SAFETY OF 8-AMINOQUINOLINE ANTIMALARIAL MEDICINES

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World Health Organization

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Contents

Abbreviations

ACT	artemisinin-based combination therapy
CI	confidence interval
CYP	cytochrome P450
G6PD	glucose-6-phosphate dehydrogenase
Hb	haemoglobin
MAO	monoamine oxidase
NAD+	nicotinamide adenine dinucleotide
NADP+	nicotinamide adenine dinucleotide phosphate
NADPH	reduced form of NADP+
PCR	polymerase chain reaction
PQ	primaquine
RBC	red blood cell
SP	sulfadoxine–pyrimethamine
US(A)	United States (of America)
WHO	World Health Organization

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This review was compiled primarily for a WHO evidence review group on the safety and effectiveness of single-dose primaquine as a *P. falciparum* gametocytocide. Contributions and recommendations made by members of the group attending a meeting in Bangkok, Thailand (13–15 August 2012), were added to the final draft.

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Preface

Primaquine is an antimalarial drug of the 8-aminoquinoline class. It has prophylactic activity, relatively weak schizontocidal activity and radical curative activity in *Plasmodium vivax* and *P. ovale* malaria and is a potent gametocytocide in *P. falciparum* infections. In this review, we focus on primaquine but also discuss pamaquine (plasmoquine), the more toxic forerunner of primaquine, which prompted drug screening for additional antimalarial agents that would be less toxic. We also describe other 8-aminoquinolines (quinocide, pentaquine, bulaquine (elubaquine) and tafenoquine). Studies of the efficacy and safety of pamaquine and primaquine, and especially their toxicity when used as antimalarial agents, are reviewed. As the main severe adverse event reported after 8-aminoquinoline use is haemolytic anaemia in glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals, G6PD deficiency and G6PD deficiency tests for use in the field are discussed in detail, as are anaemia and haemoglobinuria in relation to malaria and primaquine use. Chlorproguanil–dapsone (Lapdap®) is included as an example of a commercially developed oxidant antimalarial drug that had to be withdrawn from the market because of the adverse haemolytic effects in G6PD-deficient individuals.

Additional sections of the review cover the use of primaquine in malaria prophylaxis and treatment, radical cure of vivax malaria and as a *P. falciparum* gametocytocide. Studies in various settings of the efficacy of the different primaquine regimens used for these purposes, with various total and daily doses and intervals of administration, are presented. Primaquine has proved to be effective in curing and preventing relapse from vivax malaria, although parasite strains in certain regions (notably East Asia and Oceania) required higher dose regimens. Shortening the standard 14-day regimen to 5 days to improve adherence substantially reduced its radical curative efficacy. Primaquine was also shown to be an effective gametocytocidal drug for falciparum malaria when given as a single dose with other antimalarial agents. We found no studies in which primaquine was given to pregnant women or infants, except one study of mass drug administration which included some African children > 6 months of age (*1*). When older children were included, the age ranges and numbers (when provided by the authors) are indicated. The dose of drug used in the studies in this review is reported as the adult dose in milligrams of base equivalent, unless otherwise specified; in every study in which children were included, they received proportionally lower doses. This review spans nearly a century, during which research methodology has changed substantially and knowledge in biology and pharmacology has made great advances. The data reported are heterogeneous and not readily amenable to pooling (or weighting) as in standard meta-analyses. We therefore present the information chronologically.

Review process

We reviewed all studies to which we had access, both published (by screening the PubMed database) and unpublished (in the archives and historical collection of the World Health Organization [WHO]), in which the safety of pamaquine and primaquine was evaluated, regardless of the regimen administered. We also included published and unpublished reports of deaths, haemolysis and other severe adverse events, such as haemoglobinuria and renal failure, resulting from use of these drugs, even if they were not part of a formal study.

