Clinical Pharmacology
in Drug Development
2024, 0(0) 1–11

DOI: 10.1002/cpdd.1441

A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Single Ascending Dose Trial of AWZ1066S, an Anti-*Wolbachia* Candidate Macrofilaricide

Graham Devereux[1], Marcin Bula[2], Karen Tripp[2], Richard Fitzgerald[2], Nicola Eraut[3], Muhammad Salman Alam[4], Tomoyuki Moriyama[5], Raku Shinkyo[6], Lauren Walker[2], Duolao Wang[1], Fabian Gusovsky[7], Jeannette van der Velde[7], Joseph D. Turner[8], Weiqian David Hong[9], Paul M. O'Neill[9], Mark J. Taylor[8], and Stephen A. Ward[8]

Abstract

AWZ1066S has been developed as a potential treatment for the neglected tropical diseases lymphatic filariasis and onchocerciasis. AWZ1066S targets the *Wolbachia* bacterial endosymbiont present in the causative nematode parasites. This phase 1, first-in-human study aimed to assess the safety and pharmacokinetics of AWZ1066S in healthy human participants. In a randomized double-blind, placebo-controlled, single ascending dose study, healthy adults received a single oral dose of AWZ1066S (or placebo) and were followed up for 10 days. The planned single doses of AWZ1066S ranged from 100 to 1600 mg, and each dose was administered to a cohort of 8 participants (6 AWZ1066S and 2 placebo). In total 30 people participated, 18 (60%) female, median age 30.0 years (minimum 20, maximum 61). The cohorts administered 100, 200, 300, and 400 mg of AWZ1066S progressed unremarkably. After single 700-mg doses all 4 participants developed symptoms of acute gastritis and transient increases in liver enzymes. The severity of these adverse events ranged from mild to severe, with 1 participant needing hospital admission. Pharmacokinetic analysis indicated that AWZ1066S is rapidly absorbed with predictable pharmacokinetics. In conclusion, safety concerns prevented this study from reaching the human exposures needed for AWZ1066S to be clinically effective against lymphatic filariasis and onchocerciasis.

Keywords

AWZ1066S, lymphatic filariasis, onchocerciasis, phase 1 trial, safety pharmacokinetics

[1]Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK
[2]Clinical Research Facility, Royal Liverpool University Hospital, Liverpool, UK
[3]Fortrea Clinical Research Unit Ltd, Leeds, UK
[4]Labcorp Early Development Laboratories Limited, Huntingdon, Cambridgeshire, UK
[5]Eisai Co., Ltd. Global Drug Safety, Tsukuba, Ibaraki, Japan
[6]Drug Metabolism and Pharmacokinetics, Eisai Inc., Cambridge, MA, USA
[7]Global Public Health, Eisai Inc., Cambridge, MA, USA
[8]Centres for Drugs & Diagnostics and Neglected Tropical Disease, Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool, UK
[9]Department of Chemistry, University of Liverpool, Liverpool, UK

Submitted for publication 15 March 2024; accepted 28 May 2024.

Corresponding Author:
Graham Devereux, MD, Clinical Sciences, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK
(e-mail: graham.devereux@lstmed.ac.uk)

Trial registration: NCT05084560

The neglected tropical diseases lymphatic filariasis (LF, elephantiasis) and onchocerciasis (river blindness) are caused by parasitic filarial nematode infections and are important public health concerns in tropical regions.[1] LF affects 51 million people globally, mainly in South-East Asia and Africa. It is caused by the filarial nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* and is transmitted by mosquitoes.[2] Larval stage and adult worms parasitizing lymphatic vessels result in inflammation and lymphatic dysfunction that clinically manifests as chronic lymphoedema (elephantiasis) or scrotal swelling (hydrocoele).[3] LF is a leading cause of chronic disability globally.[4] Onchocerciasis is caused by the filarial nematode *Onchocerca volvulus* transmitted by black flies whose larvae and pupae develop in fast-flowing rivers and streams.[1] Adult worms live in subcutaneous and deep tissues and release larval microfilariae that migrate to the skin and eyes, resulting in skin disease and blindness.[1] Onchocerciasis affects 21 million people globally, mainly in Africa. It is second only to trachoma as a leading cause of infectious blindness and in endemic areas onchocerciasis accounts for 30% of blindness; in those individuals most heavily infected, over 60% are blind.[5–7]

Global programmes for the control and elimination of LF and onchocerciasis are based on the annual/biannual mass administration of anti-helminth drugs, for example, diethylcarbamazine, ivermectin, and albendazole for LF and ivermectin for onchocerciasis.[1] These drugs principally target the microfilarial stage and consequently require repeated administration and high treatment coverage to break the transmission cycle of the long-lived adult worms (onchocerciasis 10-14 years, LF 5-8 years).[1]

The nematodes that cause LF and onchocerciasis are dependent on a symbiotic intracellular bacterium, *Wolbachia*, that is essential for multiple components of nematode biology including larval growth, development, embryogenesis, fertility, transmission, and, ultimately, adult worm survival.[8,9] In proof of concept field studies, the antibiotic doxycycline has been used to target *Wolbachia* in people infected with *W. bancrofti*, *B. malayi*, and *O. volvulus*, with reductions in biomarkers, adult worms, and microfilariae.[10–17] Unfortunately, doxycycline is unsuitable for mass drug administration because it requires a relatively lengthy course of treatment and is contraindicated in young children and during pregnancy.

We report here a first-in-human trial of the anti-*Wolbachia* candidate drug AWZ1066S developed by a lead optimization program.[18–21] The azaquinazoline enantiomer AWZ1066S is highly specific for *Wolbachia* and in preclinical models is safe and showed superior efficacy to existing anti-*Wolbachia* therapies. In animal models, 7 days of oral AWZ1066S depleted *Wolbachia* by >90% with sustained sterilization of microfilariae production. This raises the prospect of AWZ1066S being used in short (7-day) courses in target populations.[19]

