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Efficacy of pyriproxyfen-pyrethroid long-lasting insecticidal nets (LLINs) and chlorfenapyr-pyrethroid LLINs compared with pyrethroid-only LLINs for malaria control in Benin: a cluster-randomised, superiority trial

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Summary

Background New classes of long-lasting insecticidal nets (LLINs) combining mixtures of insecticides with different modes of action could put malaria control back on track after rebounds in transmission across sub-Saharan Africa. We evaluated the relative efficacy of pyriproxyfen-pyrethroid LLINs and chlorfenapyr-pyrethroid LLINs compared with standard LLINs against malaria transmission in an area of high pyrethroid resistance in Benin.

Methods We conducted a cluster-randomised, superiority trial in Zou Department, Benin. Clusters were villages or groups of villages with a minimum of 100 houses. We used restricted randomisation to randomly assign 60 clusters to one of three LLIN groups (1:1:1): to receive nets containing either pyriproxyfen and alpha-cypermethrin (pyrethroid), chlorfenapyr and alpha-cypermethrin, or alpha-cypermethrin only (reference). Households received one LLIN for every two people. The field team, laboratory staff, analyses team, and community members were masked to the group allocation. The primary outcome was malaria case incidence measured over 2 years after net distribution in a cohort of children aged 6 months–10 years, in the intention-to-treat population. This study is ongoing and is registered with ClinicalTrials.gov, NCT03931473.

Findings Between May 23 and June 24, 2019, 53 854 households and 216 289 inhabitants were accounted for in the initial census and included in the study. Between March 19 and 22, 2020, 115 323 LLINs were distributed to 54 030 households in an updated census. A cross-sectional survey showed that study LLIN usage was highest at 9 months after distribution (5532 [76·8%] of 7206 participants), but decreased by 24 months (4032 [60·6%] of 6654). Mean malaria incidence over 2 years after LLIN distribution was 1·03 cases per child-year (95% CI 0·96–1·09) in the pyrethroid-only LLIN reference group, 0·84 cases per child-year (0·78–0·90) in the pyriproxyfen-pyrethroid LLIN group (hazard ratio [HR] 0·86, 95% CI 0·65–1·14; p=0·28), and 0·56 cases per child-year (0·51–0·61) in the chlorfenapyr-pyrethroid LLIN group (HR 0·54, 95% CI 0·42–0·70; p<0·0001).

Interpretation Over 2 years, chlorfenapyr-pyrethroid LLINs provided greater protection from malaria than pyrethroid-only LLINs in an area with pyrethroid-resistant mosquitoes. Pyriproxyfen-pyrethroid LLINs conferred protection similar to pyrethroid-only LLINs. These findings provide crucial second-trial evidence to enable WHO to make policy recommendations on these new LLIN classes. This study confirms the importance of chlorfenapyr as an LLIN treatment to control malaria in areas with pyrethroid-resistant vectors. However, an arsenal of new active ingredients is required for successful long-term resistance management, and additional innovations, including pyriproxyfen, need to be further investigated for effective vector control strategies.

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Introduction

Pyrethroid-treated long-lasting insecticidal nets (LLINs) are the main malaria prevention intervention in sub-Saharan Africa and have been the major contributor to the estimated 1·5 billion malaria cases and 7·6 million malaria deaths averted in the past two decades. However, since 2015, the decline in malaria cases has stalled, and between 2019 and 2020, a rebound in malaria transmission was reported in some areas of sub-Saharan Africa.[1] This rebound is likely to be a consequence of the continued spread of resistance to pyrethroid insecticides in malaria-transmitting mosquitoes, coinciding with a plateau in malaria control investment, leading to suboptimal coverage of interventions. Urgent actions are needed to prevent malaria resurgences, which were previously observed in sub-Saharan Africa in the 1980s after malaria control measures were scaled down.[2]

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Research in context

Evidence before this study

There is a paucity of evidence concerning the efficacy of new classes of insecticidal-treated nets against malaria, especially in west Africa, where vectors usually have high insecticide resistance. On March 31, 2022, we searched PubMed, with no language or date restrictions, using the terms "randomised controlled trial" AND "malaria" AND "insecticide-treated net" OR "long-lasting insecticidal net", combined with either "pyriproxyfen" OR "chlorfenapyr" OR "piperonyl butoxide". The search yielded four studies, of which two were directly relevant to our study. The effectiveness of pyriproxyfen-pyrethroid long-lasting insecticidal nets (LLINs) was assessed in Burkina Faso and Tanzania, and chlorfenapyr-pyrethroid nets were evaluated in Tanzania.

The trial in Burkina Faso used a step-wedge design to compare the pyriproxyfen-pyrethroid LLINs to standard pyrethroid-only LLINs over 18 months, whereas the Tanzanian study was a four-arm superiority trial comparing chlorfenapyr-pyrethroid LLINs, pyriproxyfen-pyrethroid LLINs, and piperonyl-butoxide (PBO)-pyrethroid LLINs versus standard pyrethroid-only LLINs over 2 years. In the Burkina Faso trial, the authors showed a 12% reduction in malaria case incidence in children receiving pyriproxyfen-pyrethroid LLINs compared with those who received standard pyrethroid-only LLINs, and observed no effect of the pyriproxyfen-pyrethroid LLINs on malaria prevalence. In the Tanzania trial, there was no evidence of superior effect of the pyriproxyfen-pyrethroid LLINs on any malaria outcomes over 2 years; children had 55% lower odds of malaria infection and 44% lower risk of malaria incidence over 2 years in villages that received chlorfenapyr-pyrethroid LLINs compared with villages that received pyrethroid-only LLINs. PBO-pyrethroid LLINs were consistently found to be more effective than pyrethroid-only LLINs; however, the duration of the superior effect varied from 12 months to 21 months according to the different trials.

Added value of this study

To our knowledge, this is the second study aiming to compare the efficacy of the next generation of LLINs combining pyriproxyfen or chlorfenapyr and pyrethroid versus standard pyrethroid-only LLINs, and the first of its kind to be conducted in west Africa. This trial confirmed the superior efficacy of chlorfenapyr-pyrethroid LLINs, in terms of malaria case incidence, prevalence, and transmission in children, over 2 years of use in the community, in an area of moderate malaria transmission and with high insecticide resistance intensity in malaria vectors, in Benin. However, pyriproxyfen-pyrethroid LLINs did not offer additional protection against malaria outcomes compared with pyrethroid-only LLINs.

Implications of all the available evidence

Given the positive findings, both in Benin and Tanzania, for chlorfenapyr-pyrethroid LLINs, they are likely to become the first WHO-recommended LLINs impregnated with an insecticide class other than pyrethroids. The absence of superior efficacy of pyriproxyfen-pyrethroid LLINs compared with standard pyrethroid-only LLINs is consistent with the results of the previous Tanzania study and calls into question the role of the current pyriproxyfen-pyrethroid LLINs in future malaria vector strategies.

