Artesunate–pyronaridine–atovaquone–proguanil and artesunate–fosmidomycin–clindamycin compared with standard artesunate–pyronaridine for the treatment of uncomplicated malaria (MultiMal): a randomised, controlled, clinical, phase 2 trial in Gabon and Ghana

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Summary

Background The emergence of *Plasmodium falciparum* strains with reduced susceptibility to the artemisinin component of artemisinin combination therapies poses a serious threat to the treatment and control of malaria in sub-Saharan Africa. Regimens consisting of combinations of three or more conventional antimalarials have been proposed as a new treatment paradigm to overcome the impending problem of drug-resistant malaria. It was the aim of the MultiMal study to assess the safety, tolerability, and efficacy of the two novel multidrug antimalarial combination therapies, artesunate–pyronaridine–atovaquone–proguanil (APAP) and artesunate–fosmidomycin–clindamycin (AFC), in comparison with standard artesunate–pyronaridine (AP).

Methods This open-label, randomised, controlled, clinical, phase 2 trial was done in Lambaréné, Gabon, and Kumasi, Ghana. Patients with uncomplicated malaria who had fever or a history of fever in the preceding 24 h and a parasitaemia in the range of 1000–100 000 per µL of blood were enrolled. Random permuted blocks of variable block sizes stratified by country were computed to generate a treatment allocation sequence. Recruitment was done across three age groups: children aged 6 months to 10 years, adolescents aged 11–17 years, and adults aged 18–65 years. Weight-adjusted oral, once-daily therapy was administered for 3 consecutive days for AP and APAP regimens dosed according to the recommendations of the manufacturer and twice daily for AFC (dose: artesunate 2 mg/kg, fosmidomycin 30 mg/kg, and clindamycin 10 mg/kg). Participants were followed up over a 42-day period. The primary endpoints of the trial, related to pharmacokinetic analyses, are being reported elsewhere; this Article reports the secondary endpoints—safety, tolerability, and efficacy of the treatment regimens (defined as adequate clinical and parasitological response [ACPR]) at days 28 and 42 after treatment initiation. ACPRs were calculated in the intention-to-treat and PCR-corrected per-protocol populations at these timepoints, whereas safety and tolerability outcomes were assessed continuously over the 42-day follow-up period in the safety population. This trial is registered with pactr.samrc.ac.za, PACTR202008909968293 and is complete.

Findings Recruitment and follow-up took place between Jan 5 and Nov 5, 2021. Of 309 screened individuals, 100 patients with uncomplicated malaria were recruited into this clinical trial: 20 semi-immune patients aged 18–65 years, 40 adolescents aged between 11 and 17 years, and finally 40 patients aged 6 months to 10 years. PCR-corrected ACPR in the per-protocol set was 100% (95% CI 80–100) for AP, 100% (90–100) for APAP, and 97% (86–100) for AFC for day 28, and 87·5% (62–98) for AP, 85·3% (69–95) for APAP, and 94·4% (81–99) for AFC on day 42. Uncorrected ACPR in the intention-to-treat set was 85% (95% CI 62–97%) for AP, 87·5% (73–96) for APAP, and 82·5% (67–93) for AFC on day 28, and 70% (46–88) for AP, 75% (59–87) for APAP, and 75% (59–87) for AFC on day 42. There was no evidence for a differential efficacy across AP, APAP, and AFC. The proportion of patients with treatment-emergent adverse events (TEAEs) did not differ across study groups (p=0·37) and all treatment regimens were safe. Three (7%) of 46 TEAEs in the APAP group were severe compared with two (10%) of 20 in the AP control group and zero of 56 in the AFC group; all severe TEAEs were haematological alterations. The other TEAEs were mild or moderate. Moreover, there were two serious adverse events (SAEs) in the APAP group (peptic ulcer disease and chest contusion) and none in the other groups; these SAEs were rated as not related to the study medication.

Interpretation Antimalarial regimens of APAP and AFC have unique characteristics to tackle the development and spread of drug-resistant *P falciparum* malaria. Given that APAP and AFC were safe, well tolerated, and highly

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efficacious in this clinical phase 2 study, they constitute promising multidrug combination regimens for further clinical development.

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Introduction

Antimalarial drug resistance constitutes a major challenge in the control and elimination of malaria.[1,2] To date, WHO recommends artemisinin combination therapy (ACT) for the treatment of uncomplicated malaria in all malaria-endemic countries.[1] One factor that potentially explains the failure of classical ACT to prevent the emergence of resistance to the drugs included in the regimen is their pharmacokinetic mismatch. Although an artemisinin derivative has an elimination half-life of below 3 h, partner drugs exhibit longer elimination half-lives of several weeks.[3] Owing to this pharmacokinetic mismatch, there is only a brief period during which the two drugs adequately protect each other. However, this period is followed by a prolonged time during which the slowly eliminated partner drug is at sub-therapeutic levels and unprotected by the rapidly eliminated artemisinin derivative. This period might facilitate the selection of drug-resistant mutants of *Plasmodium falciparum* strains in recrudescent or new infections. To overcome this situation, multidrug ACT, and in particular triple ACT, has been proposed. Multidrug ACT is postulated to increase the barriers to resistance if partner drugs with matched half-lives are combined.[4,5] This possibility and several other features makes pyronaridine, atovaquone–proguanil, and fosmidomycin highly attractive combination partners for novel multidrug ACT regimens.

A combination of artesunate–pyronaridine–atovaquone–proguanil (APAP) exerts independent modes of action for artesunate, pyronaridine, and atovaquone–proguanil, thereby potentially providing a strong barrier against drug resistance development. Pyronaridine was developed as a fixed-dose treatment with artesunate to treat uncomplicated *P falciparum* malaria and is highly effective, well tolerated, and safe.[6] Atovaquone targets the cytochrome bc1 complex of *Plasmodium* spp and inhibits several metabolic enzymes, rendering the parasite inactive.[7]

Research in context

Evidence before this study

To address resistance development in artemisinin combination therapy (ACT), triple and multidrug antimalarial combination therapy have been proposed. Multidrug ACT containing mefloquine, piperaquine, and amodiaquine has been studied extensively over the past years. Combinations with pyronaridine, atovaquone-proguanil, or fosmidomycin that have better-matched elimination half-lives with artemisinins have not yet been evaluated in novel multidrug ACT regimens. Using the terms ("artesunate" AND "pyronaridine" AND "atovaquone-proguanil") OR ("artesunate" AND "fosmidomycin" AND "clindamycin") AND ("uncomplicated malaria") AND ("treatment"), we searched PubMed for peer-reviewed clinical trials published between database inception and Sept 1, 2019. No language restrictions were applied. We identified no studies evaluating either artesunate–pyronaridine–atovaquone–proguanil (APAP) or artesunate–fosmidomycin–clindamycin (AFC) as a multidrug ACT regimen. An updated search from Sept 2, 2019, to Aug 29, 2025, also identified no studies.