In humans, based on the following unpublished preclinical studies, CYP3A has been identified as the main metabolic enzyme of AWZ1066S: (1) a greater than 90% CYP3A4 contribution was suggested in a preclinical reaction phenotyping study using 7 major recombinant cytochrome P450 (CYP) isoforms; (2) metabolism of AWZ1066S in human liver microsomes was predominantly inhibited by CYP3A selective chemical inhibitors; and (3) the primary metabolites of AWZ1066S identified in human hepatocytes were all oxidative. In ex vivo mouse, rat, monkey, and human hepatocytes, AWZ1066S is mainly metabolized via oxidation through dealkylation, oxygenation, and dehydrogenation followed by conjugation via sulphation and glucuronidation. As a perpetrator of drug-drug interactions AWZ1066S is characterized as: (1) a potential reversible inhibitor of CYP2C8, 2C9, 2C19, and 2D6 with IC50 values of 32.8, 11.1, 48.0, and 83.5 μM, respectively; (2) a time-dependent inhibitor of CYP3A as indicated by IC50 shift assay; (3) an inducer of CYP2B6 with E_{max} and EC50 values of 7.72 and 4.14 μmol/L, respectively; and (4) an inducer of CYP3A4, with E_{max} and EC50 values in the range of 58.7-227 and 23.4-29.7 μmol/L, respectively. No concentration-dependent change of plasma protein binding (86.7%-87.9%) and blood to plasma ratio (0.76-0.80) in human samples was observed in the expected exposure range in humans (up to 100 μM).

Unpublished preclinical studies identified the following potential adverse events (AEs): (1) gastrointestinal toxicity – in rats there were mild changes in the gastrointestinal tract and in monkeys there was frequent vomiting at the highest dose (300 mg/kg/day); (2) QTcF prolongation – in monkeys at the highest dose of 300 mg/kg/day; (3) hypotension – in monkeys there were dose-dependent decreases in diastolic blood pressure with doses ≥100 mg/kg/d; and (4) potential phototoxicity – evaluation of light absorption demonstrated that the greatest molar extinction coefficient between 290 and 700 nm was 1.51×10^4L/mol/cm at 361 nm, which was indicative of photoreactive potential.

The aim of the phase 1 trial presented here was to evaluate the safety and pharmacokinetics of AWZ1066S in healthy adults.

Methods

Trial Design and Oversight

This was a first-in-human, single-center, randomized, double-blind, placebo-controlled, single, and multiple ascending oral dosing study of AWZ1066S. The primary objective was to assess the safety of ascending

single and multiple oral doses of AWZ1066S. The secondary objective was to characterize the single and multiple oral dose pharmacokinetics of AWZ1066S. An exploratory objective was to determine the effect of food on AWZ1066S pharmacokinetics. In the single ascending dose (SAD) phase, cohorts of 8 participants were administered a single dose of AWZ1066S or placebo, with 6 sequential cohorts taking increasing doses of AWZ1066S (Figure S1). The protocol included a multiple ascending dose (MAD) phase and an investigation of the effect of food on AWZ1066S pharmacokinetics (cohort 4). These will not be described further because safety issues stopped the study before the food effect could be investigated and the start of the MAD phase.

The Liverpool School of Tropical Medicine (LSTM) sponsored the study and a Dose Escalation Committee (DEC) oversaw the trial. The trial was approved by the North-West Greater Manchester Central Research Ethics Committee (21/NW/0239) and the Medicines and Healthcare products Regulatory Agency (EudraCT 2021-002891-37). The study was registered on October 20, 2021 (www.clinicaltrials.gov NCT05084560). Participants provided written informed consent. The study was conducted in the Phase 1 Clinical Research Facility (CRF), Royal Liverpool University Hospital, Liverpool, UK.

The study protocol and statistical analysis plan are available in the Supplemental Information.

The Global Health Innovation Technology Fund funded the trial and in-kind contributions were received from Eisai CL, Japan. The funder had input into trial design through peer review of the proposal but had no role in data collection, analysis, or manuscript preparation.

Participants

Participants were healthy volunteers recruited by the CRF. The pertinent inclusion criteria were aged 18-65 years, body mass index 18.0-35.0 kg/m^2, and in good health. The notable exclusion criteria were alcohol consumption >14 units/week, current smoker, and a history of drug allergy or anaphylaxis. The only concomitant medications permitted were contraception, paracetamol, inhaled treatments for mild asthma, and topical treatments for mild atopic dermatitis. The protocol lists the full inclusion and exclusion criteria (Supplemental Information).

Randomization/Treatment Allocation/Blinding

A randomization schedule was produced by the LSTM Biostatistics Unit, Liverpool, UK using SAS PROC PLAN (SAS version 9.4, SAS Institute Inc.). For each cohort, 6 participants were randomly assigned to receive a single dose of AWZ1066S and 2 were randomly assigned to receive placebo.

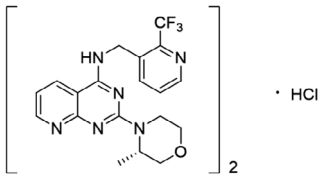

Figure 1. Structural formula of AWZ1066S-HCL.

The study was double-blind, with AWZ1066S and placebo tablets being identical in appearance. The investigators, clinical staff, participants, laboratory staff, and DEC were blinded to allocation. For practical reasons, the clinical trials pharmacy staff and those conducting the pharmacokinetic analysis were unblinded. Sentinel dosing was undertaken for the first 2 participants in each cohort.

Intervention

AWZ1066S was formulated as tablets containing either 100 or 400 mg of AWZ1066S (2-[(3*S*)-3-methyl morpholin-4-yl]-*N*-{[2-(trifluoromethyl)pyridin-3-yl]methyl}pyrido[2,3-d]pyrimidin-4-amine hemihydrochloride ($(C_{19}H_{19}F_3N_6O)_2HCl$ [Figure 1]). The study drugs were supplied by Eisai CL, Bunkyo-ku, Tokyo, Japan.

The planned maximum single doses are presented in Figure S1. Decisions to proceed to the next cohort dose were made by the DEC after a review of the safety and pharmacokinetic data. The starting dose was chosen based on the no observed adverse event level (NOAEL) determined in preclinical safety studies. The 100-mg starting dose based on body surface area gave a 10-fold safety margin over the NOAEL (100 mg/kg/day) in the most sensitive species (rat) and a 20-fold safety margin over the NOAEL (100 mg/kg/day) in monkeys. The maximum dose to be studied was 1600 mg unless mean systemic exposure from a further increment was predicted to exceed the maximum plasma concentration (C_{max}) of 10,400 ng/mL and/or an area under the concentration-time curve from time 0 extrapolated to 24 hours ($AUC_{0\text{-}24}$) of 60,700 h·ng/mL (based on a preclinical 28-day monkey toxicology study and representing the exposure [$AUC_{0\text{-}24}$] and C_{max} on week 4 at the NOAEL). The maximum 1600-mg dose was based on predicted human pharmacokinetics by modeling data from preclinical AWZ1066S studies and clinical trials targeting *Wolbachia*.[17,19,22,23] This modeling predicted that with a human AWZ1066S AUC_τ exposure of 56,200 h·ng/mL, >90% of patients would achieve a clinically efficacious ≥90% reduction in *Wolbachia* within 7 days and that a daily AWZ1066S dose of 620-1850 mg (10-30 mg/kg) would be required to achieve this.