In the past 10 years, new insecticide-treated nets containing non-pyrethroid insecticides and insecticide synergists, in combination with pyrethroids, have been shown to be safe and efficacious against malaria mosquito vectors.[3,4] The first net combined a pyrethroid insecticide and the synergist piperonyl butoxide (PBO) and, following a randomised controlled trial,[5] received a WHO policy recommendation in 2017. Other next-generation LLINs have shown encouraging results against resistant vectors in laboratory and small-scale entomological studies.[4,6,7] One of these nets is a dual active-ingredient LLIN combining a pyrethroid and a pyrrole (chlorfenapyr). Both insecticides lead to mosquito mortality, with chlorfenapyr disrupting the production of cellular energy rather than targeting the nervous system, as pyrethroids do. Another dual active-ingredient LLIN combines a pyrethroid with an insect growth regulator (pyriproxyfen), which leads to sterility in exposed adult mosquitoes.[8]

The first randomised trial of these two products was conducted in Tanzania and showed that the chlorfenapyr-pyrethroid LLINs nearly halved malaria infection prevalence and clinical cases compared with the pyrethroid-only LLINs over 2 years of use.[9] Using a pyriproxyfen-pyrethroid LLIN reduced the odds of malaria infection prevalence by 41% during the first year of use but the effect was not sustained during the second year, and no significant effect was seen on malaria cases in either year. Another trial conducted in Burkina Faso with a different brand of pyriproxyfen-pyrethroid LLIN showed a 12% reduction in malaria case incidence compared with the standard pyrethroid-only LLIN group, with no effect on malaria infection prevalence.[10]

To obtain a WHO public health recommendation, new vector control product classes need to be evaluated in two randomised trials in areas with differing malaria endemicity, insecticide resistance intensity, and malaria vector populations.[11] We assessed the relative efficacy of pyriproxyfen-pyrethroid LLINs and chlorfenapyr-pyrethroid LLINs compared with standard pyrethroid-only LLINs against malaria case incidence, infection prevalence, and transmission, in an area of pyrethroid resistance in Benin.

Methods

Study design and participants

We conducted a three-group, cluster-randomised, superiority trial in three districts (Covè, Zagnanado, and Ouinhi) in Zou Department, central Benin. Malaria is highly endemic in the region, with a peak during the wet season (May–October). The main vector control strategy in the study area is use of pyrethroid-only LLINs distributed en masse once every 3 years, and through routine service delivery by antenatal clinics and vaccination programmes. The primary vectors in the setting are *Anopheles coluzzii* and *Anopheles gambiae*. There is a high intensity of pyrethroid resistance in the main local vector populations.[12]

A demographic census of all 123 villages in the study area was done in June, 2019. Clusters consisted of villages, or several villages. To reduce contamination between study groups, clusters consisted of a core (minimum 100 households) surrounded by a buffer, ensuring that core areas of neighbouring clusters were separated by at least 1000 m. Households in the buffer and core received the intervention, but measurement of outcomes only took place in core areas. A detailed description of the study protocol is published elsewhere.[13]

Ethics approval was obtained from the Benin Ministry of Health ethics committee (6/30/MS/DC/SGM/DRFMT/CNERS/SA), the London School of Hygiene & Tropical Medicine ethics committee (16237), and the WHO Research Ethics Review Committee (ERC.0003153). The trial was independently monitored by a data safety monitoring board and a trial steering committee.

Epidemiological effect was estimated through measuring malaria case incidence in the 2 years after net distribution by active case detection in a cohort of children. A cohort of approximately 30 children aged 6 months–9 years randomly selected (by simple random sampling using a random number generator) from each cluster (1800 in total) was enrolled in July, 2020. Children were eligible for inclusion if they were permanent residents in the cluster, had no serious illnesses, and written informed consent was obtained from their guardians.

Malaria infection prevalence (in all ages) was measured by cross-sectional surveys at 6 months and 18 months after net distribution. 72 individuals residing in the core of each cluster were randomly selected (using a random number generator) from the census for each cross-sectional survey. Each prevalence survey collected data on malaria infection, measured using malaria rapid diagnostic tests (CareStart malaria HRP2/pLDH [pf/pan] combo, DiaSys, UK), net ownership and use, sex, and household assets (as a proxy for socioeconomic status).

Entomological effects were measured using human landing catches in four randomly selected houses every 3 months in each cluster. Insecticide resistance intensity was measured in two clusters per group (six clusters total) at baseline and in each follow-up year.

Written informed consent was obtained from all participants, or from guardians for participants younger than 18 years. Assent was sought for children aged between 10 and 18 years. Written consent was also obtained from volunteer mosquito collectors who were all aged 18 years or older and who were vaccinated against yellow fever. All participation was voluntary, and participants could withdraw at any time. Study investigators sought consent in French or local languages.

Randomisation and masking

We used restricted randomisation to randomly assign 60 clusters to one of three LLIN groups (1:1:1), to receive nets containing either pyriproxyfen and alpha-cypermethrin (pyrethroid), chlorfenapyr and alpha-cypermethrin, or alpha-cypermethrin only (reference). Restricted randomisation was used to ensure balanced cluster allocation between study groups with respect to population size, malaria infection prevalence (measured in the baseline survey), district (n=3), and socioeconomic status.

To mask the net types from the participants and the field workers, the nets were designed to look as similar as possible. Each net was rectangular, was requested to be the same size (1·8 m length, 1·9 m width, and 1·8 m height), and blue. To differentiate the nets in the field, a colour-coded loop was attached to the net. All data analyses were performed masked.

Procedures

The nets tested in the trial were: Royal Guard (Disease Control Technologies, Greer, SC, USA), polyethylene netting (120 deniers incorporating 220 mg/m² pyriproxyfen and 220 mg/m² alpha-cypermethrin); Interceptor G2 (BASF SE, Ludwigshafen, Germany), polyester netting (100 deniers coated with 200 mg/m² chlorfenapyr and 100 mg/m² alpha-cypermethrin); and the reference net, Interceptor (BASF SE, Ludwigshafen, Germany), polyester netting (100 deniers coated with 200 mg/m² of alpha-cypermethrin). Nets were distributed in collaboration with the Benin National Malaria Control Program. Households were asked to collect their nets from a central point and received one net per every two residents in their household, rounded up for odd numbers. Nets already in houses were not removed but householders were encouraged to use the new study nets. Additionally, net hang-up campaigns to encourage net use took place at 1 month and 7 months after distribution. Net coverage surveys to assess net ownership and usage were done at 1, 9, and 24 months after distribution. Throughout the follow-up period, children enrolled in the analysis cohort and participants enrolled in the cross-sectional surveys were also asked about their net use.

Insecticide content at baseline was assessed on 30 randomly selected new nets per LLIN brand, by gas chromatography with Flame Ionisation Detection, at the

Centre Wallon de Recherches Agronomiques, Gembloux, Belgium. The insecticidal and physical durability of the study nets is being assessed in a separate study.

Due to the COVID-19 pandemic, there was a 3-month gap between the distribution of the nets and the enrolment of children in the cohort. At enrolment and at 1 year after distribution (April, 2021), children were treated with antimalarial drugs (artemether–lumefantrine) to clear any underlying infection. The cohort was monitored from August, 2020, to April, 2022, a 21-month period, which encompassed the first 2 years after net distribution. Study nurses visited children every 2 weeks during the transmission season (April–October) and every 1 month in the dry season (November–March). At each visit, children were clinically examined and if they were febrile or had a history of fever in the past 48 h, they were tested for malaria using a malaria rapid diagnostic test. If the test was positive, the child was treated with artemether–lumefantrine, according to national guidelines.