PubMed was searched with the terms "primaquine", "pamaquine", "plasmoquine", "G6PD", "G6PD deficiency" and "human", with no date limits. Additional searches were made with the 8-aminoquinoline drug terms (individually) and including "animal", "pharmacokinetics", "haemolysis", "gametocytocide" and other terms relevant to specific sections of the review. Articles were also identified in searches of the authors' personal files, in Google Scholar, the Springer Online Archives Collection (1 March 2012) and Google. Searches were conducted with international and United States (US) spelling (e.g. "anaemia" and "anemia") and Spanish. Articles resulting from these searches and relevant references cited in those articles were reviewed. Articles published in English, French, Spanish, Italian and German were included. As most of the publications on quinocide, pamaquine, and pentaquine preceded PubMed and other searchable databases, relevant articles were sought in reference lists and obtained from libraries. We have also included unpublished observations, information from doctoral theses and reports and manuscripts shared with us by individuals, including members of the WHO expert review group to whom this review is submitted. In addition, WHO internal files in Geneva were systematically searched by Elizabeth Ashley, as were reports on adverse events due to primaquine kindly provided by the Uppsala Monitoring Centre.

We have not attempted to grade or order the quality of the evidence. Nor did we conduct a meta-analysis of the few relevant randomized controlled trials, as there would have been too many potential confounders. Most studies included gametocytaemia as a primary end-point, which may have resulted in substantial underestimation of the effects on transmissibility, as clearance of gametocytaemia is delayed with respect to their killing by 8-aminoquinolines. There are, however, sufficient data on the dose–response relations of efficacy and toxicity, and specifically on the kinetics of gametocyte killing and thus transmission-blocking properties, to form the basis for treatment recommendations (*2*).

The details of studies on the safety of pamaquine and primaquine are given in annexes 1 and 2, which summarize:

- 53 studies of primaquine in which G6PD-deficient individuals were not evaluated,
- 28 studies of primaquine in which adverse events in G6PD-deficient individuals were included and evaluated; and
- 21 studies of pamaquine.

Twelve studies of mass drug administration in which primaquine was used are included in the annexes.

Definition of "severe adverse events"

The severe adverse events described in this review include both "serious adverse events" (i.e. resulting in death, life-threatening or requiring hospitalization) and other significant adverse events of severe intensity, such as anaemia (haemoglobin (Hb), <5 g/dL), non-life-threatening events leading to treatment discontinuation, e.g. neurological adverse effects, and any event defined as "severe" by the investigators, even if additional supporting information was not available.

Executive summary

Conclusions

Primaquine currently holds a unique place in antimalarial therapeutics. It is the only generally available drug that kills hypnozoites (radical curative activity) in vivax or ovale malaria and the only drug with potent activity against mature gametocytes of *P. falciparum*. It also has causal prophylactic and weak asexual stage activity. The antimalarial activity of primaquine results from its metabolism to reactive intermediate compounds, which have not been well characterized. In addition to its therapeutic effect, primaquine induces haemolysis among people with reduced defences against oxidant compounds, mainly those with the common X-linked genetic defect of glucose-6-phosphate dehydrogenase (G6PD). Over 180 genetic variants of G6PD deficiency have been described, each of which confers a different level of deficiency. Some haemolysis always occurs when primaquine is given to G6PD-deficient individuals, but the extent of haemolysis depends on the dose and duration of exposure and the degree of deficiency.

Although primaquine has been used widely for over 60 years, estimates of the risks remain imprecise. In total, 14 deaths have been ascribed to primaquine, all following treatment with multiple doses. If the population denominator is all patients given any dose of primaquine or during mass drug administration in published studies, the risk for death associated with primaquine treatment would be 1 in 621 428, with an upper 95% confidence limit of 1 in 407 807. In studies involving testing for G6PD, the incidence of severe adverse events (nearly all related to severe haemolysis) was 11.2% (27/241) in G6PD-deficient individuals and almost zero in G6PD-normal people.

The gametocytocidal effect of primaquine requires only a single dose, and a review of dose–response relations suggests that, when given with artemisinin-based combination therapy (ACT), a single dose of 0.25 mg of base/kg (adult dose, 15 mg) has maximum effects. Current evidence suggests that this dose is unlikely to result in dangerous haemolysis, even in people with severe G6PD deficiency. For radical cure of vivax or ovale malaria, a 2-week course of treatment is required (current recommendations are 0.25 mg base/kg per day for temperate strains and 0.5 mg base/kg for tropical strains). Shorter, higher-dose regimens may be as effective, but all regimens carry a risk for inducing potentially dangerous haemolysis in G6PD-deficient individuals, and G6PD testing is required (but is seldom available in endemic areas). The simple NADPH “spot test” identifies deficiency that is <30% of normal activity and thus identifies people at haemolytic risk. Semi-quantitative rapid tests are being developed, but more information is needed on the relations between dose, genotype, phenotype, degree of haemolysis and consequent risk.