Participants were admitted to the CRF the day before dosing (day −1) and discharged 24 hours postdose. All doses were administered orally in a fasted state whilst resident in the CRF and adherence was 100%.

Safety Outcomes

Full details of data collection are documented in the protocol.

Data were collected by CRF research staff at screening, day −1, day of dosing (day 1), and day 2 whilst resident on the CRF and on days 3, 4, and 10 during outpatient visits.

Vital Signs. Pulse, blood pressure, and oral body temperature were recorded predose, 0.5, 1, 2, 4, 8, and 24 hours postdose and on days 3, 4, and 10.

Electrocardiograms. Electrocardiograms were recorded predose and 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours postdose and on days 3, 4, and 10.

Biochemistry and Hematology. Blood samples were taken predose and on days 3, 4, and 10 for clinical biochemistry and hematology, samples were analyzed by clinical laboratories at Royal Liverpool University Hospital, Liverpool, UK.

Adverse Events. AEs were recorded from the time of consent through to the last visit. AEs were summarized by severity, MedDRA Primary System Organ Class (SOC), and Preferred Term (PT).[24]

Pharmacokinetic Outcomes

Venous blood samples were taken pre- and postdose at 15, 30, and 60 minutes and after 2, 3, 4, 6, 8, 12, and 24 hours and on days 3, 4, and 10.

Plasma AWZ1066S was quantified using a method validated at Labcorp Early Development Laboratories Ltd, Huntingdon, UK. The compound AWZ1066S was extracted from human plasma (K_2EDTA) using protein precipitation extraction whilst protected from light. Analysis was performed by liquid chromatography (LC) with tandem mass spectrometry (MS) detection.[25,26] The method involved the protein precipitation of human plasma followed by LC-MS/MS detection using electrospray in the positive ionization mode using as an internal standard the d4-form of AWZ1066S prepared by Eisai Inc. with high-performance liquid chromatography (HPLC) purity of 99.54%. The LC system used mobile phase A (water/formic acid, 100/0.2 v/v) and mobile phase B (acetonitrile/water/formic acid, 90:10:0.2 v:v:v), and methanol was used as needle wash. The HPLC column used was a Waters BEH C18 1.7 µm, 50 × 2.1 mm, PN 18802350 column. The m/z values monitored for AWZ1066S were precursor ion (Q1) 405.2 m/z and product ion (Q3) 160.3 m/z.

The MS conditions used were atmospheric pressure ionization 4500; synchronization, LC Sync; ionization, electrospray positive; source temperature, 500°C; curtain gas, 30; ion spray voltage, 5200 volts; nebulizing gas, 50; auxiliary gas, 60; collisionally activated dissociation (CAD) gas, 8; collision gas, nitrogen; multiple reaction (MR) pause, 5 milliseconds; MS acquisition time, 3.0 minutes; entrance potential, 10.

The lower limit of quantification was 0.5 ng/mL. The response for each sample injection was at least 5 times greater than the baseline response in the control matrix. The relationship between the peak area ratio of the reference standard (AWZ1066S) to internal standard and the concentration of reference standard in plasma was linear over the calibration range 0.5-1000 ng/mL. A $1/x^2$ weighted least squares linear regression analysis of the data was used to calculate the slope, intercept, and coefficient of determination (r^2).

Pharmacokinetic analysis was performed by Fortrea Clinical Pharmacology Services, Leeds, UK. The pharmacokinetic parameters were determined from the plasma AWZ1066S concentrations using non-compartmental analysis performed using Phoenix WinNonlin Version 8.3.5 (Certara USA, Inc.). Plasma AWZ1066S concentrations below the low limit of quantification were entered into the database and analyzed as 0.

Statistical Methods

Sample Size. No formal statistical assessment, in terms of sample size, was conducted as this was the first administration of AWZ1066S to humans. The number of participants in each cohort was considered adequate to meet the study objectives and is a design commonly used in first-in-human studies.

Analysis. All analyses were governed by a statistical analysis plan. Given the study's exploratory nature, no formal statistical hypothesis testing was performed. The analysis of safety and pharmacokinetic data was descriptively summarized. The statistical analysis plan includes a detailed description of the pharmacokinetic analyses undertaken. SAS version 9.4 (SAS Institute Inc, country Cary, NC, USA) was used to analyze the data.

Results

Participants

The first and last participants were dosed on December 21, 2021, and January 6, 2023, respectively, and the disposition of participants is detailed in Figure S2. Recruitment of cohorts 1 (100 mg), 2 (200 mg), and 3 (400 mg) proceeded as planned. Based on pharmacokinetic considerations of cohort 3 (400 mg), the DEC decided that cohort 4 would be dosed at 300 mg and cohort 5 at 700 mg. However, because of difficulties in recruitment and funding timelines, and a pressing need

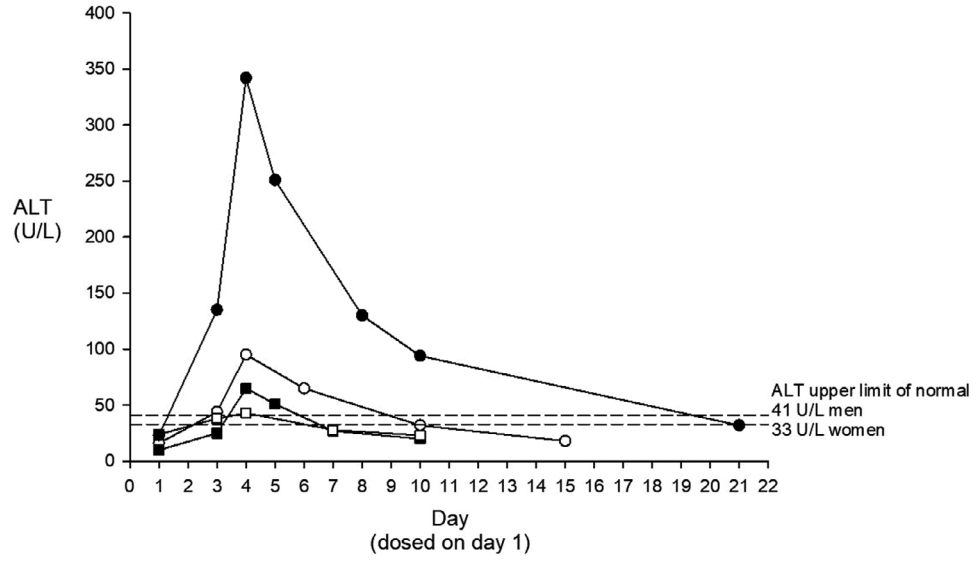

Figure 2. Time course of serum alanine aminotransferase (ALT) of 4 participants dosed with 700 mg of AWZ1066S.