During cross-sectional surveys, all participants were tested for malaria using a malaria rapid diagnostic test and received treatment if the test was positive, and children younger than 5 years were tested for anaemia. Information regarding net ownership and usage, and other household-level indicators were collected at the same time.

Entomological monitoring was also delayed and took place every 3 months between June, 2020, and April, 2022. Volunteers recruited from study clusters collected mosquitoes that landed on their legs between 1900 h and 0700 h for 1 night at four randomly selected houses in each cluster at each timepoint. Mosquitoes were morphologically identified to species and a subsample of *Anopheles* spp was tested for molecular species using PCR.[14] A random sample of *Anopheles* spp (up to 30% from each nightly catch in each cluster) were tested for sporozoites using the ELISA circumsporozoite protein technique.[15]

Outcomes

The primary outcome was malaria case incidence (infrared frontal temperature ≥37·5°C or reported fever in the previous 48 h, and positive malaria rapid diagnostic test) in children enrolled in the active case detection cohort in the 2 years after net distribution. Visits of children in the cohort at health facilities were monitored and the results of malaria rapid diagnostic tests and any treatment given were recorded. However, it was difficult to assess with certainty if malaria rapid diagnostic tests were used only if the child was symptomatic (as our protocol specified); therefore, the passive data was not included in the primary analyses.

Secondary clinical outcomes were malaria infection prevalence in all age groups and anaemia (defined as haemoglobin concentration <10 g/dL) prevalence in children aged 5 years or younger at 6 months and 18 months after net distribution.

Type and duration of adverse events related to usage of nets were recorded using a prespecified questionnaire at each cohort visit and during net usage and cross-sectional surveys. Data on hospitalisation and death in children in the cohort were collected by interviewing the child's guardian and reviewing the hospital record, following receipt of consent.

The primary entomological outcome was entomological inoculation rate, measured as the mean number of *Plasmodium* spp infective malaria vectors collected per person per night measured indoors and outdoors. Secondary entomological outcomes were vector density (number of mosquitos caught per person per night), sporozoite rate, and species composition. Centers for Disease Control and Prevention bottle bioassays were performed by exposing adult female *An gambiae*, collected as larvae, to alpha-cypermethrin for 30 min (1, 2, 5, and 10 times the diagnostic dose), and to chlorfenapyr (100 µg/bottle) and pyriproxyfen (100 µg/bottle) for 60 min each year.[16] Mortality was recorded at 30 min for all four doses of alpha-cypermethrin (insecticide resistance intensity measurement), and at 24, 48, and 72 h after exposure for chlorfenapyr. The reduction in fecundity rate induced by pyriproxyfen relative to unexposed mosquitoes was assessed by ovarian dissection, 3 days after pyriproxyfen exposure. Other outcomes included in the protocol (parity, resting behaviour, survivorship, and other resistance measures) are still to be analysed and will be published elsewhere.

Statistical analysis

The sample size calculations for epidemiological data collection were calculated using the method of Hayes and Moulton.[17] The study was designed to detect a 30% difference in malaria case incidence, assuming a control group incidence of 1 malaria case per child-year and a coefficient of variation of 0·3 between clusters. This design required 20 clusters per group and 25 (20+5 allowing for loss to follow-up) children per cluster followed up for 2 years to give 80% power. Due to the delay in enrolment, which resulted in a shorter follow-up time, the number of children per cluster was increased to 30 (1800 children total). This sample size calculation includes adjustment for multiple testing (allowing for the three groups) using a Bonferroni-corrected two-sided α of 2·5%.

For malaria infection prevalence, it was assumed that the prevalence in the reference group was 40%, with a coefficient of variation between clusters of 0·3. With 72 individuals per cluster, the study had 80% power to detect a 30% lower prevalence in the intervention groups compared with the reference group, using a Bonferroni-corrected α for multiple comparisons.

The primary intention-to-treat analysis was a comparison of incidence of clinical malaria episodes between each dual active-ingredient LLIN group and the