Added value of this study

To the best of our knowledge, the MultiMal study is the first clinical trial to assess the efficacy, safety, and tolerability of two novel multidrug ACT regimens, APAP and AFC, in comparison with standard artesunate–pyronaridine (AP) treatment in patients with uncomplicated malaria. The study was done in two African populations in Ghana and Gabon and suggested high efficacy for the APAP and AFC multidrug ACT regimens, similar to the excellent efficacy of standard ACT with AP. However, it is important to note that, given the phase 2 nature of this trial, the sample size was quite small and the study was not powered to robustly detect differences in efficacy. Notably, the proportion of patients with treatment-emergent adverse events did not statistically differ across study groups.

Implications of all the available evidence

Multidrug ACT, such as triple ACT, is an innovation that fits into the framework of the WHO Strategy to Respond to Antimalarial Drug Resistance in Africa. If implemented early in national treatment programmes, the efficacy of multidrug or triple ACT regimens would be expected to potentially remain highly efficacious for a longer period than currently used ACT. However, despite promising findings supporting the future use of the multidrug ACT regimens APAP and AFC in the fight against anti-malarial drug resistance development, multidrug ACT is still a fairly novel treatment approach. Therefore, future research is warranted and the efficacy we observed should be confirmed in larger phase 3 trials. In addition to efficacy evaluations, APAP and AFC multidrug ACT research should also monitor tolerability and safety of these regimens and assess their additional, unique benefits conferred (eg, the favourable transmission blocking properties of APAP and the dual anti-malarial and anti-bacterial activity of AFC).

Atovaquone–proguanil is characterised by high efficacy, favourable tolerability and safety profiles, and no epidemiologically relevant drug resistance.[8] Furthermore, the half-lives of individual APAP drugs are fairly evenly matched.[3] Lastly, the combination of artesunate with atovaquone–proguanil is expected to convey favourable antitransmission properties, on the basis of the finding that artesunate eliminates young stage gametocytes and atovaquone–proguanil acts against the sexual development of *P falciparum*.[9,10] This combined antitransmission effect of the APAP regimen could be a crucial feature to avoid the selection of drug resistance in high-transmission settings.

Also, the drugs in the artesunate–fosmidomycin–clindamycin (AFC) combination have independent mechanisms of action that might decrease the risk of selection for drug resistance. Whereas fosmidomycin selectively inhibits the non-mevalonate pathway of isoprenoid synthesis, clindamycin acts directly on the apicoplast of *Plasmodium* parasites.[11–13] Important features of the AFC regimen are that all partner drugs have a matched half-life of less than 5 h[5] and that they are well tolerated and safe.[3,13] Moreover, fosmidomycin has a proven collateral activity on a broad range of Gram-positive and Gram-negative bacterial pathogens,[12,14] and clindamycin is effective in moderate-to-severe infections caused by Gram-positive bacteria and anaerobic pathogens.[15] The development of antimalarials with antibiotic properties is clinically promising on the basis of the fact that malaria cannot be clinically distinguished from bacterial diseases and laboratory-based confirmation is often unavailable in regions most affected by malaria.[16] Importantly, patients with bacterial sepsis often also harbour concomitant, asymptomatic *Plasmodium* infections in regions of high malaria transmission, which are readily detected by rapid diagnostic tests and can confuse the clinical diagnosis.[17] Thus, the AFC combination could have strong antimalarial activity while also exerting antimicrobial activity against the most important Gram-positive and Gram-negative bacterial pathogens causing bloodstream infections, although the efficacy of this dual action at the doses used for multidrug ACT should be confirmed in future, dedicated studies.[11,14,15]

The aim of the MultiMal study was to assess the pharmacokinetic characteristics, as well as the efficacy, safety, and tolerability of the two novel multidrug ACT regimens APAP and AFC in comparison with standard artesunate–pyronaridine (AP) treatment in African patients with uncomplicated malaria. The primary objective of this analysis was to report the clinical and parasitological efficacy, safety, and tolerability of APAP and AFC multidrug ACT regimens compared with AP.

Methods

Study design and participants

This study was designed as a randomised, controlled, open-label, clinical, phase 2 trial and investigated the efficacy, safety, and tolerability of APAP and AFC multidrug antimalarial combination therapy (MDACT) regimens in comparison with AP. The study was done at the Centre de Recherches Médicales de Lambaréné in Lambaréné, Gabon, and at the Kumasi Center for Collaborative Research in Tropical Medicine, in Kumasi, Ghana. At both sites, malaria transmission is perennial with little seasonal variation and endemicity of a hyper-to-holoendemic transmission intensity. Artemether–lumefantrine and artesunate–amodiaquine are recommended as the first-line treatments in Gabon and artesunate–amodiaquine in Ghana. AP is recommended as an alternative treatment in a number of countries but is not widely available.

This trial recruited male and female African patients aged older than 6 months to younger than 66 years (weight >5 and <90 kg), presenting with uncomplicated *P falciparum* malaria. Patients presenting with microscopically confirmed *P falciparum* mono-infection of 1000–100 000 asexual parasites per μL of blood, and with fever (axillary temperature ≥37·5°C), or history of fever in the previous 24 h, were included after provision of written informed consent by themselves or their legal representatives. Important exclusion criteria were the presence of criteria indicative of severe malaria, haemoglobin below 8 g/100 mL, clinically significant medical disorders, pregnancy, previous antimalarial treatment in the past 6 weeks, and receipt of any malaria vaccine (appendix pp 30 and 57).