to identify doses for inclusion in the MAD phase, the DEC authorized the suspension of cohort 4 (300 mg) after the dosing of 1 participant and the starting of cohort 5 (700 mg). After the 5th participant in cohort 5 (700 mg) had been dosed, the study was stopped because of safety concerns. In total, 30 participants were dosed: cohort 1 (n = 8), cohort 2 (n = 8), cohort 3 (n = 8), cohort 4 (n = 1), and cohort 5 (n = 5). The median age of participants was 30.0 years (minimum 20 years, maximum 61 years), 18 (60%) were female, mean (SD) body mass index was 25.1 (4.18) kg/m^2, and 26 participants (87%) were white. Table S1 presents the demographic details of the participants administered single doses of AWZ1066S/placebo.

Safety

Single dosing with 100, 200, 300, and 400 mg of AWZ1066S proceeded uneventfully. AE and serious AE data are presented in Tables 1, 2, and S2. Headache was the most frequently reported AE, being reported by 9 participants (30%).

After single 700-mg doses, all 4 participants on the active drug developed symptoms consistent with acute gastritis (epigastric discomfort/pain, gastro-esophageal reflux, anorexia) 12 hours postdose and increases in serum levels of the liver enzyme alanine transaminase (ALT) that peaked on day 4, returning to normal after 10-21 days (Tables 1 and 2, Figure 2). For 2 participants, the ALT increases were considered mild (1.1×, 2.0× upper limit of normal [ULN]), for 1 moderate (2.9 × ULN), and for 1 severe (10.4 × ULN). Three participants also had increases in aspartate aminotransferase (AST), for 1 the increase was considered mild, for 1 moderate, and for 1 severe. In 2 participants the ALT and AST increases were considered to be related to AWZ1066S. Two participants had mild increases in gamma-glutamyl transferase and 1 had a mild increase in alkaline phosphatase. There were no clinically significant changes in bilirubin, clotting, hematology, urinalysis, or ECG. The sentinel safety data (mild epigastric discomfort, mild increase in serum ALT) from this cohort were reviewed prior to commencing dosing of the reminder of the cohort.

The most severely affected participant reported severe epigastric pain 12 hours after dosing with 700 mg and this was associated with a brief syncopal episode. The next day the participant was unable to tolerate oral fluids or solids because they exacerbated the pain and the epigastric pain was associated with abdominal tenderness on examination. The participant was admitted to hospital because of the severity and persistence of the pain. In hospital, the participant was treated with analgesia, intravenous fluids, and proton pump inhibitor therapy. The symptoms settled and she was discharged 2 days after dosing. Rises in ALT and AST were noted on day 3 that peaked on day 4 at 10.4 and 8.6 times ULN, respectively. AST and ALT returned to normal by day 21. Despite extensive clinical investigation for possible biliary obstruction, viral hepatitis, autoimmune hepatitis, Wilson's disease, hemochromatosis, and α1-antitrypsin deficiency, no cause other than AWZ1066S was found to explain the increases in ALT/AST. The epigastric pain and ALT/AST increases were considered severe, related to the study medication, and fulfilled the criteria for a suspected unexpected serious AE. The study was stopped because it met the predefined safety stopping criterion of 1 participant having a serious AE considered to be related to the study drug.

Table 1. Adverse Events, Preferred Term Descriptors, and Incidence

Preferred term descriptor	Dose of AWZ1066S n (%)					Placebo (N = 7)
	100 mg (N = 6)	200 mg (N = 6)	300 mg (N = 1)	400 mg (N = 6)	700 mg (N = 4)	
Headache	2 (33%)	1 (17%)	1 (100%)	3 (50%)		2[a] (29%)
Epigastric pain not food related					4[b] (100%)	
Raised liver function test[c]					3[b] (75%)	
Raised liver enzymes[d]					3[b] (75%)	
Catheter site bruise	1 (17%)			1(17%)		1 (14%)
Nausea					1 (25%)	1 (14%)
Abdominal discomfort					1 (25%)	
Aches and pains in legs					1 (25%)	
Acid reflux (esophageal)					2 (50%)	
Cold symptoms					1 (25%)	
Cough	1 (17%)					
Creatinine kinase elevated	1 (17%)					
Decreased appetite					1 (25%)	
Dizzy					1 (25%)	
Dry lips						1 (14%)
Dry mouth						1 (14%)
Fall						1 (14%)
Hematuria microscopic	1 (17%)					
Hemospermia	1 (17%)					
Hoarse voice						1 (43%)
Hypertension			1 (100%)			
Hypoglycemic episode		1 (17%)				
Itchy legs	1 (17%)					
Neutrophilia						1 (14%)
Neutropenia					1 (25%)	
Syncope					1 (25%)	
Tiredness		1 (17%)				
Urinary tract infection				1 (17%)		

N, number of participants in group; n, number of participants with event; %, percentage of participants in group with event.
[a] One participant had 2 headaches.
[b] In 1 participant, 3 events classed as serious adverse events.
[c] Preferred term for raised aspartate aminotransferase.
[d] Preferred term for raised aspartate alanine aminotransferase.

