	
		160 mg BID (fed) Male N=6	200 mg BID (fed) Male N=6
$C_{max, 0–12}$ (ng/mL)	geo mean[a]	492	665
	95% CI	(333, 727)	(394, 1122)
	Range	263–788	383–1280
$C_{max, 12–24}$ (ng/mL)	geo mean[a]	377	966
	95% CI	(204, 696)	(580, 1609)
	Range	123–638	548–1970
$t_{max, 0–12}$ (h)	Median	2.42	2.00
	Range	1–6	1–6
$t_{max, 12–24}$ (h)	Median	4.03	5.00
	Range	4–12	2–8
$t_{½}$ (h)	mean[b]	7.87[c]	8.55
	SD (%CVb)	1.77 (22.5)	1.68 (19.6)
	Range	5.83–9.98	6.96–11.1
AUC_{inf} (ng·h/mL)	geo mean[a]	7885[c]	12513
	95% CI	(4571, 13599)	(7502, 20870)
	Range	4015–12117	7432–26994

BID: twice daily (2 doses 12 h apart); N: number of subjects receiving treatment; SD: standard deviation; %CVb: between-subject coefficient of variation; CI: confidence interval; AUC_{inf}: area under the concentration time curve (AUC) from time 0 to infinity; $C_{max,12–24}$: maximum plasma concentration from time 12 to time 24; $t_{max,0–12}$: time at which C_{max} is observed from time 0 to time 12; $t_{max,12–24}$: time at which C_{max} is observed from time 12 to time 24; $t_{½}$: terminal elimination half-life; a: geometric mean; b: arithmetic mean; c: n=5

Table S8: Summary of AUT00201 plasma pharmacokinetic parameters in Parts A2 and A3: PK parameter population; N=6 (Part A2) and 8 (Part A3) per group

AUT00201 parameter		Part A2		Part A3	
		160 mg Female N=6	140 mg BID (fed) Female N=6	80 mg (fed) Male N=8	80 mg (fasted) Male N=8
C_{max} (ng/mL)	geo mean[a]	352	–	227	130
	95% CI	(278, 446)	–	(153, 336)	(68.7, 247)
	Range	264–521	–	108–447	39.3–310
$C_{max, 0–12}$ (ng/mL)	geo mean[a]	–	506	–	–
	95% CI	–	(428, 598)	–	–
	Range	–	373–598	–	–
$C_{max, 12–24}$ (ng/mL)	geo mean[a]	–	581	–	–
	95% CI	–	(382, 883)	–	–
	Range	–	368–1000	–	–
t_{max} (h)	Median	2.00	–	3.50	3.50
	Range	1.00–3.00	–	0.50–8.00	1.00–4.00
$t_{max, 0–12}$ (h)	Median	–	2.50	–	–
	Range	–	1.00–3.98	–	–

AUT00201 parameter		Part A2		Part A3	
		160 mg Female N=6	140 mg BID (fed) Female N=6	80 mg (fed) Male N=8	80 mg (fasted) Male N=8
$t_{max,\ 12–24}$ (h)	Median	–	4.83	–	–
	Range	–	1.83–7.83	–	–
$t_{½}$ (h)	mean[b]	9.97	8.52	6.10	7.37
	SD (%CVb)	1.77 (17.7)	2.85 (33.5)	2.43 (39.8)	2.36 (32.1)
	Range	8.0–12.7	5.1–12.5	2.9–9.9	4.2–9.9
AUC_{inf} (ng·h/mL)	geo mean[a]	4033	9338	1407	1012
	95% CI	(3134, 5191)	(6820, 12785)	(797, 2484)	(596, 1718)
	Range	3121–6229	6442–13589	708–3179	498–2059

BID: twice daily (2 doses 12 h apart); N: number of subjects receiving treatment; SD: standard deviation; %CVb: between-subject coefficient of variation; CI: confidence interval; AUC_{inf}: area under the concentration time curve (AUC) from time 0 to infinity; C_{max}: maximum plasma concentration; $C_{max,0–12}$: maximum observed concentration from time 0 to time 12; ; $C_{max,\ 12–24}$: maximum observed concentration from time 12 to time 24; t_{max}: time at which C_{max} is observed; $t_{max,0–12}$: time at which C_{max} is observed from time 0 to time 12; $t_{max,\ 12–24}$: time at which C_{max} is observed from time 12 to time 24; a: geometric mean; b: arithmetic mean.