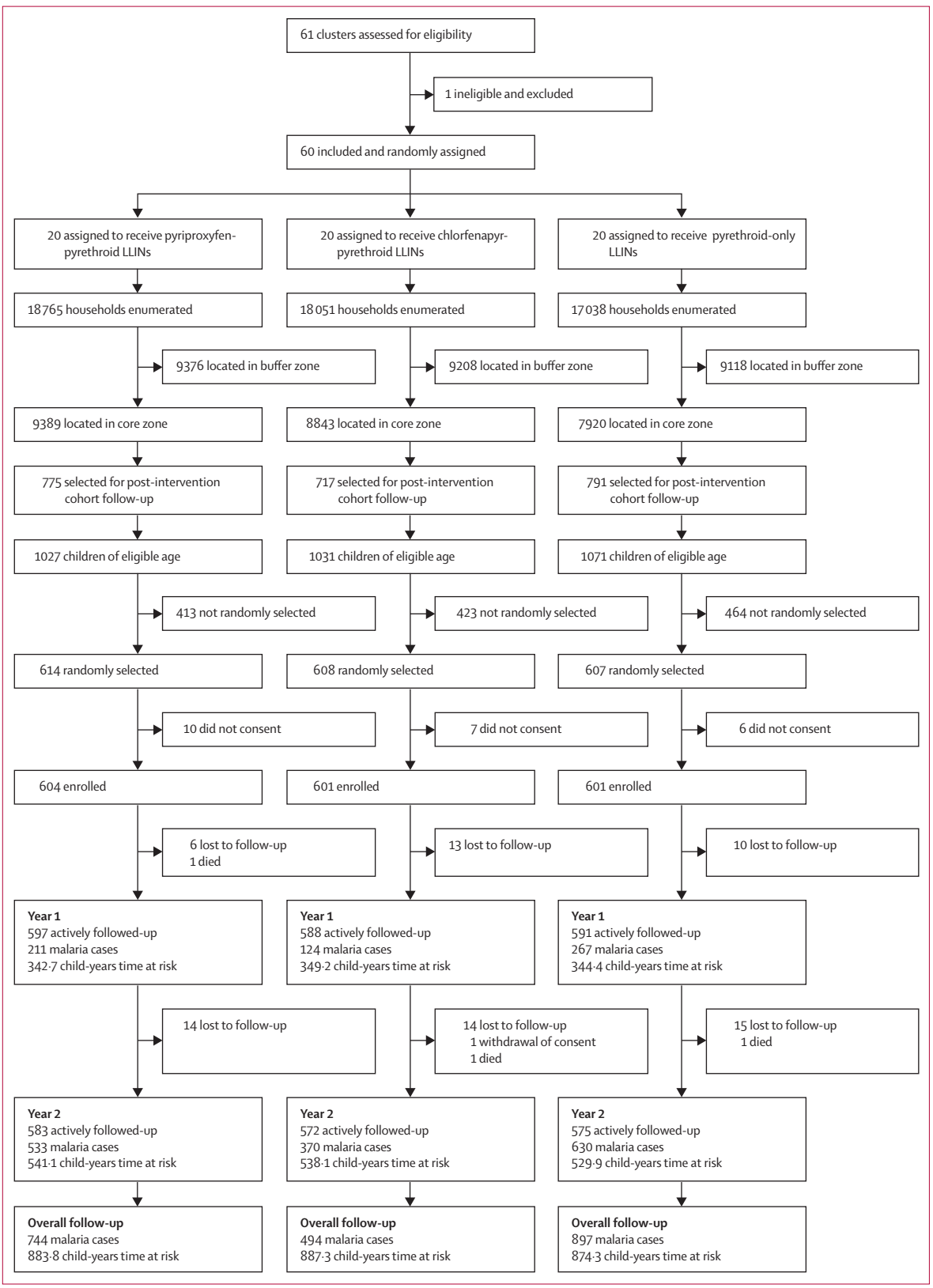

***Figure*: Trial profile**
LLIN=long-lasting insecticidal net.

reference group (pyrethroid-only LLIN). A child was not considered at risk for 2 weeks after treatment and malaria cases detected within 2 weeks of a previous malaria case were not counted, to allow for circulating histidine-rich protein after parasite clearance. We performed sensitivity analyses increasing the censoring period after treatment to 4 weeks or requiring at least one negative visit after a positive diagnosis before follow-up time was uncensored. Survival analysis was used to compare the risk of having a malaria case in each group, using a Cox proportional hazards model allowing for multiple events per child and using cluster-robust estimates of variance by adjusting the SEs. The effect of intervention nets on malaria infection prevalence and anaemia was estimated using mixed-effects logistic models in intention-to-treat analyses with cluster included as a random effect. In per-protocol analyses, only participants who reported using the appropriate study nets in each group were included. Missing data was assessed and found to be missing at random (data not shown), so no imputation was performed.

Indoor and outdoor malaria vector density was calculated for each household visit. A pooled entomological inoculation rate was calculated for each cluster at each visit (eight visits per cluster, 480 visits total). Nightly entomological inoculation rate was calculated as the mean number of mosquitoes per household multiplied by the proportion of sporozoite-positive mosquitoes. Mixed-effect generalised linear models with a negative binomial distribution were used to analyse entomological inoculation rate and mosquito density and a mixed-effect logistic regression for sporozoite rate. Vector density and sporozoite rate were calculated at the household level, with collection timepoint and cluster as random effects. Entomological inoculation rate was calculated at the cluster level for each timepoint, with cluster included as a random effect in the models.

Post-hoc sensitivity analyses for malaria incidence and prevalence included adjusting for baseline cluster-level variables used in restricted randomisation and for entomological outcomes, adjusting for their respective value in the baseline survey. Time-by-study group interactions were examined for each model. Stata (version 16) was used to analyse epidemiological and entomological data.

This study is registered with ClinicalTrials.gov, NCT03931473.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit for publication.

	Pyriproxyfen-pyrethroid LLIN group	Chlorfenapyr-pyrethroid LLIN group	Pyrethroid-only LLIN group
Clusters			
Number of clusters	20	20	20
Total population in core and buffer areas	74 822	70 989	69 239
Mean population in core area of clusters (range)	1096·1 (225–4524)	1120·5 (243–5040)	1058·1 (252–5217)
Number of people per household, mean (SD)	4·0 (2·3)	3·9 (2·3)	4·1 (2·4)
Number of sleeping spaces per household, mean (SD)	2·2 (1·2)	2·1 (1·1)	2·2 (1·2)
Household and participant characteristics in the baseline cross-sectional survey			
Low socioeconomic status*	35·8%; 529/1479	36·2%; 533/1474	29·0%; 431/1487
LLIN ownership (at least one LLIN in the household)	96·4%; 1426/1479	97·1%; 1431/1474	95·1%; 1415/1488
LLIN usage in all age groups the night before survey	95·8%; 1312/1370	94·9%; 1258/1326	96·5%; 1343/1392
Malaria infection prevalence in all age groups	43·1%; 636/1475	40·7%; 598/1468	46·5%; 690/1485
Anaemia prevalence in children aged 6 months–4 years†	53·3%; 136/255	53·3%; 131/246	50·2%; 122/243
Entomological characteristics			
Human biting density per person per night indoors‡, mean (SD)	32·9 (28·9)	21·6 (21·0)	29·2 (25·3)
Human biting density per person per night outdoors‡, mean (SD)	20·3 (17·6)	16·4 (17·4)	20·6 (20·2)
Indoor EIR per person per night, mean (SD)	0·62 (0·93)	0·48 (0·65)	0·96 (1·18)
Outdoor EIR per person per night, mean (SD)	0·27 (0·45)	0·17 (0·44)	0·33 (0·45)
Children (aged 6 months–10 years) at cohort enrolment			
Proportion of children younger than 5 years	52·3%; 316/604	50·6%; 304/601	53·6%; 322/601
Proportion of female children	47·2%; 285/604	48·4%; 291/601	48·8%; 293/601
Net usage the night before survey	99·0%; 598/604	98·7%; 593/601	99·2%; 596/601

Data are n or %; n/N unless otherwise stated. EIR=entomological inoculation rate. LLIN=long-lasting insecticidal net. *Proportion of households in the poorest tercile based on the wealth index of the entire study area. †Anaemia defined as haemoglobin concentration of <10 g/dL. ‡Malaria vectors included *Anopheles gambiae*, *Anopheles funestus*, and *Anopheles nili*.

Table 1: **Baseline characteristics**

Results

Between May 23 and June 24, 2019, 53 854 households and 216 289 inhabitants were accounted for in the initial census and included in the study. The households were delineated into 61 clusters, with one subsequently excluded due to extensive seasonal flooding (figure). Baseline cross-sectional epidemiological and entomological surveys were conducted between October and November, 2019 (table 1). The malaria infection prevalence was 43·5% (1924 of 4428 participants, cluster range 15·1–72·7%) and population self-reported LLIN usage was 95·7% (3913 of 4088 participants). Cluster demographics and malaria prevalence were similar between groups (table 1). Entomological inoculation rate was 0·68 *Plasmodium* spp infective bites per person per night (cluster range 0·00–3·80) indoors and 0·26 per person per night (0·00–1·88) outdoors.

Between March 19 and 22, 2020, 115 323 LLINs were distributed to 54 030 households in an updated census, with 97·1% of households receiving at least one net per household. Active ingredients in the new nets were found to be within or higher than the acceptable target limits depending on LLIN brand (appendix p 4). Study net usage was highest (5532 [76·8%] of 7206 participants) at 9 months after distribution, following a second hang-up campaign, but had decreased by 24 months (4032 [60·6%] of 6654). Study net coverage and usage indicators were similar between study groups up to 18 months after net distribution. At 24 months, pyriproxyfen-pyrethroid LLIN usage and ownership was the lowest (appendix pp 5–6). Use of any type of LLIN (study LLINs and others) remained greater than 80% up to 24 months after distribution (appendix p 7).