Background

Primaquine is an antimalarial drug of the 8-aminoquinoline class. It has prophylactic activity and radical curative activity in *Plasmodium vivax* and *P. ovale* malaria and is a potent gametocytocide in *P. falciparum* infections. Efforts to limit the spread of artemisinin resistance and to eliminate malaria in some parts of the world have renewed interest in the use of single-dose primaquine because of its transmission-blocking effects in falciparum malaria. A significant obstacle to its use is concern about its safety in populations with G6PD deficiency, in whom it may precipitate acute haemolytic anaemia of variable severity. This is the only significant toxic effect associated with therapeutic use of this drug. G6PD deficiency is relatively common in malaria-endemic countries; its prevalence, as predicted in a Bayesian geostatistical model, is highest in sub-Saharan Africa and the Arabian Peninsula, peaking at 32.5%. The median prevalence was estimated to be lower in Asia (≤20%) but with many more people affected because of the higher population density. People with much rarer congenital methaemoglobinaemia (an elevated blood level of methaemoglobin, an oxidized form of Hb that has decreased affinity for oxygen and is formed when its ferrous iron (Fe^{2+}) is oxidized to ferric iron (Fe^{3+})) or nicotinamide adenine dinucleotide (NAD^+) methaemoglobin reductase deficiency are also at risk for haemolysis. The objective of this review is to present the evidence on the risks associated with primaquine use.

The 8-aminoquinolines

The first 8-aminoquinoline developed for the treatment of malaria, in the mid-1920s, was pamaquine (plasmoquine, plasmochin). It had weak asexual stage activity against *P. falciparum* but was gametocytocidal, even at low doses, and had radical curative activity in vivax malaria. Pamaquine was used extensively, including in mass drug administration, but it was not well tolerated. The main adverse effects were abdominal pain, vomiting, methaemoglobinaemia and haemolytic anaemia in some people. Seventeen deaths were reported in association with its use, mainly due to acute haemolysis.

Primaquine was developed in the 1940s during screening for safer, more effective alternatives, and it has been the standard treatment for radical cure of vivax and ovale malaria for more than 60 years. Primaquine has also been used in addition to the standard treatment of falciparum malaria in areas of low transmission to reduce transmissibility of the treated infection. It has sometimes been been used as chemoprophylaxis and in mass treatment campaigns.

A closely related 8-aminoquinoline, quinocide, a positional isomer of primaquine, was developed and used in the former Soviet Union. It was realized relatively recently that quinocide was a manufacturing contaminant in primaquine formulations, which had gone undetected for decades. Bulaquine (elubaquine), now discontinued, is an 8-aminoquinoline that was developed and marketed only in India in 1980 and was administered in combination with chloroquine. Primaquine is a major metabolite of bulaquine.

Tafenoquine is a slowly eliminated, more potent 8-aminoquinoline in an advanced stage of development (phase IIb) by GlaxoSmithKline and the Medicines for Malaria Venture. All 8-aminoquinolines cause oxidant haemolysis in people with G6PD deficiency.

The principal biological activity of 8-aminoquinolines is thought to be due to highly reactive metabolites such as the 5-methoxy metabolite, which are short-lived in vivo. It has not been possible to dissociate the antimalarial properties of these drugs from their oxidant toxicity, which suggests that they have a common mechanism.

Pharmacokinetics of primaquine

After oral administration, primaquine is absorbed quickly, reaching peak plasma concentrations within approximately 2 h. It has a large volume of distribution. In studies with healthy volunteers, the terminal elimination half-life was estimated at 4–6 h. Peak concentrations of the principal metabolite, carboxyprimaquine (considered to be biologically inert), occur approximately 6 h after drug administration. Carboxyprimaquine reaches substantially higher levels and is eliminated more slowly than the parent compound. Recent studies have implicated cytochrome P450 (CYP), notably 2D6, and monoamine oxidase (MAO) A as the main enzymes associated with primaquine metabolism, the CYP 2D6 route creating the active metabolites, and the MAO route producing the inert carboxyprimaquine.