In Part A2, AUT00201 PK parameters in female subjects who received 160 mg (fasted) and 140 mg BID (fed) differed little from those in males receiving the same or similar doses.

In Part A3, AUT00201 absorption in male subjects was unaffected by food: t_{max} was 3.5 h after 80 mg in both fasted and fed states. However, geometric mean C_{max} was greater after dosing in the fed state (227 ng/mL) than in the fasted state (130 ng/mL). Statistical analysis showed that food increased the bioavailability of 80 mg AUT00201 in male subjects: geometric mean ratios for fed-fasted comparisons of C_{max}, AUC_{inf}, and AUC_{last} were 1.4–1.7; and 90% CI were above the range 0.8–1.25. Arithmetic mean $t_{½}$ was shorter (6.1 h) after fed doses than after fasted (7.4 h). That phenomenon was also seen in Part A1: after 160 mg doses in the fed (6.7 h) and fasted states (9.1 h), but not after higher doses (300 mg fasted: 8.4 h; 320 mg fed: 8.3 h).

After the 3 mg dose of AUT00201, arithmetic mean $t_{½}$ was artefactually short (3.5 h) owing to the brief persistence of quantifiable drug concentrations in plasma. In contrast, in Part A arithmetic mean $t_{½}$ was 5.7 to 9.1 h after 6–320 mg doses in fasted and fed states in male subjects; 7.9 and 8.6 h, respectively, after fed-state 160 and 200 mg BID in male subjects; and 10.0 and 8.5 h, respectively, after 160 mg (single dose; fasted) and 140 mg BID (fed) in female subjects. Low plasma concentrations probably led to the over-estimation of λ_Z in some male subjects who received 3–12 mg AUT00201.

In Part A1, arithmetic mean MRT_{inf} and MRT_{last} increased with dose after AUT00201 in the fasted state: from 4.53 and 3.24 h after 3 mg to 17.2 and 16.6 h after 300 mg, respectively; that probably reflects the brief persistence of quantifiable drug concentrations in plasma after the lower doses. Fed-state dosing in Part A1 yielded MRT about 60% of that after the same or similar doses in the fasted state. MRT in female subjects (Part A2) was slightly longer than after the same dose in male subjects. In Part A3, 80 mg AUT00201 in the fasted state yielded higher arithmetic mean MRT_{inf} and MRT_{last} (9.92 and 8.37 h) than after the same dose in the fed state (7.79 and 7.25 h).

Why MRT increased with dose more than did $t_{½}$ may be explained by how each parameter is derived: MRT is dependent upon AUC, whereas $t_{½}$ is derived from λ_Z plots. The widely-spaced sampling timepoints > 24 h postdose, coupled with unexpectedly high plasma concentrations at those timepoints, increased MRT without reducing λ_Z and thereby increasing $t_{½}$.

Part B

Selected PK parameters of AUT00201 are summarised in Table S9.

Table S9: Summary of plasma pharmacokinetic parameters in Part B after 1 and 14 days' repeated twice-daily (BID) with 40 and 100 mg AUT00201: PK parameter population; N=6 per group