In the 2283 households that were randomly selected for post-intervention cohort follow-up, 3129 children were eligible, 1829 of whom were randomly selected, and consent was obtained for 1806 (figure). Among the 2849 and 2771 households randomly selected at 6 months and 18 months after LLIN distribution for the malaria prevalence cross-sectional surveys, 4781 (85·1%) of 5620 households consented, with a similar proportion in the two surveys. The remaining households were either not available during the visit (775 [13·8%]) or declined to participate (64 [1·1%]; appendix p 8).

Children aged 6 months–9 years enrolled for active case detection were monitored for 21 months, for a total follow-up time of 2645·4 child-years at risk. Loss to follow-up was similar between groups (figure). At enrolment, children were balanced on age, sex, and net usage. During the 21-month follow-up period, we detected 2135 malaria cases through active case detection (table 2). The mean malaria case incidence over 21 months of follow-up was 1·03 cases per child-year (95% CI 0·96–1·09) in the pyrethroid-only LLIN reference group, 0·84 cases per child-year (0·78–0·90) in the pyriproxyfen-pyrethroid LLIN group (hazard ratio [HR] 0·86, 95% CI 0·65–1·14; p=0·28), and 0·56 cases per child-year (0·51–0·61) in the chlorfenapyr-pyrethroid LLIN group (HR 0·54, 95% CI 0·42–0·70; p<0·0001; table 2). The strongest effect was observed in the first year of follow-up in the chlorfenapyr-pyrethroid group (HR 0·46, 95% CI 0·30–0·72; p=0·0005; table 2). When combining active and passive visits, the number of cases detected nearly doubled; however, the effect size in comparison to the reference group was similar for both intervention nets (appendix p 9).

Malaria infection prevalence was 23·5% (1037 of 4409 participants) at 6 months and 34·9% (1554 of 4450) at 18 months after net distribution (table 3). There was strong evidence for a reduction of malaria infection prevalence in the chlorfenapyr-pyrethroid LLIN group at 6 months (15·7%; odds ratio [OR] 0·47, 95% CI 0·32–0·69; p=0·0002) and at 18 months (27·9%; OR 0·60, 0·43–0·85; p=0·0041) compared with the reference group (28·0% at 6 months and 38·7% at 18 months; table 3). In the pyriproxyfen-pyrethroid group, there was no evidence for a reduction in malaria prevalence at 6 months (26·9%; OR 0·92, 95% CI 0·63–1·35; p=0·67) or 18 months (38·2%; OR 0·97, 0·69–1·37; p=0·87) compared with the reference group. Results were similar for the per-protocol analysis (appendix p 11). There was no evidence of a reduction in moderate to severe anaemia prevalence in either intervention group (table 3). The post-hoc analysis adjusting for covariates used in the randomisation did not change the interpretation of the results (appendix pp 12–13).

	Number of clinical malaria episodes	Child-years of follow-up	Incidence, cases per child-year (95% CI)	Hazard ratio	95% CI	p value*
Overall						
Pyrethroid-only LLIN group	897	874·3	1·03 (0·96–1·09)	1 (ref)	..	..
Pyriproxyfen-pyrethroid LLIN group	744	883·8	0·84 (0·78–0·90)	0·86	0·65–1·14	0·28
Chlorfenapyr-pyrethroid LLIN group	494	887·3	0·56 (0·51–0·61)	0·54	0·42–0·70	<0·0001
Year 1						
Pyrethroid-only LLIN group	267	344·4	0·77 (0·69–0·87)	1 (ref)	..	..
Pyriproxyfen-pyrethroid LLIN group	211	342·7	0·62 (0·54–0·70)	0·83	0·51–1·35	0·46
Chlorfenapyr-pyrethroid LLIN group	124	349·2	0·36 (0·30–0·42)	0·46	0·30–0·72	0·0005
Year 2						
Pyrethroid-only LLIN group	630	529·9	1·19 (1·10–1·29)	1 (ref)	..	..
Pyriproxyfen-pyrethroid LLIN group	533	541·1	0·98 (0·90–1·07)	0·88	0·68–1·13	0·31
Chlorfenapyr-pyrethroid LLIN group	370	538·1	0·69 (0·62–0·76)	0·57	0·45–0·73	<0·0001

Each intervention was compared with the pyrethroid-only LLIN group for the same timepoint. LLIN=long-lasting insecticidal net. *A p value of <0·025 was considered significant after Bonferroni correction.

Table 2: **Malaria case incidence in children aged 6 months–10 years per year of follow-up and overall (including active visits only)**

See Online for appendix

	Malaria infection					Anaemia in children younger than 5 years				
	n/N	Prevalence	OR	95% CI	p value*	n/N	Prevalence	OR	95% CI	p value*
6 months after net distribution										
Pyrethroid-only LLIN group	412/1471	28·0 %	1 (ref)	..	..	99/241	41·1 %	1 (ref)	..	..
Pyriproxyfen-pyrethroid LLIN group	394/1463	26·9 %	0·92	0·63–1·35	0·67	117/250	46·8 %	1·24	0·71–2·18	0·45
Chlorfenapyr-pyrethroid LLIN group	231/1475	15·7 %	0·47	0·32–0·69	0·0002	82/241	34·0 %	0·71	0·40–1·26	0·24
18 months after net distribution										
Pyrethroid-only LLIN group	576/1489	38·7 %	1 (ref)	..	..	118/252	46·8 %	1 (ref)	..	..
Pyriproxyfen-pyrethroid LLIN group	564/1478	38·2 %	0·97	0·69–1·37	0·87	108/245	44·1 %	0·84	0·40–1·79	0·65
Chlorfenapyr-pyrethroid LLIN group	414/1483	27·9 %	0·60	0·43–0·85	0·0041	118/246	48·0 %	1·08	0·51–2·28	0·84

Anaemia was defined as a haemoglobin concentration of <10 g/dL. LLIN=long-lasting insecticidal net. OR=odds ratio. *A p value of <0·025 was considered significant after Bonferroni correction.

Table 3: **Malaria infection and anaemia prevalence in the study population at 6 months and 18 months after net distribution (intention-to-treat analysis)**

Adverse events related to study nets were reported in 528 (45·4%) of 1162 participants surveyed at 1 month after net distribution. The highest proportion of adverse events was reported in the pyrethroid-only LLIN group (247 [63·8%] of 387 participants), followed by the pyriproxyfen-pyrethroid LLIN group (212 [52·5%] of 404) and then the chlorfenapyr-pyrethroid LLIN group (69 [18·6%] of 371). Facial burning (392 [33·7%] of 1162 participants), and skin irritation or itchiness (244 [20·9%]) were the most common adverse events in all groups. Adverse events were rare in all three groups at all later timepoints (appendix pp 14–15). We recorded 44 serious adverse events (including three deaths) in the cohort children, with 32 (72·7%) documented as severe malaria (11 cases in the pyriproxyfen-pyrethroid LLIN group, ten in the chlorfenapyr-pyrethroid LLIN group, and 11 in the pyrethroid-only LLIN group). No serious adverse events related to net use were reported.