See Online for appendix

The study conformed to the Declaration of Helsinki and guidelines laid down by the International Conference on Harmonisation for good clinical practice. The clinical trial was approved by the relevant Independent Ethics Committees and local regulatory authorities (Ethics Committee Hamburg, Germany [PV7228], Committee on Human Research, Publication and Ethics, Kumasi, Ghana [CHRPE/AP/197/20], Food and Drugs Authority Ghana Ghana [FDA/CT/204], Institutional Review Board, Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon [CEI-006/2020], National Ethics Committee Gabon [009/CNER/SG/P]). The study protocol was registered online before recruitment, with, pactr.samrc.ac.za PACTR202008909968293.

Randomisation and masking

Random permuted blocks of variable block sizes were computed by the trial statistician to generate a treatment allocation sequence, which was stored in sequentially numbered envelopes. Allocation sequences were computed separately for Ghana and Gabon with Stata17 by use of the ralloc command. Eligible patients were randomly assigned to the study groups AP, APAP, and AFC within three age groups according to an age-step-down recruitment procedure, beginning with 20 adults (18–65 years), followed by 30 adolescents (11–17 years), and concluded with 50 children (6 months–10 years). A data safety monitoring board assessed all relevant data before authorising recruitment of the next age cohort. Allocation ratios of AP:APAP:AFC were 0:1:1 in the adult group, 1:1:1 in the adolescent group, and

1:2:2 in the children group. No adults were recruited in the AP group because of the extensive, existing evidence regarding the favourable safety, efficacy, and pharmacokinetics of the regimen.[6] It was ensured that most recruited participants were African children, who constitute the most important target group for antimalarial treatment because they are disproportionally affected by malaria. Only laboratory analysts were masked to treatment allocation; study participants and clinical personnel were not masked in this open-label study.

Procedures

For AP and APAP, weight-adjusted oral doses were calculated according to the summary of product characteristics of the respective standard treatment, and dosing was done under direct observation once daily for 3 days. Artesunate–pyronaridine and atovaquone–proguanil were administered as fixed-dose regimens as adult and paediatric dosing formulations as applicable (appendix pp 56–57 and 29–30). For AFC, weight-adjusted oral doses were administered under direct observation twice daily for 3 days: artesunate at 2 mg/kg, fosmidomycin at 30 mg/kg, and clindamycin at 10 mg/kg. AP and AFC were dosed independently of food and APAP was given with food or milk. Artesunate–pyronaridine was manufactured and provided by ShinPoong (Seoul, South Korea) free of charge; atovaquone–proguanil (Glenmark, Gröbenzell, Germany) and clindamycin (Ratiopharm, Ulm, Germany) were procured commercially from a Hamburg-based pharmacy (Bavaria-Apotheke, Hamburg, Germany); and artesunate and fosmidomycin were manufactured by and purchased from RenaClinical (Horley, UK) and Nextpharma (Göttingen, Germany), respectively.

Patients who vomited within 30 min of start of dosing were re-dosed once. In case of further vomiting, patients were excluded, and rescue treatment was administered. Following drug administration, patients were actively followed up for 42 days. Patients remained hospitalised for at least 48 h and were then discharged, provided that parasite and fever clearance had been achieved. Patients returned to the clinical research centre for assessment on days 3, 7, 14, 21, 28, 35, and 42. Blood films (thick and thin), dried blood spots, and axillary temperature measurements were taken at screening (pre-dose), at 8, 12, 24, 36, 48, 60, and 72 h, and at all later follow-up visits until day 42. Assessments for safety included haematology, clinical chemistry, urinalysis, and a single 12-lead electrocardiogram (ECG). Furthermore, safety and tolerability were assessed in accordance with good clinical practice guidelines. Any untoward medical or laboratory finding not present at baseline or explained by the natural course of disease was recorded as an adverse event. Adverse events were ascertained actively by a study physician each time that a participant visited the clinical research centre by doing a physical examination of the whole body, by use of a standardised questionnaire including common signs and symptoms of malaria, and by documenting any other possible complaint. In case of a minor participant (particularly in the case of young children), a primary caregiver was consulted additionally. In addition, adverse events were recorded retrospectively if they occurred between two study visits.

Blood films were stained with 3–4% Giemsa solution (Sigma-Aldrich, Darmstadt, Germany) for 45–60 min and then read by a first microscopist. A second microscopist, masked to initial microscopy results, re-read all slides, and a third microscopist resolved potential discrepancies. A slide was considered negative in the absence of asexual parasites per 1000 counted leucocytes by use of a 100 × magnification oil immersion objective.[18,19] Parasitaemia was calculated as follows: (number of counted parasites/counted leucocytes) × most recent absolute leucocyte count per µL. External quality control for malaria microscopy by the Bernhard Nocht Institute for Tropical Medicine (Hamburg, Germany) was done and applied to a subset of blood films and a favourable concordance rate was set at higher than 80% for quantification of parasitaemia and at 100% for positivity or negativity.

Dried blood spots prepared concurrently with blood films were used for molecular analysis. *Plasmodium* DNA was extracted from dried blood spots with the Mag Mini Kit (LGC Biosearch Technology, Middleton, WI, USA). Genotyping was done first by PCR with a T3 Thermocycler (Biometra, Göttingen, Germany) to detect *Plasmodium* genes *MSP-1*, *MSP-2*, and *GLURP*, and then by a nested PCR to detect markers pertaining to the allelic families of *MSP-1* (ie, *RO33*, *MAD20*, and *K1*) and *MSP-2* (ie, *3D7*, and *FC27*). Subsequently, the PCR products were transferred to a gel matrix for identification of genetic polymorphisms by measurement of base-pair length. This step was done on a Applied Biosystems 3130xl Genetic Analyzer (Thermo Fisher Scientific, Foster City, CA, USA) by use of the analytical software GeneMapper 4.1 (Thermo Fisher Scientific).

Outcomes

The primary endpoints of the trial, related to pharmacokinetic analyses, will be reported elsewhere. This Article focuses on the secondary endpoints: safety, tolerability, and efficacy of the study regimens, assessed at days 28 and 42.

Efficacy was established via the PCR-corrected and crude adequate clinical and parasitological response (ACPR) on days 28 and 42. ACPR was defined according to WHO recommendations as the absence of parasitaemia irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure, or late parasitological failure (appendix pp 14 and 120).[19] We also assessed the incidence rate of reappearance, re-infection, and recrudescence over 42 days and parasite clearance time, which was defined as the first timepoint with a negative measurement followed by concordant negative measurements.