Table 2. Summary of Adverse Events by Severity and Causality

Adverse events	Dose of AWZ1066S[a]					Placebo (N = 7)
	100 mg (N = 6)	200 mg (N = 6)	300 mg (N = 1)	400 mg (N = 6)	700 mg (N = 4)	
AE	8 (4, 66.7%)	3 (2, 33.3%)	5 (3, 50.0%)	2 (1, 100%)	20 (4, 100%)	10 (6, 85.7%)
SAE	0	0	0	0	3 (1, 25%)	0
Life-threatening AE	0	0	0	0	0	0
Death related AE	0	0	0	0	0	0
Treatment-related AE	0	0	0	0	5 (2, 50%)	0
Treatment-related SAE	0	0	0	0	3 (1, 25%)	0
Intensity of AEs						
Mild	8 (4, 66.7%)	3 (2, 33.3%)	5 (3, 50.0%)	2 (1, 100%)	14 (4, 100%)	10 (6, 85.7%)
Moderate	0	0	0	0	3 (2, 50%)	0
Severe	0	0	0	0	3 (1, 25%)	0

N, number of participants in group. AE, adverse event; SAE, serious adverse event.
[a] Events expressed as: number of events (number of participants with event, percentage of participants with event).

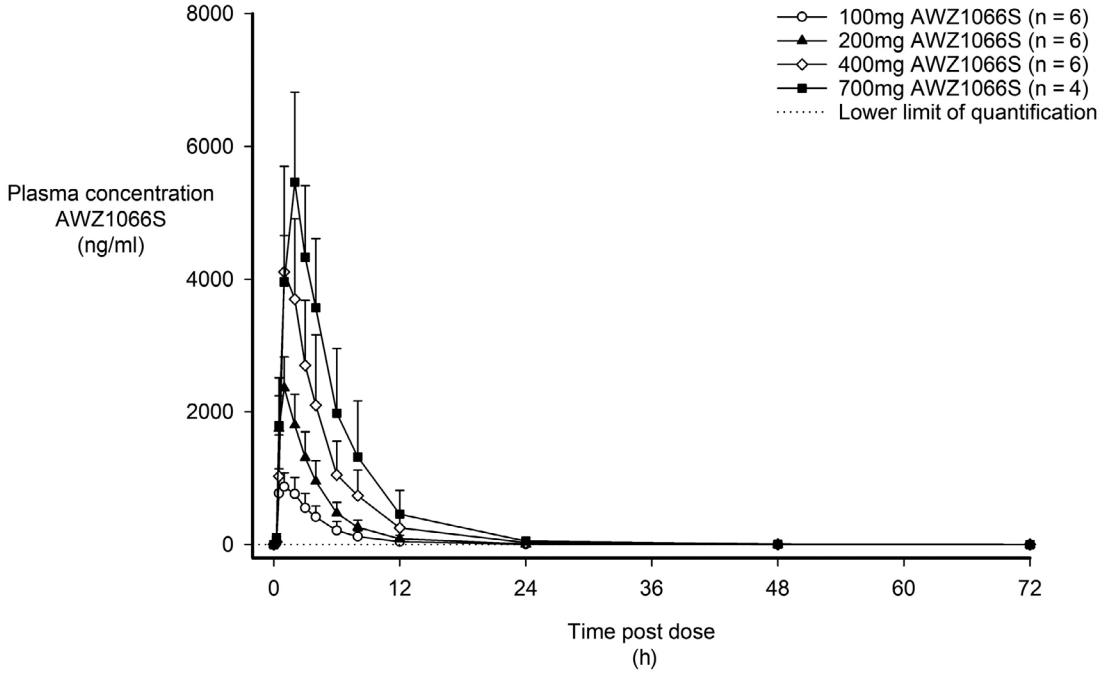

Figure 3. Mean AWZ1066S plasma concentrations after single doses of 100, 200, 400, and 700 mg of AWZ1066S. Error bars ± standard deviation (SD).

Pharmacokinetics

Figure 3 and Table 3 present the plasma profiles and calculated pharmacokinetic parameters for AWZ1066S after single oral doses. The protocol-defined pharmacokinetic stopping exposures ($AUC_{0\text{-}24}$ of 60,700 h·ng/mL or C_{max} of 10,400 ng/mL) were not exceeded in any participant. In addition, the geometric mean $AUC_{0\text{-}\infty}$ observed at the highest 700-mg dose (28,400 h·ng/mL) did not achieve the target exposure of 56,200 h·ng/mL anticipated to be required for clinical efficacy.

The plasma concentration-time profiles for all doses were characterized by a rapid absorption phase. Median t_{max} was similar at each dose, ranging from 1.00 to 2.00 hours postdose with an overall range of 0.5-2 hours postdose. After reaching C_{max}, plasma AWZ1066S concentrations appeared to decline in a multi-phasic manner and remained quantifiable until 24-72 hours postdose for 100 and 200 mg, 48-72 hours postdose for 400 mg, and 24-72 hours postdose for 700 mg. CL/F ranged between 21.9 and 27.5 L/h. There was no clear trend with dose for either of these parameters. The

Table 3. Calculated Pharmacokinetic Parameters for AWZ1066S Following Single Oral Doses

Pharmacokinetic parameter	Statistic	AWZ1066S dose			
		100 mg (n = 6)	200 mg (n = 6)	400 mg (n = 6)	700 mg (n = 4)
$AUC_{0\text{-tlast}}$	Geomean (gCV)	3630 (42.6)	8440 (32.2)	18,200 (34.1)	28,300 (40.8)
(ng.h/mL)	mean [SD]	3890 [1550]	9190 [2620]	19,100 [6560]	30,000 [10,900]
$AUC_{0\text{-}24}$	Geomean (gCV)	3610 (41.7)	8810 (31.9)	18,000 (33.3)	27,900 (39.4)
(ng.h/mL)	mean [SD]	3850 [1500]	9150 [2580]	18,800 [6330]	29,400 [10,400]
$AUC_{0\text{-}\infty}$	Geomean (gCV)	3640 (42.6)	8850 (32.3)	18,200 (34.0)	28,400 (40.8)
(ng.h/mL)	mean [SD]	3890 [1550]	9200 [2620]	19,100 [6560]	30,000 [10,900]
C_{max}	Geomean (gCV)	1020 (15.4)	2500 (22.2)	4510 (13.1)	5580 (21.9)
(ng/mL)	mean [SD]	1030 [159]	2550 [508]	4540 [628]	5690 [1280]
t_{max}	Median	1.01	1.00	1.02	2.00
(hour)	min-max	0.500-2.00	0.550-1.15	1.00-2.00	1.00-2.00
$t_{1/2}$	Geomean (gCV)	3.61 (37.3)	3.68 (69.2)	5.07 (40.1	5.44 (73.3
(hour)	mean [SD]	3.83 [1.52]	4.47 [3.54]	5.42 [2.32]	6.25 [3.32]
CL/F	Geomean (gCV)	27.5 (42.6)	22.6 (32.3)	21.9 (34.0	24.7(40.8)
(L/h)	mean [SD]	29.4 [11.7]	23.6 [7.86]	22.9 [7.21]	26.2 [10.5]

n, number of subjects. $AUC_{0\text{-}24}$, area under the plasma concentration versus time curve from time of last dose to 24 hours postlast dose; $AUC_{0\text{-}\infty}$, area under the plasma concentration versus time curve from 0 to infinity; $AUC_{0\text{-tlast}}$, area under the plasma concentration versus time curve from time 0 to the last quantifiable concentration; C_{max}, observed maximum drug plasma concentration; CL/F, apparent total clearance following extravascular administration; gCV, geometric coefficient of variation (%); Geomean, geometric mean; Min, minimum; Max, maximum; SD, standard deviation; $t_{1/2}$, the terminal elimination half-life; t_{max}, time to reach maximum plasma concentration.