AUT00201 parameter		40 mg AUT00201 BID N=6		100 mg AUT00201 BID N=6	
		Day 1	Day 14	Day 1	Day 14
C_{max} (AM) (ng/mL)	geo mean[a]	103	173	373	994
	95% CI	(57.3, 184)	(88.2, 341)	(213, 651)	(592, 1671)
	Range	48.9–242	87.4–566	171–604	539–2060
C_{max} (PM) (ng/mL)	geo mean[a]	134	159	564	796
	95% CI	(66.8, 270)	(81.9, 308)	(330, 962)	(420, 1507)
	Range	61.9–432	72.5–501	266–1110	444–1790
t_{max} (AM) (h)	Median	4.00	3.50	3.00	1.50
	Range	2.0–5.8	1.0–6.0	1.0–4.1	1.0–4.0
t_{max} (PM) (h)	Median	1.99	6.00	3.01	4.00
	Range	1.0–6.0	2.0–6.0	1.0–8.0	2.0–6.0
$t_{½}$ (h)	mean[b]	–	6.92	–	6.86
	SD (%CVb)	–	3.0 (43.3)	–	1.7 (25.2)
	Range	–	4.7–12.4	–	4.4–9.1
AUC_{inf} (ng·h/mL)	geo mean[a]	–	3011	–	15347
	95% CI	–	(1368, 6630)	–	(8183, 28784)
	Range	–	1310–12164	–	7796–41272
AUC_{tau} (AM) (ng·h/mL)	geo mean[a]	515	1370[c]	1820	6245
	95% CI	(256, 1036)	(370, 5070)	(1108, 2990)	(3545, 11001)
	Range	254–1751	578–4171	826–2986	3255–15158
AUC_{tau} (PM) (ng·h/mL)	geo mean[a]	766	1198	3037	5971
	95% CI	(394, 1490)	(596, 2407)	(1887, 4889)	(3278, 10875)
	Range	388–2497	537–4006	1355–4432	3211–14962

%CVb: between-subject coefficient of variance; AUC_{inf}: area under the concentration time curve (AUC) from time 0 to infinity; AUC_{tau}(AM): AUC during a dosing interval (tau) after a morning dose; AUC_{tau}(PM): AUC during a dosing interval (tau) after an evening dose; BID: twice daily; CI: confidence interval; C_{max}(AM): maximum observed concentration after a morning dose; C_{max}(PM): maximum observed concentration after an evening dose; $t_{½}$: terminal elimination half-life; SD: standard deviation; t_{max}(AM): time at which C_{max} is observed after a morning dose; t_{max}(PM): time at which C_{max} is observed after an evening dose; a: geometric mean; b: arithmetic mean; c: n=4.

In Part B, 40 and 100 mg AUT00201 BID were rapidly absorbed – most t_{max} values were 1.0−6.0 h across dosing groups, though 1 subject had t_{max}(PM) of 8 h on Day 1, and there was no clear relationship with dose. As expected, C_{max} was greater at the higher dose level: geometric mean C_{max}(AM) and C_{max}(PM) were, respectively, 103 and 134 ng/mL (Day 1) and 173 and 159 ng/mL (Day 14) after 40 mg, and 373 and 564 ng/mL (Day 1) and

994 and 796 ng/mL (Day 14) after 100 mg doses. Accumulation after 14 days' repeated dosing is discussed below.

C_{trough} showed that plasma concentrations of AUT00201 had reached steady state after 3 days of 40 mg AUT00201 BID (Day 4) and 6 days of 100 mg BID (Day 7).

As expected, AUC was greater at the higher dose level: for example, after 14 days' repeated AUT00201 BID dosing, geometric mean AUC_{inf} (Day 14) was 3011 and 15,347 ng·h/mL after 40 and 100 mg doses, respectively. When compared with single doses received by male subjects in Part A, AUC_{inf} (Day 14) after 40 mg BID was similar to that after 160 mg in the fasted (3405 ng·h/mL) and fed state (2988 ng·h/mL), and AUC_{inf} (Day 14) after 100 mg BID was about twice that after a single dose of 300 mg in the fasted state (7051 ng·h/mL).

Additionally, though dose proportionality could not be formally assessed in Part B, greater than dose-proportional increases in geometric mean C_{max} and AUC, of 3.5 to 5.7-fold, were observed between the 40 mg and 100 mg AUT00201 dosing groups.

After 14 days' repeated dosing with 40 and 100 mg AUT00201 BID, accumulation was moderate and increased with dose: geometric mean R_{ac}(AUC) and $R_{ac}(C_{max})$ were, respectively, 2.33 and 1.55 after 40 mg, and 3.28 and 2.13 after 100 mg doses.

After 14 days' repeated dosing with 40 and 100 mg AUT00201 BID, arithmetic mean $t_{½}$ was 6.9 h in both dosing groups – broadly similar to that after single doses in Part A.

Arithmetic mean MRT_{inf} and MRT_{tau} were similar across BID dosing groups in Part B: about 17 and 5.8 h, respectively. Those MRT_{inf} values were similar to that after a single 300 mg dose in the fasted state in male subjects (17.2 h; Part A).