The derivation of crude and PCR-adjusted ACPR and of recrudescence and new infection adhered to the principles set down by WHO and Medicines for Malaria Venture.[19,20] *MSP-1*, *MSP-2*, and *GLURP* were used to distinguish

between recrudescence and re-infection for cases in which reappearing asexual parasitaemia was detected microscopically any time during follow-up. A reappearing parasitaemia was classified as recrudescence if at least one allele at each locus was common to the pretreatment and the corresponding post-treatment samples. The definition of re-infection was applied if all the alleles from the post-treatment sample were different from those in the pretreatment sample, for one or more of the tested loci.

Safety and tolerability endpoints included incidence of adverse events and serious adverse events (SAEs) and cases fulfilling Hy's law definition. Only treatment-emergent adverse events (TEAEs) are visualised and discussed in this Article, other adverse events will not be reported. TEAEs are the adverse events that were rated by the medical investigator as "possibly related", "probably related", or "definitely related" to the study treatment.

Although we had planned to analyse the proportion of patients with gametocytes, we chose not to present this outcome because of data sparsity.

Statistical analysis

The sample size was calculated to ensure adequate precision for the pharmacokinetic characterisation of the study drugs to detect drug clearance differences of 20% between study groups and was determined to be 100 participants. Results of the pharmacokinetic characterisation will be reported elsewhere, whereas this Article focuses on secondary endpoints that are related to the safety, tolerability, and efficacy of the experimental treatment regimens.

This clinical phase 2 trial assessed these endpoints descriptively in an age-step-down process to generate first pivotal data for the further clinical development. By use of a method described by Huber,[21] we calculated that an experimental study group size of 40 would yield a 25% width of a 95% CI constructed around an assumed ACPR of 95% (appendix p 11).

As the main efficacy endpoint, the PCR-corrected ACPR was evaluated at days 28 and 42 in the per-protocol analysis set. The crude (PCR-uncorrected) ACPR was assessed at days 28 and 42 in the intention-to-treat analysis set. Reappearing parasitaemias, re-infections, and recrudescences over 42 days were evaluated with the Kaplan–Meier method in the per-protocol analysis set. For analysis of the parasite clearance time, the per-protocol analysis set was used. Safety and tolerability were evaluated in the intention-to-treat set.

The intention-to-treat analysis set included all participants who received at least one study drug dose and had a confirmed positive blood film for *P falciparum* asexual parasitaemia at inclusion. The per-protocol set was the primary analysis set and included all patients in the intention-to-treat set who correctly received the entire treatment schedule over 3 days and completed follow-up assessments to ascertain ACPR at days 28 and 42.

ACPRs at days 28 and 42 are presented with 95% CIs. This clinical phase 2 trial was not designed to statistically test for differences in efficacy, tolerability, and safety between treatment groups, but to provide the first descriptive data in the respective treatment regimens by use of a conservative age-step-down approach. The results shall therefore serve as a basis for further clinical development in larger clinical phase 2 and 3 trials. Descriptive summary statistics were produced for all endpoints. Time to reappearance of parasitaemia was visualised with Kaplan–Meier plots and the log-rank test to test for differences between study groups. Data were managed with REDCap10 (Vanderbilt, TN, USA) and statistical analyses were done with Stata 17. This study adhered to the CONSORT statement (appendix pp 2–3).

Missing data were handled differently within the per-protocol and intention-to-treat analytical approaches. In the per-protocol analysis, only available data were used. In the intention-to-treat analysis, we imputed an unfavourable outcome in the case of missing data (eg, for missing data related to the ACPR at day 42, treatment failure was assumed in the intention-to-treat analysis whereas the same record was censored in the per-protocol analysis).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Patient recruitment and follow-up was done between Jan 5, 2021, and Nov 5, 2021. Of 309 screened individuals, 100 participants fulfilled the inclusion and exclusion criteria and were recruited (figure 1); 53 were male and 47 were female. Following the predefined age-step-down procedure, 20 adults, 30 adolescents, and 50 children were recruited (table 1). Owing to logistical constraints, the AFC group was only active in Gabon, whereas the other treatment groups were active at both recruitment sites. The study-group-specific numbers of participants recruited in Gabon were 11 ([55%] of 20) and in Ghana were nine ([45%] of 20) in the AP group, 13([33%] of 40) and 27 ([68%] of 40) in the APAP group, and 40 ([100%] of 40) and 0 (of 40) in the AFC group, respectively (table 1 and appendix p 4).

All patients received at least one dose of study medication; therefore, the intention-to-treat and safety population were identical (figure 1). 88 participants received all doses and completed follow-up for assessment of day 28 and 86 participants for assessment of day 42 (ie, per-protocol populations). External quality control for malaria microscopy yielded a favourable concordance rate of 101 (88% of 115) for the quantification of parasitaemia and full concordance (115 of 115) for positivity or negativity.

During follow-up, there were 13 microscopically detected re-appearances of parasitaemia (figure 2 and appendix p 16). The first reappearance occurred on day 22 of follow-up in the AFC study group compared with reappearances on day 35 in AP and APAP study groups. PCR analyses indicated that four reappearing parasitaemias were