Efficacy of primaquine as an antimalarial drug

Primaquine is an effective prophylactic drug for both vivax and falciparum malaria when given at 0.5 mg/kg (adult dose, 30 mg) daily in endemic areas. Travellers start treatment 1 day before departure and continue for 1 week after returning from an endemic area. Longer courses are not needed, because primaquine acts on liver stages (causal activity). Primaquine is used mainly for radical cure of *P. vivax* or *P. ovale* malaria with a blood stage schizonticide such as chloroquine or an ACT. It is also used for terminal prophylaxis and for presumptive therapy to prevent relapses of *P. vivax* or *P. ovale* malaria arising from hypnozoites. As a gametocytocidal agent for prevention of *P. falciparum* transmission, primaquine has usually been administered in a single dose of 0.5–0.75 mg base/kg, but recent recommendations are for a lower dose of 0.25 mg/kg without G6PD testing in combination with ACTs.

The efficacy of primaquine for radical cure of *P. vivax* depends predominantly on the total dose given. Poor adherence to currently recommended 14-day radical treatment courses is a major issue. Short-course (7–10 days), high-dose regimens (>0.5 mg/kg per day) may be as effective as standard 14-day courses. In 14-day regimens, a daily dose of 0.5 mg/kg per day is optimal against tropical strains, whereas 0.25 mg/kg per day is sufficient for temperate strains. Short regimens of 0.25 mg/kg per day for 5 days, used extensively in some countries, are ineffective for preventing vivax relapse.

Risks for acute haemolytic anaemia associated with primaquine among G6PD-deficient individuals

Much of our knowledge about the risk for acute haemolytic anaemia associated with administration of primaquine comes from studies performed between the late 1940s and the early 1960s. The University of Chicago–Army Medical Research Unit was established at the Illinois State Penitentiary in the United States of America (USA) in 1944, with the aim of evaluating the therapeutic and toxicological properties of antimalarial drugs. It was well known that pamaquine caused haemolysis in some people, particularly those of African or Asian descent. In 1952, researchers at this institution reported haemolysis in healthy African American men who received primaquine. This led within a few years to the identification of G6PD deficiency, an X-linked genetic abnormality prevalent throughout malaria-endemic countries. G6PD is the first step in the pentose phosphate shunt, which generates the NADPH required to regenerate reduced glutathione and is also necessary for the function of catalase. These are the principal antioxidant defences of red cells. In G6PD deficiency, the mutant enzyme usually degrades more rapidly than the normal enzyme, so that older red cells become increasingly vulnerable to oxidant drugs and foods. Primaquine-induced haemolysis was studied extensively in volunteers with the A– (African) variant of G6PD deficiency. Clinically evident haemolysis typically appeared 2–3 days after the first dose of primaquine and continued with daily drug administration for a further 3–7 days. Only the older red cells are haemolysed in the A– variant, which is among the mildest of the 186 known genetic variants. The haematocrit began to rise on days 7–10, despite continued drug administration, due to an influx of young, relatively primaquine-resistant erythrocytes into the circulation that exceeded the loss of older red blood cells (RBCs). The degree of haemolysis was related to the dose of 8-aminoquinoline given. In hemizygote men with the G6PD A– variant, daily doses of <15 mg primaquine resulted in mild haemolysis without anaemia, 15 mg/day caused moderate haemolysis with mild anaemia, 30 mg/day caused severe haemolysis with acute anaemia, and 45 mg/day caused dangerous haemolytic anaemia. In view of the haematological toxicity associated with primaquine and the findings of studies in rhesus monkeys that showed the efficacy of intermittent treatment regimens for curing *P. cynomolgi* malaria, studies were conducted to evaluate the effect on radical cure of vivax malaria of intermittent twice-weekly and weekly therapy. An 8-week course of 45 mg primaquine given with chloroquine cured > 90% of experimental infections with the *P. vivax* Chesson strain and showed negligible haematological toxicity in African American men with G6PD deficiency. This regimen was therefore advocated for patients with "mild" G6PD deficiency variants.

Other drugs also cause haemolysis when administered to G6PD-deficient individuals. An example of a temporarily approved antimalarial agent that posed a considerable risk for haemolysis in this population was chlorproguanil–dapsone (Lapdap®), marketed as a fixed-dose combination and later combined with artesunate. Dapsone is an oxidant drug. This

antimalarial was withdrawn after haematological toxicity (haemolytic anaemia) was observed in G6PD-deficient individuals in several studies.