A total of 259 265 mosquitoes were collected over 3840 collection nights indoors and outdoors, of which 20·9·% (29 814 from indoors and 24 436 from outdoors) were malaria vectors, with *An gambiae* sensu lato the most predominant. Overall, indoor entomological inoculation rate was lower in both intervention groups than in the reference group, with a mean entomological inoculation rate of 0·09 infectious bites per night per person in the chlorfenapyr-pyrethroid LLIN group (rate ratio [RR] 0·34, 95% CI 0·18-0·62; p=0·0005), 0·12 in the pyriproxyfen-pyrethroid LLIN group (RR 0·42, 0·23-0·74; p=0·0028), and 0·28 in the pyrethroid-only LLIN group (table 4). Mean indoor vector density appeared to be lower in the chlorfenapyr-pyrethroid LLIN group (10·1 bites per person per night, density ratio 0·44, 95% CI 0·23–0·84; p=0·014), and in the pyriproxyfen-pyrethroid LLIN group (13·6 bites per person per night, density ratio 0·58, 0·30–1·12; p=0·11) than in the reference group (23·0 bites per person per night), but the latter difference was not statistically significant (table 4). Adjusting for baseline vector density in the models gave similar results (appendix p 16). There was no significant difference in sporozoite rate in any of the intervention groups compared with the reference group (table 4).

Reduction in outdoor entomological inoculation rate compared with the reference group was observed only in the chlorfenapyr-pyrethroid LLIN group (RR 0·30, 95% CI 0·13–0·67; p=0·0035; appendix p 17). There was very weak evidence for a reduction in outdoor entomological inoculation rate in the pyriproxyfen-pyrethroid LLIN group compared with the pyrethroid-only group (RR 0·58, 0·30–1·13; p=0·11). Similar effects were observed for outdoor vector density for both intervention nets (appendix p 17).

Post-intervention pyrethroid resistance intensity was high across the study groups in *An gambiae* sensu lato, with mean mortality of 85% or less after exposure to 10 times the diagnostic concentrations of alpha-cypermethrin in year 2 (appendix p 18). No resistance to chlorfenapyr was observed during either year after net distribution (appendix p 18). Exposure of *An gambiae* sensu lato to pyriproxyfen led to a high reduction in fecundity rate relative to unexposed control mosquitoes over the 2 years after net distribution (73·2%, 95% CI 62·2–82·4 in year 1, and 76·6%, 66·6–84·9 in year 2).

Discussion

This trial assessed the efficacy of two dual active-ingredient LLINs in an area of Benin with malaria vectors that are highly resistant to pyrethroids and found that chlorfenapyr-pyrethroid LLINs provided significantly better protection against malaria for up to 2 years after net distribution compared with pyrethroid-only LLINs. Children aged 6 months–10 years living in clusters that received chlorfenapyr-pyrethroid LLINs had a 46% lower incidence of malaria over 2 years after LLIN distribution;

participants of any age had 53% lower odds of malaria infection at 6 months and 40% lower odds at 18 months after LLIN distribution, and were exposed to a 66% reduction in entomological inoculation rate compared with those living in clusters that received pyrethroid-only LLINs. Similar gains were not observed with the pyriproxyfen-pyrethroid LLIN, with a small reduction in malaria incidence in the first year of the study that was attenuated in year 2, and no effect on malaria infection prevalence in either year, compared with the pyrethroid-only reference group. However, a 58% reduction in indoor entomological inoculation rate was detected with the pyriproxyfen-pyrethroid LLIN. Both dual active-ingredient LLINs provided a similar safety profile compared with standard pyrethroid-only LLINs, with short-lasting skin irritation and facial burning (commonly associated with the pyrethroid alpha-cypermethrin) most frequently reported in participants using pyrethroid-only LLINs and pyriproxyfen-pyrethroid LLINs. A similar observation has been reported in another randomised controlled trial evaluating the same LLINs,[9] and this is likely to be associated with the high concentration of alpha-cypermethrin in those nets compared with the chlorfenapyr-pyrethroid LLINs.

To our knowledge, this is the second cluster-randomised trial to provide strong evidence of increased efficacy of chlorfenapyr-pyrethroid LLINs relative to pyrethroid-only LLINs for malaria control in areas with pyrethroid-resistant vectors. Chlorfenapyr insecticide on nets has already shown improved control of pyrethroid-resistant *An gambiae* in laboratory and semi-field experimental huts against resistant malaria vectors.[18–21] The previous trial in Tanzania reported a malaria case incidence reduction of 44%, a 55% decrease in odds of malaria prevalence, and 85% reduction in entomological inoculation rate compared with the pyrethroid-only group, consistent with these results, despite the differing malaria vector populations and intensity of pyrethroid resistance in the mosquitoes.[9] In both trials, per-protocol analyses suggested that individuals living in the chlorfenapyr-pyrethroid clusters benefited regardless of whether they were using a study net, suggesting a community effect of the net, which was likely to be obtained by the overall reduction of mosquito density, which remained considerably lower than in the pyrethroid-only group in both years of this trial after net distribution. Community effect is also indicated by the reduction in both indoor and outdoor entomological indices in the chlorfenapyr-pyrethroid group in the present study.

Similar to the trial in Tanzania,[9] the pyriproxyfen-pyrethroid LLINs we tested did not provide additional protection against malaria infection or disease compared with pyrethroid-only LLINs. However, there was evidence of an effect on indoor transmission, with the strongest effect seen in the first year after net distribution. Tiono and colleagues[10] have previously reported a 12% reduction in malaria incidence and 49% reduction in entomological

	Number of households analysed	Vector density per night				Sporozoite rate					Entomological inoculation rate		
		Number of female *Anopheles* vectors	Mean *Anopheles* bites per night per person	Density ratio (95% CI)	p value	Number of sporozoite-positive female *Anopheles* vectors	Number of *Anopheles* vectors tested	Sporozoite rate (%)	Odds ratio (95% CI)	p value	Mean infectious bites per night per person	Rate ratio (95% CI)	p value
Overall													
Pyrethroid-only LLIN group	640	14 709	23·0	1 (ref)	..	68	4812	1·6%	1 (ref)	..	0·28	1 (ref)	..
Pyriproxyfen-pyrethroid LLIN group	640	8670	13·6	0·58 (0·30–1·12)	0·11	42	3283	1·0%	0·88 (0·58–1·34)	0·56	0·12	0·42 (0·23–0·74)	0·0028
Chlorfenapyr-pyrethroid LLIN group	640	6435	10·1	0·44 (0·23–0·84)	0·014	37	3602	1·0%	0·70 (0·45–1·09)	0·11	0·09	0·34 (0·18–0·62)	0·0005
Year 1													
Pyrethroid-only LLIN group	320	7371	23·0	1 (ref)	..	42	2898	2·0%	1 (ref)	..	0·25	1 (ref)	..
Pyriproxyfen-pyrethroid LLIN group	320	4537	14·2	0·50 (0·26–0·96)	0·039	15	1621	0·7%	0·61 (0·32–1·15)	0·13	0·08	0·32 (0·13–0·81)	0·016
Chlorfenapyr-pyrethroid LLIN group	320	3572	11·2	0·47 (0·24–0·90)	0·023	17	2130	0·8%	0·53 (0·29–0·98)	0·042	0·08	0·30 (0·12–0·78)	0·013
Year 2													
Pyrethroid-only LLIN group	320	7338	22·9	1 (ref)	..	26	1914	1·3%	1 (ref)	..	0·33	1 (ref)	..
Pyriproxyfen-pyrethroid LLIN group	320	4133	12·9	0·67 (0·32–1·37)	0·27	27	1662	1·4%	1·18 (0·61–2·29)	0·63	0·17	0·52 (0·24–1·12)	0·095
Chlorfenapyr-pyrethroid LLIN group	320	2863	8·9	0·41 (0·20–0·84)	0·015	20	1472	1·2%	0·96 (0·47–1·97)	0·91	0·13	0·39 (0·17–0·89)	0·025

Each intervention group was compared with the pyrethroid-only LLIN group for the same timepoint. Entomological inoculation rate was calculated as the product of human biting density multiplied by sporozoite rate at cluster level. LLIN=long-lasting insecticidal net.