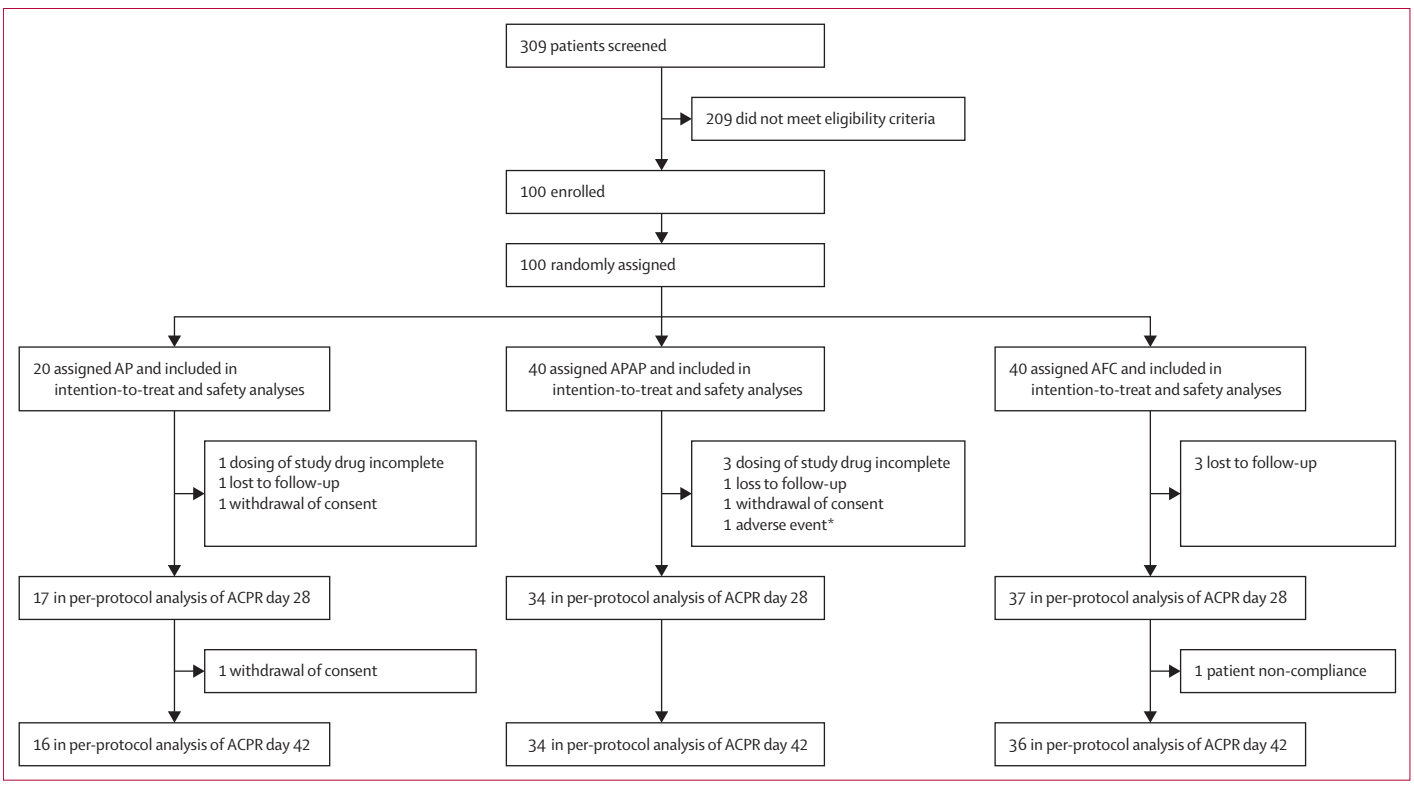

Figure 1: **Trial profile**
ACPR=adequate clinical and parasitological response. AFC=artesunate-fosmidomycin-clindamycin. AP=artesunate–pyronaridine. APAP=artesunate–pyronaridine–atovaquone–proguanil. *Serious adverse event (chest pain) rated as not related to the study medication by the medical investigator. See case description in the Results section.

re-infections and nine were classified as recrudescence (figure 3 and appendix pp 5–6).

Overall, PCR-corrected ACPR in the per-protocol analysis was higher than 95% in all study groups on day 28 whereas on day 42, ACPR was 87·5% in the AP control group, 85·3% in the APAP group and 94·4% in AFC (table 2). Furthermore, uncorrected ACPR on day 28 in the intention-to-treat set was higher than 83% for all groups and higher than ACPR on day 42, which ranged from 70% to 75%. There were no relevant differences of ACPR among the three treatment and age groups.

Furthermore, the stratified presentation of crude and corrected ACPR per treatment group and age group showed similar results (table 2); results in both countries were also similar (appendix pp 7–8). In the per-protocol population, the median parasite clearance time in all age groups combined was 24 h in all study arms (appendix p 9).

The highest frequency of TEAEs were in the gastrointestinal tract category for all study groups (table 3 and appendix p 10). The highest percentage occurred in the AFC study group (34 [61%] of 56 TEAEs) followed by the APAP study group (24 [52%] of 46) and the AP study arm (six [30%] of 20). The higher number and proportion of gastrointestinal tract TEAEs in the AFC study group becomes more pronounced when restricting these TEAEs to "diarrhoea" (appendix pp 11–12). Diarrhoea frequency was highest in the AFC group (21 [38%] of 56 TEAEs) compared with similar proportions in the AP (two [10%] of 20) and APAP (four [9%] of 46) study groups. All episodes of diarrhoea were rated as "mild" and the median duration of diarrhoea was less than 1 day, with only one participant in the AFC group having diarrhoea for 41 days. Other unspecific gastrointestinal TEAEs occurred slightly more often in the APAP group than in the AP group.

The highest frequency of TEAEs in the category "haematological and metabolic" occurred in the AP (five [25%] of 20 TEAEs) and APAP (seven [15%] of 46) study groups followed by the AFC study group (one [2%] of 56; table 3). Within this category, haematological alterations constituted the majority of TEAEs. Anaemia or decrease in haemoglobin from baseline occurred most frequently in the AP group (three [15%] of 20 TEAEs), followed by the APAP group (three [7%] of 46) and the AFC group (one [2%] of 56). Neutropenia occurred three times (three [7%] of 46) in the APAP group, and not at all in the AP and AFC groups (appendix p 9).