Testing for glucose-6-phosphate dehydrogenase deficiency at points of care

G6PD deficiency is diagnosed in the laboratory by molecular genotyping or phenotypic assays, which may be qualitative or quantitative (G6PD enzyme activity expressed in U/g Hb). Genotyping is available only in specialized centres with site-specific polymerase chain reaction (PCR) tests to detect known variants. The phenotype still varies within a single genotype, and levels of G6PD activity fluctuate in time. Red cells from hemizygotes or homozygotes with G6PD deficiency typically have <30% of the enzyme activity of normal red cells, and, in the more severe variants (such as the Mediterranean type), enzyme activity may be barely detectable. Female heterozygotes are mosaics and typically have half the degree of deficiency of homozygotes or hemizygotes but, because of lyonization, may sometimes have a predominantly deficient red cell population. The gold standard for quantitative assessment of G6PD deficiency is a spectrophotometric assay, which requires moderate laboratory expertise and quality assurance. Fluorescence spot tests and cytochemical assays are also used. All the phenotypic assays require some laboratory skill and experience, appropriate equipment and a functioning cold chain.

The main test currently used at points of care is the fluorescence spot test, which is based on the Beutler method from the 1960s to detect the production of reduced $NADP^+$ (NADPH). It detects deficiency <30% of normal and thus identifies people at risk for haemolysis. Rapid qualitative and semi-quantitative diagnostic tests are being developed.

Interactions between anaemia, malaria, antimalarial drugs and glucose-6-phosphate dehydrogenase deficiency

Evaluating the risk for haemolytic anaemia of G6PD-deficient individuals who receive primaquine as an antimalarial drug is not straightforward, as many patients presenting with falciparum or vivax malaria are already anaemic, and the illness itself may provoke haemolysis independently of the drug. Most malaria-associated anaemia is found in children. Thus, when evaluating the haemolytic risk presented by primaquine, it is critical to know the Hb levels before treatment. Certain anaemias may confer some protection from the haemolytic effects of primaquine and its pathological consequences, because a younger circulating RBC population has higher G6PD activity and is therefore more resistant to drug-induced haemolysis. Furthermore, there may have been time for an adaptive right shift in the oxygen dissociation curve.

Safety and tolerability of primaquine

Evidence for the safety of primaquine comes from case reports, clinical studies and observations during mass drug administration. The most commonly reported minor adverse effect associated with primaquine is

gastrointestinal intolerance (mainly abdominal discomfort), which can be overcome by co-administration with food. The main concern is the risk for acute haemolytic anaemia, which is largely confined to G6PD-deficient individuals.

Fourteen deaths associated with primaquine administration have been reported over the past six decades. One death due to acute renal failure in 2002 was reported in an 18-year-old man with G6PDd Mediterranean variant who had received at least one dose of 45 mg primaquine and had been hospitalized for haemolytic anaemia requiring blood transfusion. The other deaths all followed administration of multiple doses. Four were in G6PD-deficient Ceylonese children and were a result of acute intravascular haemolysis. The dose of primaquine they had received could not be ascertained, but they were likely to have been overdosed. In a brief internal WHO report in 1978, five patients in Turkey who had been treated for vivax malaria were reported to have died. One death from hepatic necrosis was reported in association with primaquine use in the United Kingdom. Two deaths in G6PD-deficient Brazilians were attributed at autopsy to acute haemolytic anaemia caused by primaquine. One death in the USA was reported in 1997 to the Uppsala Monitoring Centre. With a population denominator of all patients given any dose of primaquine or in mass drug administration in published studies, the risk for death associated with primaquine ingestion would be 1 in 621 428, with an upper 95% confidence limit of 1 in 407 807. As most of these reported deaths occurred in countries with a minority of the global malaria burden, there is some doubt about the generalizability of this estimate.

We found *69 studies and case reports* (excluding mass drug administration) for evaluating adverse events; 20 included G6PD-deficient individuals, while in 49 studies G6PD-deficient individuals were excluded or G6PD deficiency status was unknown. Several earlier reports preceded the identification of G6PD deficiency and involved African American or "primaquine-sensitive" individuals, who were likely to have had the G6PD A– variant. No severe adverse events were reported in G6PD-normal individuals, with the possible exception of a psychotic reaction in an individual with undetermined G6PD status.