Table 4: **Indoor entomological outcomes per year of collection and overall (year 1 and 2 combined).**

inoculation rate with a pyriproxyfen-pyrethroid LLIN compared with pyrethroid-only LLINs over 18 months in a stepped-wedge randomised trial in Burkina Faso. The different study design, length of follow-up, and brand of net might explain the differences seen between the two studies. In Benin, laboratory and semi-field experimental hut studies have shown the superior efficacy of pyriproxyfen-pyrethroid nets on entomological indicators compared with standard pyrethroid-only LLINs,[6,22,23] and are consistent with the decrease in indoor vector density observed in our trial. However, the decrease in indoor vector density did not translate into significant disease reduction, suggesting that a larger effect on malaria transmission (entomological inoculation rate) is crucial to provide community protection. Although lower net usage in the pyriproxyfen-pyrethroid LLIN group could have partially contributed to the lack of effect, we are also assessing textile durability, sterilisation effects, and chemical content of pyriproxyfen-pyrethroid LLINs to fully understand these results.

With several trials now showing the superior efficacy of next-generation LLINs over pyrethroid-only nets, the importance of developing new active ingredients to use on nets in the future is brought to the fore. The distribution of next-generation LLINs across sub-Saharan Africa has already begun, with the development of other brands of chlorfenapyr-pyrethroid nets underway,[24] as well as the development of chlorfenapyr product formulations for indoor residual spraying.[25] Although chlorfenapyr-pyrethroid nets offer a superior alternative to pyrethroid-only nets in areas of pyrethroid resistance, to preserve their effectiveness optimal resistance-management strategies should be used. There was no evidence of the development of resistance to chlorfenapyr during the 2 years of this trial; however, the wide-scale deployment of one type of insecticide risks the rapid development of resistance, which could result in a similar situation to the current widespread resistance to pyrethroids. The nets should be deployed ideally alongside other insecticides as part of a strategy aimed to reduce selection pressure for development of resistance in mosquito vectors. Given the significant effect of the pyriproxyfen-pyrethroid LLINs on malaria transmission during the first year after net distribution, additional studies are necessary to evaluate the potential value of active ingredients such as pyriproxyfen, which are not primarily intended to kill resistant adult mosquitoes but rather to sterilise them. There might still be a role for these hormone growth-regulator insecticides in combination with other active ingredients for net treatment in long-term resistance management.

If WHO policy recommendations are made on the basis of this trial's results, future chlorfenapyr-pyrethroid nets might not have to undergo the rigorous trial testing that Interceptor G2 has. Caution should, however, be taken to assess the comparative efficacy, quality, and durability of the second-in-class chlorfenapyr-pyrethroid nets as they might use different concentrations of chlorfenapyr, different pyrethroids, or different net materials. Considering the time and resources required to generate evidence of epidemiological effect using randomised trials, non-inferiority experimental hut trials, recently proposed by WHO,[26] might be a useful alternative for second-in-class chlorfenapyr-pyrethroid LLINs. Further work to investigate the capacity of such entomological studies to predict the performance of the trial nets against clinical malaria is ongoing.[27]

Our study has some limitations. First, net ownership and use were high throughout the study; however, this did not always equate to study net use, which was approximately 60% at 2 years after net distribution, with overall usage of greater than 80%. Similar findings have been observed in other bednet trials, and probably indicate populations choosing to discard damaged nets when other nets are readily available. Second, the three types of nets were not completely identical and differences in textile material and size might have resulted in differential net usage between the groups. As our net use indicator was primarily self-reporting, it is also possible that net usage was overestimated. Third, our primary measure of incidence was based on active detection, involving visits every 2 weeks or every month. The passive data collected alongside the trial suggests that this frequency of visit resulted in some cases being missed, meaning the absolute effect of the nets might be greater than reported here. However, the relative effect of the nets remained similar when the active and passive data were combined. Finally, cost-effectiveness was not assessed; however, the trial in Tanzania[9] showed that dual active-ingredient LLINs can be highly cost-effective and even cost-saving compared with pyrethroid-only LLINs when providing sufficient protection.

New classes of vector control interventions currently require clinical trial evaluation in two different geographical settings, after 24 months of community use.[28] Currently, the only class of next-generation LLIN to receive a WHO recommendation are the PBO synergist nets. However, previous publications have shown concerns about the durability of these nets.[29] This trial provides the second key evidence for the effectiveness of chlorfenapyr-pyrethroid nets in an area with pyrethroid-resistant vectors and will therefore support a WHO policy recommendation. However, the effect of the nets was reduced in the second year of the trial, and there is no evidence for the efficacy of the nets in their third year of use. Many studies report that the durability of nets is much less than the 3 years required to be designated as long lasting.[30,31] The next-generation LLINs might face the same problems of fabric integrity and durability of insecticidal content unless standards of manufacture are improved. Different channels of distribution (eg, school-based, antenatal care visits, or expanded programme immunisation visits) could play a key role in maintaining high levels of net use in communities.[32]

Although generating this evidence to support a WHO recommendation for another class of bednet is a key turning point in malaria control, without new insecticides and new ways to deploy them, we risk repeating the mistakes of the past. Now is the time for more innovation and less complacency.

Contributors

NP, JC, and CN conceived and designed the study, with contributions from MR, IK, LAM, and MCA. JC and MA led the development of the analysis plan, with input from BA, ASo, and NP. MA, CN, JC, NP, FT, and MCA coordinated the trial implementation with local and national authorities, RA, and AO-H. MA, BY, HA, ASo, and LA led the data collection in the field with CA and GGP, and oversight from MCA, JC, and NP. ASo, BY, ASi, and RA led the molecular laboratory work, supervised by MCA and supported by LAM and NP. MA, JC, and NP wrote the data management plan and, with ED, developed collection tools and managed the data. MA, ED, LA, BA, ASo, and JC did the statistical analysis of the epidemiological and entomological outcomes with input from MCA and NP. MA, JC, and NP wrote the first draft of the manuscript with input from ASo, CN, MR, IK, and MCA. MA and JC accessed and verified the data. MA, JC, and NP had full access to all the data and had final responsibility for the decision to submit for publication. All authors reviewed, read, and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Data collected for the study, including deidentified participant data and data dictionaries, might be made available at the end of the third year of trial follow-up upon reasonable request to the corresponding author.