When exploring the number of TEAEs per patient and across treatment groups, almost half of patients in the respective treatment group did not report any TEAEs (ten [50%] of 20 participants for AP, 24 [60%] of 40 for APAP, and 18 [45%] of 40 for AFC). A participant in the control group had the highest number of TEAEs per patient (n=9),

	Artesunate–pyronaridine (n=20)	Artesunate–pyronaridine–atovaquone–proguanil (n=40)	Artesunate–fosmidomycin–clindamycin (n=40)	Total (n=100)
Country				
Gabon	11 (55%)	13 (33%)	40 (100%)	64 (64%)
Ghana	9 (45%)	27 (68%)	0	36 (36%)
Baseline parasitaemia, parasites per µL	28 166 (5900–49 504)	20 540 (8970–43 455)	13 577 (7372–25 391)	19 015 (7581–37 156)
Axillary temperature, °C	37·3 (36·7–38·2)	37·2 (36·6–38·2)	36·9 (36·3–38·2)	37·1 (36·5–38·2)
Sex				
Male	11 (55%)	19 (47·5%)	23 (57·50%)	53 (53%)
Female	9 (45%)	21 (52·5%)	17 (42·5%)	47 (47%)
Age group				
Adult	0	10 (25%)	10 (25%)	20 (20%)
Adolescent	10 (50%)	10 (25%)	10 (25%)	30 (30%)
Children	10 (50%)	20 (50%)	20 (50%)	50 (50%)
Age by age group, years				
Adult (18–65 years)	NA	26·2 (19·7–49·8)	28·2 (19·4–35·8)	26·2 (19·6–40·2)
Adolescent (11–17 years)	14·7 (14·0–16·6)	13·8 (12·8–14·4)	14·1 (12·4–15·6)	14·2 (12·8–15·4)
Children (6 months–10 years)	7·4 (4·0–7·8)	5·7 (4·4–6·8)	6·8 (4·8–8·9)	6·0 (4·3–7·8)
Weight by age group, kg				
Adult (18–65 years)	NA	53·3 (50·7–58·2)	67·2 (53·3–73·8)	57·1 (51·6–71·5)
Adolescent (11–17 years)	52·7 (39·1–62·4)	43·1 (34·8–48·7)	40·6 (33·5–42·6)	42·7 (36·8–55·8)
Children (6 months–10 years)	19·8 (14·1–22·3)	18·1 (14·5–20·6)	22·5 (18·4–25·6)	19·3 (16–23·3)
Height by age group, cm				
Adult (18–65 years)	NA	158 (154–162)	166 (164–170)	163 (156–168·5)
Adolescent (11–17 years)	160 (151–167)	153 (149–161)	158 (146–164)	157 (149–164)
Children (6 months–10 years)	120 (99–127)	113 (103–118)	125 (110–130)	115 (103–129)

Data are n (%) or median (IQR). All variables with an intrinsic age correlation were stratified by age group. Data on ethnicity were not collected and are therefore not reported. NA=not applicable.

Table 1: **Demographics and baseline characteristics**

followed by the APAP group (n=7), and the AFC group (n=6). The proportion of TEAEs per patient across the treatment groups was similar (appendix p 13).

There were no deaths and no cases fulfilling Hy's law. Furthermore, there were two SAEs in the APAP group (peptic ulcer disease and chest contusion) and none in the AP and AFC groups. Both were rated as unrelated to the study medication by the clinical investigator and resolved fully (appendix p 18).

Although there were no severe TEAEs in the AFC treatment group, there were two severe TEAEs in the AP study group and three in the APAP study group. All severe TEAEs were noted in the category "haematological" (two patients had anaemia at day 3 after treatment in the AP group; two patients had neutropenia at day 2 and one patient had anaemia at day 11 in the APAP group). Further details on the characteristics of TEAEs are given in the appendix (p 14). There was no case of clinically significant ECG abnormality.

One paediatric participant in the APAP study group had elevated bilirubin and jaundice on day 11 classified as an episode of post-artemisinin delayed haemolysis (appendix p 18).

Most TEAEs were rated as mild by the clinical investigator (15 [75%] of 20 in the AP group; 37 [80%] of 46 in the APAP group; and 54 [96%] of 56 in the AFC study group). The median duration of TEAEs was 1 day or less (appendix p 14). Furthermore, most TEAEs were resolved at day 42 of follow-up, although two patients still had TEAEs at the end of follow-up: one patient in the APAP study group had

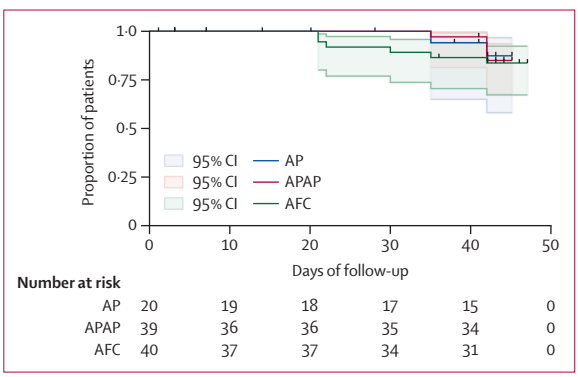

Figure 2: **Kaplan–Meier plots showing PCR-uncorrected microscopically detected reappearance of parasitaemia**

Log-rank test: p=0·88. In case of loss to follow-up participant is censored. AFC=artesunate–fosmidomycin–clindamycin. AP=artesunate–pyronaridine. APAP=artesunate–pyronaridine–atovaquone–proguanil.

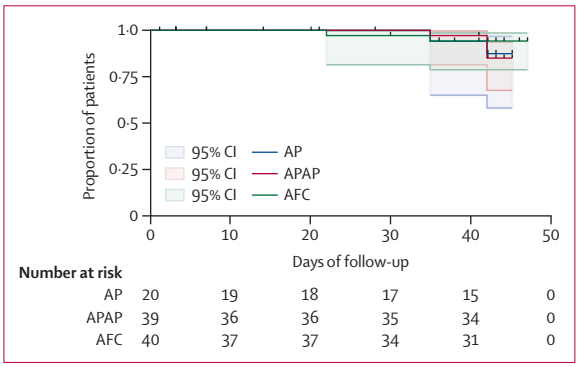

Figure 3: **Kaplan–Meier plots showing PCR-corrected microscopically detected reappearance of parasitaemia (ie, recrudescence)**
Log-rank test: p=0·47. In case of loss to follow-up participant is censored. AFC=artesunate–fosmidomycin–clindamycin. AP=artesunate–pyronaridine. APAP=artesunate–pyronaridine–atovaquone–proguanil.

anaemia and one patient had diarrhoea in the AFC study group.