intersubject variability, based on geometric CV% ranged between 31.9% and 42.6% for $AUC_{0\text{-}\infty}$, $AUC_{0\text{-tlast}}$, and $AUC_{0\text{-}24}$. The intersubject variability in C_{max} was lower and ranged between 31.1% and 22.2%. Over the dose range 100-700 mg of AWZ1066S, geometric mean $AUC_{0\text{-}\infty}$, $AUC_{0\text{-tlast}}$, and C_{max} generally appeared to increase in a dose–proportional manner. This was confirmed by statistical analysis, with the estimates of the regression coefficients for $AUC_{0\text{-}\infty}$, $AUC_{0\text{-tlast}}$, and C_{max} being 1.07, 1.07, and 0.91, respectively. The geometric mean $t_{1/2}$ tended to increase from 3.61 hours at 100 mg to 5.44 hours at 700 mg with increasing doses, perhaps reflecting a more accurate estimation of $t_{1/2}$ by having a greater number of quantifiable later time points at higher dose levels. For individual participants across all doses, the $t_{1/2}$ ranged from 2.20 to 11.4 hours.

Discussion

AWZ1066S has been designed to target the *Wolbachia* endosymbiont critical for the functioning and survival of the adult nematodes responsible for LF and onchocerciasis.[18,19] Preclinical testing has confirmed high specificity against *Wolbachia*, indicated the dose required for a 7-day treatment course in humans, highlighted potential AEs, and identified the NOAEL. In this first-in-human, phase 1 study, safety issues prevented the study from achieving the human exposure to AWZ1066S that pharmacokinetic modeling indicates will be needed for clinical efficacy in LF and onchocerciasis. In addition, the predefined pharmacokinetic stopping exposures (C_{max} 10,400 ng/mL, $AUC_{0\text{-}24}$ 60,700 h·ng/mL) based on NOAELs in nonclinical toxicology studies were not exceeded at the highest single 700-mg dose evaluated. No further dose increases, up to the highest planned dose of 1600 mg, were possible because of safety concerns with the single 700-mg dose. The study did not progress to the MAD phase or investigate the effect of food on AWZ1066S pharmacokinetics.

The planned doses for cohorts 4 and 5 were 800 and 1200 mg, respectively, but after review of cohort 3 (400 mg) the DEC decided cohort 4 would be dosed at 300 mg and cohort 5 at 700 mg. The lower doses were decided on because pharmacokinetic modeling based on the results of cohort 3 (400 mg) raised concerns about exceeding the C_{max} stopping criterion with 800 mg AWZ1066S in the highest-exposed individual(s) although predicted geometric mean exposures were acceptable. Whilst the systemic AWZ1066 exposure was dose linear and predictable and the observed exposures were within the predicted range for AUC, the C_{max} was higher than modeled due to the rapid rate of absorption. Assuming a doubling of bioavailability with food, the planned food effect dose was lowered accordingly (300 mg) to be no more than half of the highest fasted dose completed (700 mg) before dosing after food. However, because of difficulties in recruitment, funding timelines and a pressing need to identify doses for inclusion in the MAD phase, the

DEC authorized the suspension of cohort 4 (300 mg) after the dosing of 1 participant and the starting of cohort 5 (700 mg), with the intention of completing cohort 4 later.

The single 100-400-mg doses of AWZ1066S raised no concerns with no upward trends in ALT or AST. However, after single 700-mg doses of AWZ1066S all 4 participants reported symptoms consistent with acute gastritis and 1 participant was admitted to hospital as a consequence. In addition, in all 4 participants, a single dose 700-mg dose of AWZ1066S was associated with evidence of hepatocellular damage as evidenced by increasing serum ALT and in 3 participants increases in serum AST. No other cause for hepatocellular injury was identified and synthetic liver function was preserved.[27,28]

The symptoms of gastritis reported after single 700 mg AWZ1066S doses are consistent with those observed in preclinical studies but seem to have occurred in humans with single doses and at much lower AWZ1066S exposures than in animals. In a preclinical safety study of monkeys, vomiting was frequently observed throughout the 28-day dosing period at 300 mg/kg/day. In rats after 28 days of dosing, multifocal mild gastric ulceration was present in 1 male dosed at 300 mg/kg and similar changes were observed in 2 animals at 500-750 mg/kg/day. Further studies would be required to determine whether the symptoms of gastritis are a consequence of direct irritation and/or indirect mechanisms.

The hepatocellular damage observed after the single 700-mg doses of AWZ1066S was somewhat unexpected as this was not evident from preclinical studies. In rats, minimal or mild centrilobular hepatocellular hypertrophy was observed in the liver after 7 days of AWZ1066S dosed at 300 and 1000 mg/kg/day, respectively. In monkeys, no changes in liver function tests were noted during 28 days of dosing with AWZ1066S at 300 mg/kg/day and at postmortem there was minimal or mild, diffuse hepatocellular hypertrophy in 2/3 males and 1/3 females. These changes were confirmed to be reversible during a recovery study and considered adaptive changes associated with hepatic metabolizing enzyme induction potential of AWZ1066S and nonadverse. In ex vivo mouse, rat, monkey, and human hepatocytes, 15 putative metabolites were tentatively identified. In human hepatocytes after incubation at 37°C for 240 minutes, 8 metabolites were detected which were all found in at least 2 of the animal species tested, demonstrating that no unique metabolites were formed in human hepatocytes. The participant dosed with 700 mg of AWZ1066S who reported the most severe symptoms of gastritis and the highest ALT rise had the lowest systemic exposure to AWZ1066S of the whole cohort, with C_{max} and $AUC_{0\text{-}24}$ values being about the same as cohort 3, who tolerated 400 mg with no symptoms of gastritis or increase in liver enzymes.