A total of *192 severe adverse events* were reported, 25 in individuals whose G6PD status was not determined and 167 in G6PD-deficient individuals. The majority (87.4%) of the events were reported in people with confirmed G6PD deficiency (with one possible exception), some of whom had malaria. Events in individuals not tested for G6PD deficiency (13.1% of all severe adverse events) were reported in studies that were conducted before G6PD testing became available. As all the events occurred in either African American or primaquine-sensitive individuals (probably with the G6PD A– variant), we included them in the G6PD-deficient group. Thus, all the severe adverse events reported in the literature were probably in G6PD-deficient individuals. The studies (excluding mass drug administration and case

reports) comprised approximately 5000 G6PD-normal individuals, nearly 10 000 people of unknown G6PD status and 241 with confirmed G6PD deficiency. The incidence of severe adverse events in the group with unknown G6PD status was 0.25% (24/9461), but many of the studies included African Americans who were probably G6PD deficient and had acute haemolytic anaemia. The incidence of severe adverse events in the G6PD-deficient group was 11.2% (27/241), and that in G6PD-normal people was almost zero. In the case reports, for which the denominator is unknown, one severe adverse event (a psychotic reaction) was reported in a person of unknown G6PD status, and 139 severe adverse events occurred in G6PD-deficient individuals.

Of all the severe adverse events, 12% occurred after a probable overdose of primaquine: 75.5% after a daily dose of either 15 or 30 mg administered mostly for vivax malaria and 12.5% after administration of 30 or 45 mg primaquine as a single-dose gametocytocide or in weekly prophylactic or radical curative regimens. The majority of the events in which primaquine might have been administered at higher than recommended doses (91.3%) were in children; most occurred in children in Ceylon (now Sri Lanka) aged 2–12 years, whereas only 1.4% of the events occurred in children given 15 or 30 mg primaquine daily. Of the severe adverse events reported after single or weekly doses, 33.3% were in children.

With the exception of case reports of overdosing, these results are not representative of the risk of various primaquine regimens for children, as most of the studies conducted enrolled only adults. In the studies of mass drug administration, with much larger cohorts and fewer adverse events reported, the incidence was very low after either daily (2.9 per million) or single or weekly doses (only one case of severe anaemia reported). Of the events reported after a daily primaquine regimen in mass administration, 61.5% were haemolysis, resulting in an estimated incidence of 1.8 per million. In the safety studies (with smaller cohorts), the incidence of severe adverse events was 0.26% after daily regimens of primaquine and 0.42% after single or weekly doses. In the latter category, 43.8% of all events were in children <12 years. All the adverse events were acute haemolysis. In the case reports, 108 severe adverse events were associated with a daily primaquine regimen and 8 with a single dose of 30 or 45 mg. The incidence of severe adverse events clearly differs widely, depending on whether they are found by passive case detection during large-scale implementation or in prospective studies. This is not surprising, as anaemia may go unnoticed, and transient haemoglobinuria may go unreported.

In *12 mass drug administration programmes* in which primaquine was given to more than 9 million people in various regions of the world, 27 severe adverse events were reported, resulting in an estimated incidence of three events per 1 million. Most of the events (16, or 59.3%) were significant haemolysis, giving an estimated incidence of 1.8 episodes of haemolysis per 1 million people receiving primaquine. In five of the mass administration

programmes, 15 mg primaquine were given once daily for 14 days to about 9 million people in Afghanistan, Azerbaijan, the Korean peninsula and Tajikistan. In the six smaller programmes, populations were given a single dose weekly or on alternate weeks. Most of the severe adverse events reported were associated with the 15-mg daily dose regimen (in individuals in Afghanistan, Azerbaijan, the Korean peninsula and Tajikistan); one event was recorded after a 45-mg single dose administered with artesunate to 564 people in the United Republic of Tanzania.

Limitations

It was not possible to estimate the risks of pregnant and lactating women, infants and G6PD-deficient individuals who had haemolytic anaemia after administration of primaquine, because insufficient data were available. Most of the adverse events were reported in case reports, in which details of treatment were not always available. Additional questions on the effects of malaria and of anaemia on primaquine-induced haemolysis remain unanswered.

1. 8-Aminoquinolines

Six 8-aminoquinolines have been used as antimalarial agents in humans. Although most are no longer in use, all were evaluated first in animals and then in humans to assess their efficacy and toxicity in the treatment of malaria. They are structurally related (Table 1). In contrast to other antimalarial drugs, such as the 4-aminoquinolines, and with the notable exception of tafenoquine, all the 8-aminoquinolines that have been studied in detail are rapidly metabolized in humans in vivo, with only a small fraction (<1%) eliminated unchanged in the urine. The commonest side-effect of these drugs is abdominal pain; the discomfort is proportional to the dose taken but is reduced (even at higher doses) when the drug is administered with food. The main concern throughout the development and use of 8-aminoquinolines has been the risk for haemolysis of G6PD-deficient individuals.