Discussion

This phase 2 study investigated the efficacy, safety, and tolerability of APAP and AFC multidrug ACT regimens in comparison with the standard ACT of AP. All treatment groups showed rapid parasite reduction and clinical response after initiation of treatment. Efficacy as measured by PCR-corrected ACPR was higher than 95% in all study groups on day 28 and higher than 85% on day 42 after administration of study drugs. These results are indicative for excellent day-28 efficacy of the experimental treatment regimens APAP and AFC in comparison with also excellent day-28 efficacy of standard AP treatment. This puts the APAP and AFC regimen in line with essential clinical efficacy requirements suggested for new antimalarial medicines by Medicines for Malaria Venture.[22] Interestingly, day-42 efficacy was comparatively lowest for the AP control group (87·5%) and the APAP group (85·3%) and highest for the AFC group (94·4%). However, given the evidence from meta-analytical evaluations indicating that standard AP treatment has an antimalarial day-42 efficacy of higher than 95%, our comparatively lower values for AP treatment seem to most likely result from sampling variation given the quite small sample size of this phase 2 clinical trial.[23] Combining this fact with the observation that the 95% CIs of the efficacy estimates for all study groups largely overlapped, we suggest that the experimental APAP and AFC treatments are likely to have a similar efficacy to standard of care AP treatment; non-inferiority is possible but will need confirmation in phase 3 trials. There was no apparent difference between age groups, indicating that the intrinsic activity of the study drugs was adequate also in populations without substantial acquired semi-immunity.

The shorter time of reappearance of parasitaemia in the AFC group is a biologically plausible phenomenon, as it can be explained by differences in the pharmacokinetic characteristics of AFC-containing and AP-containing regimens. Although the elimination half-lives of atovaquone and pyronaridine are approximately 3 days and 7–13 days, respectively, the elimination half-lives of artesunate, fosmidomycin, and clindamycin are below 5 h.[3] Therefore, the AFC regimen does not have an equally long post-therapeutic antimalarial prophylactic effect to that of AP and APAP.

The incidence of diarrhoea was higher in the AFC group (38%) than in the AP (10%) and APAP (9%) groups. Also, one patient in the AFC group had a potentially treatment-associated episode of mild diarrhoea lasting for 41 days, which had not resolved by the end of the study. It is plausible that this episode might have been treatment associated since the antibacterial co-activity of the partner drugs fosmidomycin and clindamycin are known to occasionally induce episodes of diarrhoea. It is worth mentioning that, although there is a reported association of lincosamide antibiotics with *Clostridium difficile* colitis, to our knowledge, there is no report of a *C difficile*-associated colitis requiring medical treatment that has been associated with the antimalarial use of clindamycin.[24] Indeed, young and otherwise healthy paediatric patients with malaria have negligible risks for *C difficile* colitis. Furthermore, less favourable gastrointestinal tolerability occurred not only in the comparison between AFC and AP or APAP but also in the comparison between APAP and AP. Overall, on the basis of the small sample size of this clinical phase 2 trial, there is no evidence for a differential safety profile of APAP and AFC regimens compared with the control group of AP.

To date, three major studies on multidrug ACT regimens have been published, including combinations of artemether–lumefantrine–amodiaquine (n=156 and n=286), arterolane–piperaquine–mefloquine (n=72), and dihydroartemisinin–piperaquine–mefloquine (n=269).[25–27] These studies were done in sub-Saharan Africa (n=217), southeast Asia (n=312), and in both southeast Asia and sub-Saharan Africa (n=1100). All multidrug ACT regimens established non-inferior efficacy in comparison with a standard-of-care ACT. In line with the findings of our study, previous studies reported a trend for less favourable (particularly gastrointestinal) tolerability. Meta-analyses might yield a clearer picture on whether multidrug ACT regimens are less well tolerated than traditional ACT.

This study also has some limitations. First, this phase 2 study had a relatively small sample size, and, owing to logistical constraints, the AFC group was only active at the study centre in Gabon. Second, the study used a PCR correction method based on *MSP-1*, *MSP-2*, and *GLURP* as recommended by WHO in 2007, but during the study, WHO released a new recommendation that replaces *GLURP* with one microsatellite marker.[28] In our study, we observed nine cases of recrudescence. In light of these observations, we recognise that adopting the current interim WHO recommendation to replace *GLURP* with a microsatellite marker (Poly-α, Pfpk2, or TA1) in settings characterised by high multiplicity of infection, as outlined

	Artesunate–pyronaridine	Artesunate–pyronaridine–atovaquone–proguanil	Artesunate–fosmidomycin–clindamycin
All age groups combined			
ACPR at day 28 (intention-to-treat and PCR-uncorrected)	85% (62–97); 17/20	87·5% (73–96); 35/40	82·5% (67–93); 33/40
ACPR at day 42 (intention-to-treat and PCR-uncorrected)	70% (46–88); 14/20	75% (59–87); 30/40	75% (59–87); 30/40
ACPR at day 28 (per-protocol and PCR-corrected)	100% (80–100); 17/17	100% (90–100); 34/34	97% (86–100%); 36/37
ACPR at day 42 (per-protocol and PCR-corrected)	87·5% (62–98); 14/16	85·3% (69–95); 29/34	94·4% (81–99%); 34/36
Adult age group			
ACPR at day 28 (intention-to-treat and PCR-uncorrected)	NA	90% (55–100); 9/10	90% (55–100); 9/10
ACPR at day 42 (intention-to-treat and PCR-uncorrected)	NA	80% (44–97); 8/10	90% (55–100); 9/10
ACPR at day 28 (per-protocol and PCR-corrected)	NA	100% (66–100); 9/9	100% (66–100); 9/9
ACPR at day 42 (per-protocol and PCR-corrected)	NA	88·9% (52–100); 8/9	100% (66–100); 9/9
Adolescent age group			
ACPR at day 28 (intention-to-treat and PCR-uncorrected)	90% (55–100); 9/10	80% (44–97); 8/10	100% (69–100); 10/10
ACPR at day 42 (intention-to-treat and PCR-uncorrected)	80% (44–97); 8/10	60% (26–88); 6/10	80% (44–97); 8/10
ACPR at day 28 (per-protocol and PCR-corrected)	100% (66–100); 9/9	100% (63–100); 8/8	100% (69–100); 10/10
ACPR at day 42 (per-protocol and PCR-corrected)	100% (63–100); 8/8	75% (35–97); 6/8	100% (66–100); 9/9
Children age group			
ACPR at day 28 (intention-to-treat and PCR-uncorrected)	80% (44–97); 8/10	90% (68–99); 18/20	70% (46–88); 14/20
ACPR at day 42 (intention-to-treat and PCR-uncorrected)	60% (26–88); 6/10	80% (56–94); 16/20	65% (41–85); 13/20
ACPR at day 28 (per-protocol and PCR-corrected)	100% (63–100); 8/8	100 (80–100); 17/17	94% (73–100); 17/18
ACPR at day 42 (per-protocol and PCR-corrected)	75% (35–97); 6/8	88·2 (64–99); 15/17	88·9% (65–99); 16/18

Data are % (95% CI); n/N. There was no recruitment of adult participants in the artesunate–pyronaridine group. ACPR=adequate clinical and parasitological response. NA=not applicable.