Although increases in ALT >3 times ULN are relatively common in trials during drug development, the likelihood of these drugs being severely hepatotoxic is low. However, in the current study 25% of the 700-mg group had an ALT rise of >10 times ULN and whilst this increases the likelihood that AWZ1066S is overtly hepatotoxic, some drugs that are not severely hepatotoxic can cause similar ALT rises.[27] Our observation in the current study that AWZ1066S was associated with hepatocellular damage after a single dose and in all participants is of particular cause for concern, especially as the hepatocellular damage was associated with gastrointestinal symptoms sufficient to hospitalize 1 participant. It is possible that AWZ1066S or its metabolites are hepatotoxic and/or gastro-toxic. Subsequent work (not presented) demonstrated no differences in the plasma and urine metabolic profiles of the 700- and 200-mg cohorts. Furthermore, the plasma and urine metabolic profiles of the participant with the severest symptoms after 700-mg dosing did not differ from the rest of the 700- or 200-mg cohorts.

Recruitment to this first-in-human study proved to be somewhat more difficult than anticipated. In common with most interventional trials in the UK during and immediately after the COVID-19 pandemic, recruitment was considerably more difficult because of logistical and pandemic related factors.[29] Of the 206 potential participants expressing an interest in the study only 30 were dosed. The majority of the screen fails were due to increased diagnoses of anxiety and depression requiring medication, abnormal blood results in violation of eligibility criteria (predominantly liver function tests), raised BMI, and ineligible comorbidities. We speculate that this reflects the general population that we recruited from because Liverpool is one of the most deprived areas in the UK.[30]

The inclusion criteria included the concomitant use of estrogen-based hormonal contraception, inhaled treatments for mild asthma and topical treatments for mild atopic dermatitis. The use of hormonal contraception was primarily a consequence of the UK Regulatory Authority's requirement that all women participants used highly effective forms of contraception. Exclusion of women using hormonal contraception would have severely limited access to the study for women. The potential concern that AWZ1066S may induce CYP activity sufficiently to cause hormonal contraceptive failure was unlikely given the single doses administered and the highly effective forms of contraception required. There is in vitro, ex vivo, and in vivo evidence that hormonal contraception has the potential to be a perpetrator of drug–drug interactions by modulating the expression of various drug-metabolizing enzymes

and transporters in both the gut and liver; however, the clinical significance of these properties requires further evaluation.[31,32] Currently, the UK Summary of Product Characteristics do not highlight any potential CYP-mediated drug interactions where the hormonal contraceptive is the perpetrator drug. In the current study, all participants administered 700 mg of AWZ1066S developed abdominal pain and abnormal liver function tests, but only one was taking hormonal contraception. Although this participant had the most severe AEs she had the lowest systemic exposure to AWZ1066S of the cohort. Participants were also permitted to use low-dose inhaled corticosteroids for mild asthma and topical corticosteroids for mild atopic dermatitis because systemic absorption is considered to be negligible and a single dose of AWZ1066S is unlikely to affect corticosteroid treatment by inducing CYP activity.[33] Further evaluation of AWZ1066S would have required detailed assessment of drug-drug interactions.

Safety and tolerability concerns stopped this study from reaching the pharmacokinetic exposure that preclinical studies and modeling had indicated would be needed for AWZ1066S to be clinically effective against LF and onchocerciasis. Subsequent to the current study, further animal-based preclinical work (not presented) was conducted to investigate the utility of administering AWZ1066S at bioequivalent lower human doses, but in combination with the anthelmintic albendazole. Albendazole is a proven synergist augmenting *Wolbachia* depletion from filarial tissues by approximately 2-fold in vivo when combined with a range of oral anti-*Wolbachia* drugs, including AWZ1066S.[21,34] The results indicated that lower doses of AWZ1066S considered to be safe for repeated dosing in humans were not efficacious, with or without the addition of the albendazole synergist. Based primarily on the findings of the current study, further human studies of AWZ1066S are not planned.

Acknowledgments

The authors would like to acknowledge Dr Suzy Collins for agreeing to be the Independent Clinical expert for the Dose Escalation Committee.

Conflicts of Interest

G.D., M.B., K.T., R.F., and M.S.A. have no conflicts of interest to declare. N.E. is employed as a senior pharmacokineticist by Fortrea Clinical Trials Unit Ltd. M.S.A. is employed by Labcorp Early Development Laboratories Limited. T.M., R.S., F.G., and J.v.d.V. are employed by Eisai CL, Japan. D.W., J.D.T., W.D.H., and M.J.T. have declared funding from the Global Health Innovative Technology Fund. W.D.H., P.M.O'N., M.J.T., S.A.W., and J.D.T. are named on international patents that include the drug used in this study.

Funding

Funding was provided from the Global Health Innovative Technology Fund, G2019-202. In-kind contributions were received from Eisai CL, Japan.

References

1. Taylor MJ, Hoerauf A, Bockarie M. Lymphatic filariasis and onchocerciasis. *Lancet*. 2010;376:1175-1185.
2. Local Burden of Disease 2019 Neglected Tropical Diseases Collaborators. The global distribution of lymphatic filariasis, 2000–18: a geospatial analysis. *Lancet Glob Health*. 2020;8:e1186-e1194.
3. Furlong-Silva J, Cross SD, Marriott AE, et al. Tetracyclines improve experimental lymphatic filariasis pathology by disrupting interleukin-4 receptor-mediated lymphangiogenesis. *J Clin Invest*. 2021;131:e140853.
4. Zeldenryk LM, Gray M, Speare R, Gordon S, Melrose W. The emerging story of disability associated with lymphatic filariasis: a critical review. *PLoS Negl Trop Dis*. 2011;5:e1366.
5. Lewallen S, Courtright P. Blindness in Africa: present situation and future needs. *Br J Ophthalmol*. 2001;85:897-903.
6. Thylefors B, Negrel AD, Pararajasegaram R, Dadzie KY. Global data on blindness. *Bull World Health Organ*. 1995;73:115-121.
7. Little MP, Basanez MG, Breitling LP, Boatin BA, Alley ES. Incidence of blindness during the Onchocerciasis Control Programme in Western Africa, 1971–2002. *J Infect Dis*. 2004;189:1932-1941.
8. Quek S, Cook DAN, Wu Y, et al. Wolbachia depletion blocks transmission of lymphatic filariasis by preventing chitinase-dependent parasite exsheathment. *Proc Natl Acad Sci USA*. 2022;119:e2120003119.
9. Johnston KL, Hong WD, Turner JD, O'Neill PM, Ward SA, Taylor MJ. Anti-Wolbachia drugs for filariasis. *Trends Parasitol*. 2021;37(12):1068-1081.
10. Hoerauf A, Mand S, Adjei O, Fleischer B, Büttner DW. Depletion of Wolbachia endobacteria in Onchocerca volvulus by doxycycline and microfilaridermia after ivermectin treatment. *Lancet*. 2001;357(9266):1415-1416.
11. Hoerauf A, Mand S, Volkmann L, et al. Doxycycline in the treatment of human onchocerciasis: kinetics of Wolbachia endobacteria reduction and of inhibition of embryogenesis in female Onchocerca worms. *Microbes Infect*. 2003;5:261-273.
12. Taylor MJ, Makunde WH, McGarry HF, Turner JD, Mand S, Hoerauf A. Macrofilaricidal activity after doxycycline treatment of *Wuchereria bancrofti*:

a double-blind, randomised placebo-controlled trial. *Lancet*. 2005;365:2116-2121.
13. Debrah AY, Mand S, Specht S, et al. Doxycycline reduces plasma VEGF-C/sVEGFR-3 and improves pathology in lymphatic filariasis. *PLoS Pathog*. 2006;2:e92.
14. Supali T, Djuardi Y, Pfarr KM, et al. Doxycycline treatment of *Brugia malayi*-infected persons reduces microfilaremia and adverse reactions after diethylcarbamazine and albendazole treatment. *Clin Infect Dis*. 2008;46:1385-1393.
15. Hoerauf A, Specht S, Büttner M, et al. Wolbachia endobacteria depletion by doxycycline as antifilarial therapy has macrofilaricidal activity in onchocerciasis: a randomized placebo-controlled study. *Med Microbiol Immunol*. 2008;197:295-311.
16. Mand S, Pfarr K, Sahoo PK, et al. Macrofilaricidal activity and amelioration of lymphatic pathology in *Bancroftian filariasis* after 3 weeks of doxycycline followed by single-dose diethylcarbamazine. *Am J Trop Med Hyg*. 2009;81:702-711.
17. Hoerauf A, Specht S, Marfo-Debrekyei Y, et al. Macrofilaricidal activity after doxycycline only treatment of *Onchocerca volvulus* in an area of *Loa loa* co-endemicity: a randomized, double-blind, placebo controlled trial. *PLoS Negl Trop Dis*. 2010;4:e660.
18. Johnston KL, Cook DAN, Berry NG, et al. Identification and prioritization of novel anti-Wolbachia chemotypes from screening a 10,000-compound diversity library. *Sci Adv*. 2017;3:eaao1551.
19. Hong WD, Benayoud F, Nixon GL, et al. AWZ1066S, a highly specific anti-Wolbachia drug candidate for a short-course treatment of filariasis. *Proc Natl Acad Sci USA*. 2019;116:1414-1419.
20. Hong WD, O'Neill PM, Taylor MJ, et al. Transformation of the manufacturing process from discovery to kilogram scale for AWZ1066S: a highly specific anti-Wolbachia drug candidate for a short-course treatment of filariasis. *Org Process Res Dev*. 2023;27(1):42-53.
21. Hegde S, Marriott AE, Pionnier N, et al. Combinations of the azaquinazoline anti-Wolbachia agent, AWZ1066S, with benzimidazole anthelmintics synergise to mediate sub-seven-day sterilising and curative efficacies in experimental models of filariasis. *Front Microbiol*. 2024;15:1346068.
22. Aljayyoussi G, Tyrer HE, Ford L, et al. Short-course, high-dose rifampicin achieves Wolbachia depletion predictive of curative outcomes in preclinical models of lymphatic filariasis and onchocerciasis. *Sci Rep*. 2017;7:210.
23. Debrah AY, Mand S, Marfo-Debrekyei Y, et al. Macrofilaricidal effect of 4 weeks of treatment with doxycycline on *Wuchereria bancrofti*. *Trop Med Int Health*. 2007;12:1433-1441.
24. Putrik P, Ramiro S, Lie E, et al. Deriving common comorbidity indices from the MedDRA classification and exploring their performance on key outcomes in patients with rheumatoid arthritis. *Rheumatology*. 2018;57:548-554.
25. Pitt JJ. Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. *Clin Biochem Rev*. 2009;30:19-34.
26. Himmelsbach M. 10 Years of MS instrumental developments – impact on LC-MS/MS in clinical chemistry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2012;883-884:3-17.
27. Reuben A. Hy's Law. *Hepatology*. 2004;39:574-578.
28. Temple R. Hy's law: predicting serious hepatotoxicity. *Pharmacoepidemiol Drug Saf*. 2006;15:241-243.
29. Lorenc A, Rooshenas L, Conefrey C, et al. Non–COVID–19 UK clinical trials and the COVID–19 pandemic: impact, challenges and possible solutions. *Trials*. 2023;24:424.
30. Sherratt FC, Field JK, Marcus MW. Association between smoking and health outcomes in an economically deprived population: the Liverpool Lung Project. *J Epidemiol Community Health*. 2017;71:806-810.
31. Rodrigues AD. Drug interactions involving 17α-ethinylestradiol: considerations beyond cytochrome P450 3A induction and inhibition. *Clin Pharmacol Ther*. 2022;111:1212-1221.
32. Shelepova T, Nafziger AN, Victory J, et al. Effect of a triphasic oral contraceptive on drug-metabolizing enzyme activity as measured by the validated Cooperstown 5+1 cocktail. *Clin Pharmacol*. 2005;45:1413-1421.
33. Wilson AM, Brewster HJ, Lipworth BJ. Dose-response comparison of systemic bioactivity with inhaled budesonide and triamcinolone acetonide in asthmatic adults. *J Allergy Clin Immunol*. 1998;102:751-756.
34. Turner JD, Sharma R, Al Jayoussi G, et al. Albendazole and antibiotics synergize to deliver short-course anti-Wolbachia curative treatments in preclinical models of filariasis. *Proc Natl Acad Sci USA*. 2017;114:E9712-E9721.

Supplemental Information

Additional supplemental information can be found by clicking the Supplements link in the PDF toolbar or the Supplemental Information section at the end of web-based version of this article.