Parts A and B

The amount of administered AUT00201 excreted unchanged in urine was negligible.

AUT00208, a synthetic-route intermediate of AUT00201, could not be detected in plasma at any dose level after single or repeated doses of AUT00201.

Pharmacodynamics

phEEG (Part A1 only): 17 subjects across Groups 2 and 3 (receiving doses as described in Table S1) had at least 1 evaluable post-baseline quantitative (qEEG) measurement. No qEEG assessments were done after 20 mg AUT00201. No relevant age difference was found between the 2 groups.

While some effects on power in the alpha and theta frequency bands were observed, there was no evidence of a relationship with AUT00201 plasma concentration. Therefore, it is likely those effects are chance findings. While some effects of AUT00201 were observed on the power in the other frequency bands of the EEG spectrum, there did not appear to be any robust patterns with regard to time, dose, or electrode location.

LSEQ (Part B only): LSEQ scores > 50 mm indicate an improvement on the sleep parameter, compared with the subject's usual experience. By that measure, subjects overall experienced slight improvements from normal in getting to sleep, quality of sleep, awakening from sleep,

and behaviour following wakefulness, except in the 100 mg AUT00201 BID dosing group, where the mean LSEQ score was 46.8 mm across timepoints (including baseline). However, there was no evident effect on either dose of AUT00201 on any of the sleep parameters: changes from baseline were minimal across parameters, timepoints and dosing groups, showed no consistent difference between active and placebo dosing, and could be positive or negative with no clear pattern.

Audiology (Part B only): Overall, there was no evident effect of either dose of AUT00201 on any of the OAE or PART parameters. Changes from baseline were minimal across parameters, timepoints and dosing groups, showed no consistent difference between active and placebo dosing, and could be positive or negative with no clear pattern.

Conclusions:

Safety

- Single and repeated oral doses of AUT00201 were well tolerated in healthy subjects. There were no deaths, SAEs, otherwise significant AEs, or TEAEs leading to subject withdrawal during the study. All TEAEs were mild or moderate in severity.
- AUT00201 was found to be safe after both single and repeated doses: there were no clinically significant findings or changes in clinical laboratory variables, the results of physical examinations, vital signs, or ECGs, during the study. There were no positive results on C-SSRS. EEG abnormalities in 2 subjects were short-lived with no clear association with AUT00201 administration.

Pharmacokinetics

- AUT00201 was rapidly absorbed after single and repeated doses: t_{max} was mostly 1–6 h across single and repeated dose levels.
- $t_{½}$ was 5.7–10.0 h across all dose regimens.
- PK parameters in women were similar to those in men.
- C_{max} and AUC increased with dose. Statistical analysis showed with > 90% confidence that the increases in AUC were greater, and increases in C_{max} smaller, than dose-proportional after single doses of 3−300 mg AUT00201. Greater than dose-proportional increases in C_{max} and AUC also occurred between 40 mg and 100 mg AUT00201 BID repeated dosing groups.
- Plasma concentrations were generally higher after dosing in the fed state than in the fasted state. Statistical analysis showed that prior feeding increased the bioavailability of 80 mg AUT00201 compared with the same dose in the fasted state.
- C_{trough} showed that plasma concentrations of AUT00201 had reached steady state after 3 days of 40 mg AUT00201 BID (Day 4) and 6 days of 100 mg BID (Day 7).
- Accumulation increased with dose: ≤ 2.3- and ≤ 3.3-fold after 40 and 100 mg AUT00201 BID, respectively, for 14 days.

- Variation in C_{max} was high: there were 4.9 to 6.5-fold ratios between subjects at each extreme after 40 mg AUT00201 BID, and 3.5 to 4.2-fold ratios after 100 mg BID.
- Excretion of AUT00201 in urine was negligible.

Pharmacodynamics

- 40 and 100 mg AUT00201 BID for up to 14 days had no evident effect on LSEQ sleep parameters. Statistical analysis of audiology data (OAE and PART-spatial release) did not reveal any clear effect of AUT00201 administration.
- There was no clear evidence of a relationship between qEEG and AUT00201 administration.
- Statistical analysis of audiology parameters (OAEs and PART-spatial release) did not reveal a clear association with AUT00201 administration.