Table 2: **ACPR at day 42 and day 28 after treatment (including 95% CIs and absolute numbers)**

in the 2021 consultation document, would be beneficial. This shift is particularly pertinent in high-transmission areas, such as those included in our study, where the accuracy of distinguishing between recrudescence and re-infection might be compromised owing to the limitations associated with *GLURP*.[29] Third, although both APAP and AFC were shown to be efficacious, safe, and tolerable, our study could not investigate all unique features of APAP and AFC regimens comprehensively. For APAP, artesunate eliminates young stage gametocytes, and atovaquone–proguanil exerts a delayed effect on the sexual development of *Plasmodium* spp. In addition, AP is shown to preclude onwards transmission of sporadic isolates with specific drug resistance.[30] Although this might theoretically lead to an enhanced antitransmission property, this cannot be proven by the current study, owing to the sparsity of incidental gametocyte detection (data not shown). Costs for atovaquone–proguanil have decreased substantially over the past decade but are still higher than for other antimalarial drugs. Unless further reductions of costs are achieved, atovaquone–proguanil might at present make APAP a less attractive regimen for

	Artesunate–pyronaridine (20)	Artesunate–pyronaridine–atovaquone–proguanil (n=40)	Artesunate–fosmidomycin–clindamycin (n=40)
Cardiovascular	1 (5%)	0	0
Gastrointestinal	6 (30%)	24 (52%)	34 (61%)
Haematological and metabolic	5 (25%)	7 (15%)	1 (2%)
Musculoskeletal	1 (5%)	1 (2%)	1 (2%)
Neurological	5 (25%)	7 (15%)	16 (29%)
Skin	2 (10%)	7 (15%)	4 (7%)
Total	20	46	56

Data are n or n (%). Percentages are calculated as proportions of the total number of TEAEs in each group. TEAEs=treatment-emergent adverse events.

Table 3: **Summary of TEAEs occurring during 42 days of follow-up by treatment group and organ class**

implementation in malaria control programmes. For AFC, the collateral antibacterial activity might predispose its further development into a broad-spectrum therapy used for the treatment of malaria and bacterial diseases. However, the current study only focused on clinical and *Plasmodium*-related endpoints. Therefore, future AFC studies should attempt to incorporate further microbiological efficacy endpoints. Given the favourable antimalarial efficacy of AFC as estimated in this study, the conduct of future, larger AFC studies should be pursued. Fourth, the favourable results for APAP and AFC regimens, such as the high day-28 efficacies and day-42 efficacy, which is similar to standard AP treatment, need to be interpreted in the context of being produced by a clinical phase 2 study, requiring confirmation by larger phase 3 studies. In this regard, future phase 3 studies should not only assess efficacy up to day 28 but also include later timepoints (eg, day 42 or 63) to verify whether standard-of-care AP treatment and experimental APAP treatment in particular retain excellent efficacy beyond day 28.

Despite promising preliminary findings favouring the multidrug ACT regimens APAP and AFC, multidrug ACT is still a relatively novel treatment concept that, to the best of our knowledge, has not yet been operationally implemented in any malaria-endemic country. In this context, authors have mentioned that the potential effect of triple or multidrug ACT regimens in halting the development of drug-resistance development would be highest when being implemented in routine malaria control before the development of clinically important partner drug resistance.[5,31] Only in such a setting would multidrug ACT be able to effectively prevent the selection of drug-resistant strains, when still both long-acting partner drugs remain mutually protective. In other words, multidrug ACT would need to be implemented during periods of still highly efficacious ACT. To confirm the efficacy and safety of the multidrug ACT regimens assessed and thus support their broader implementation in malaria-endemic regions, large phase 3 trials are needed.

Contributors

JM, MR, CP, and SGW substantially contributed to analysis and interpretation of data. JCDA, OM-A, AAA, FNS, WL, JK, JMB, JRE, IDA, RBA, EYB-P, EA, DEM, EP, DKK, PB, SK, BL, STA, JHA, RZ-M, GM-N, and PGK substantially contributed to acquisition of data. MR, JM, and SGW substantially contributed to conception and design. JM and MR participated in drafting the manuscript. SGW, CP, JCDA, OM-A, and AAA critically revised the manuscript for important intellectual content. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication, and JM, DEM, JCDA, and PB directly assessed and verified the data of the study.

Declaration of interests

The authors have no competing interests directly related to this study. SGW reports the following unrelated interests: over the past 36 months, he has received research grants or contracts from Boehringer Ingelheim for developing novel methodology to analyse exposure–response data, from AqVida for the development of dosing software for anticancer drugs, and InfectoPharm for modelling preclinical combination therapy data; he has provided consulting services for Merck and Medicines for Malaria Venture, both related to modelling preclinical combination therapy data; has received payment from GSK for a lecture on combination modelling; and has held leadership roles, including serving as the President of the International Society of Anti-Infective Pharmacology from 2020 to 2022 (unpaid), and as a member of the EC of the pharmacokinetics–pharmacodynamics study group of the European Society of Clinical Microbiology and Infectious Diseases since 2019, and as a Fellow starting in 2024.

Data sharing

Individual participant data underlying the results reported in this article, including text, tables, figures, and appendices, will be available after de-identification. Interested parties can also access the study protocol. Data will be shared starting 9 months and ending 36 months following the publication of the Article. Access will be granted to investigators whose proposed use of the data has been approved by an independent review committee (a learned intermediary) identified for this purpose. The data will be available for individual participant data meta-analysis. Proposals for data access can be submitted up to 36 months after the Article's publication. After this period, the data will be stored in our Institute's data warehouse and will be accessible without investigator support, other than the deposited metadata. To submit proposals and access the data, interested individuals should contact the corresponding author for additional information and guidance.

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