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References

1 WHO. World malaria report 2021. Geneva: World Health Organization, 2021.

2 Cohen JM, Smith DL, Cotter C, et al. Malaria resurgence: a systematic review and assessment of its causes. *Malar J* 2012; **11:** 122.

3 WHO. Report of the 15th WHOPES Working Group meeting. Geneva: World Health Organization, 2012.

4 WHO. Report of the twentieth WHOPES Working Group meeting, WHO/HQ, Geneva, 20–24 March 2017: review of Interceptor G2LN, DawaPlus 3.0 LN, DawaPlus 4.0 LN, SumiLarv 2 MR, Chlorfenapyr 240 SC. Geneva: World Health Organization, 2017.

5 Protopopoff N, Mosha JF, Lukole E, et al. Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. *Lancet* 2018; **391:** 1577–88.

6 Ngufor C, Agbevo A, Fagbohoun J, Fongnikin A, Rowland M. Efficacy of Royal Guard, a new alpha-cypermethrin and pyriproxyfen treated mosquito net, against pyrethroid-resistant malaria vectors. *Sci Rep* 2020; **10:** 12227.

7 N'Guessan R, Boko P, Odjo A, Knols B, Akogbeto M, Rowland M. Control of pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes with chlorfenapyr in Benin. *Trop Med Int Health* 2009; **14:** 389–95.

8 Harris C, Lwetoijera DW, Dongus S, et al. Sterilising effects of pyriproxyfen on *Anopheles arabiensis* and its potential use in malaria control. *Parasit Vectors* 2013; **6:** 144.

9 Mosha JF, Kulkarni MA, Lukole E, et al. Effectiveness and cost-effectiveness against malaria of three types of dual-active-ingredient long-lasting insecticidal nets (LLINs) compared with pyrethroid-only LLINs in Tanzania: a four-arm, cluster-randomised trial. *Lancet* 2022; **399:** 1227–41.

10 Tiono AB, Ouédraogo A, Ouattara D, et al. Efficacy of Olyset Duo, a bednet containing pyriproxyfen and permethrin, versus a permethrin-only net against clinical malaria in an area with highly pyrethroid-resistant vectors in rural Burkina Faso: a cluster-randomised controlled trial. *Lancet* 2018; **392:** 569–80.

11 WHO. The evaluation process for vector control products. Geneva: World Health Organization, 2017.

12 Yovogan B, Sovi A, Padonou GG, et al. Pre-intervention characteristics of the mosquito species in Benin in preparation for a randomized controlled trial assessing the efficacy of dual active-ingredient long-lasting insecticidal nets for controlling insecticide-resistant malaria vectors. *PLoS One* 2021; **16:** e0251742.

13 Accrombessi M, Cook J, Ngufor C, et al. Assessing the efficacy of two dual-active ingredients long-lasting insecticidal nets for the control of malaria transmitted by pyrethroid-resistant vectors in Benin: study protocol for a three-arm, single-blinded, parallel, cluster-randomized controlled trial. *BMC Infect Dis* 2021; **21:** 194.

14 Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. *Malar J* 2008; **7:** 163.

15 Wirtz RA, Zavala F, Charoenvit Y, et al. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. *Bull World Health Organ* 1987; **65:** 39–45.

16 WHO. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd edn. Geneva: World Health Organization, 2016.

17 Hayes RJ, Moulton LH. Cluster randomised trials, 2nd edn. London: Chapman and Hall, 2017.

18 N'Guessan R, Ngufor C, Kudom AA, et al. Mosquito nets treated with a mixture of chlorfenapyr and alphacypermethrin control pyrethroid resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes in west Africa. *PLoS One* 2014; **9:** e87710.

19 Camara S, Ahoua Alou LP, Koffi AA, et al. Efficacy of Interceptor G2, a new long-lasting insecticidal net against wild pyrethroid-resistant *Anopheles gambiae* s.s. from Côte d'Ivoire: a semi-field trial. *Parasite* 2018; **25:** 42.

20 Bayili K, N'do S, Namountougou M, et al. Evaluation of efficacy of Interceptor G2, a long-lasting insecticide net coated with a mixture of chlorfenapyr and alpha-cypermethrin, against pyrethroid resistant *Anopheles gambiae* s.l. in Burkina Faso. *Malar J* 2017; **16:** 190.

21 Tungu PK, Michael E, Sudi W, Kisinza WW, Rowland M. Efficacy of interceptor G2, a long-lasting insecticide mixture net treated with chlorfenapyr and alpha-cypermethrin against *Anopheles funestus*: experimental hut trials in north-eastern Tanzania. *Malar J* 2021; **20:** 180.

22 Djènontin A, Ahoua Alou LP, Koffi A, et al. Insecticidal and sterilizing effect of Olyset Duo, a permethrin and pyriproxyfen mixture net against pyrethroid-susceptible and -resistant strains of *Anopheles gambiae* s.s.: a release-recapture assay in experimental huts. *Parasite* 2015; **22:** 27.

23 Ngufor C, N'Guessan R, Fagbohoun J, et al. Efficacy of the Olyset Duo net against insecticide-resistant mosquito vectors of malaria. *Sci Transl Med* 2016; **8:** 356ra121.

24 WHO. Vector control products pipeline. Prequalification of medical products (IVDs, medicines, vaccines and immunization devices, vector control). 2022. https://extranet.who.int/pqweb/vector-control-products/prequalification-pipeline (accessed Sept 1, 2022).

25 Ngufor C, Fongnikin A, Hobbs N, et al. Indoor spraying with chlorfenapyr (a pyrrole insecticide) provides residual control of pyrethroid-resistant malaria vectors in southern Benin. *Malar J* 2020; **19:** 249.

26 WHO. Data requirements and protocol for determining non-inferiority of insecticide-treated net and indoor residual spraying products within an established WHO intervention class. Geneva: World Health Organization, 2019.

27 Sherrard-Smith E, Ngufor C, Sanou A, et al. Inferring the epidemiological benefit of indoor vector control interventions against malaria from mosquito data. *Nat Commun* 2022; **13:** 3862.

28 WHO. Norms, standards and processes underpinning development of WHO recommendations on vector control. Geneva: World Health Organization, 2020.

29 Lukole E, Cook J, Mosha JF, et al. Protective efficacy of holed and aging PBO-pyrethroid synergist-treated nets on malaria infection prevalence in north-western Tanzania. *PLoS Glob Public Health* 2022; **2:** e0000453.

30 Briet O, Koenker H, Norris L, et al. Attrition, physical integrity and insecticidal activity of long-lasting insecticidal nets in sub-Saharan Africa and modelling of their impact on vectorial capacity. *Malar J* 2020; **19:** 310.

31 Lorenz LM, Bradley J, Yukich J, et al. Comparative functional survival and equivalent annual cost of 3 long-lasting insecticidal net (LLIN) products in Tanzania: a randomised trial with 3-year follow up. *PLoS Med* 2020; **17:** e1003248.

32 Stuck L, Chacky F, Festo C, et al. Evaluation of long-lasting insecticidal net distribution through schools in Southern Tanzania. *Health Policy Plan* 2022; **37:** 243–54.