Table 1. Primaquine-related 8-aminoquinolines used as antimalarial agents

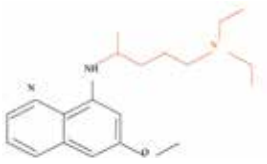
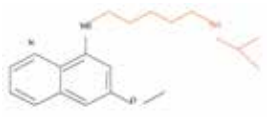
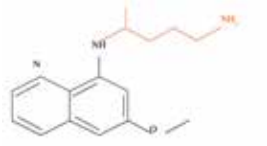
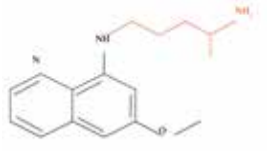
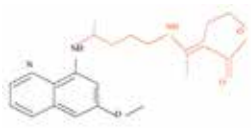
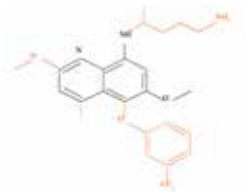
Drug name (year developed)	Chemical structure (conserved structure in black, variable parts in red)	Pharmacokinetics	Toxicity as compared with primaquine and current status as antimalarial
Pamaquine (1924), first 8-aminoquinoline used and first synthetic antimalarial drug		Peak serum levels achieved at –3 h, then decline with a half-life of –2 h	More toxic than primaquine, especially in G6PD-deficient individuals; higher incidences of methaemoglobinaemia and cyanosis in comparative studies; more severe adverse effects and deaths reported No longer used
Pentaquine (– 1945)		Similar to pamaquine: peak serum levels achieved at –3 h, then decline with a half-life of –2 h	More toxic than primaquine; some comparative studies showed similar toxicity to pamaquine when given at doses > 45 mg daily No longer used
Primaquine (1945)		Elimination half-life in humans –4–6 h; mean peak plasma concentration, 100–200 ng/ml 2–3 h after a 30-mg dose	Commonest side-effects are gastrointestinal, reduced when administered with food; haemolytic risk for G6PD-deficient individuals Only 8-aminoquinoline widely used as antimalarial agent
Quinocide (1949); developed and used mainly in the former USSR		Elimination half-life in humans, 4–6 h, peaking at 3 h at 37 µg/L in plasma after a single dose of 23 mg	Similar to primaquine No longer available

Table 1. Primaquine-related 8-aminoquinolines used as antimalarial agents (cont'd)

Drug name (year developed)	Chemical structure (conserved structure in black, variable parts in red)	Pharmacokinetics	Toxicity as compared with primaquine and current status as antimalarial
Elubaquine or bulaquine (1980); marketed only in India as Aablaquine (combined with chloroquine) to prevent vivax relapse		Elimination half-life, –1.2 h in rats and rabbits, –3.7 h in monkeys. In monkeys, after a 10-mg/kg oral dose, bulaquine shows two-peak maximum concentration, reached at 0.25 and 2 h (159 ± 4 ng/mL and 183 ± 46 ng/mL, respectively); converted to primaquine with a C_{max} of 383 ± 132 ng/mL 12 h after oral administration	Similar to primaquine for G6PD-deficient individuals; more studies needed on possible reduced effect on methaemoglobin increase No longer commercially available
Tafenoquine (1978)		Elimination half-life in humans, 14 days, reaching peak blood concentration of 417–489 ng/mL in healthy volunteers after a dose of 600 mg	More potent than primaquine in some activities Under evaluation as a substitute for primaquine

1.1 Pamaquine, the predecessor of primaquine

Pamaquine has pre-erythrocytic, radical curative and gametocytocidal properties. It has weak but significant activity against asexual stages of *P. vivax* and *P. malariae* but is ineffective against *P. falciparum* trophozoites. It was less effective in terms of radical curative activity than primaquine and was more toxic.

Pamaquine (also known as plasmochin, pamaquine, plasmocide, rhodoquine and praequine) is an 8-aminoquinoline identified in Germany in the 1920s during an early "high-throughput screen" for antimalarial agents performed in canaries infected with *P. relictum*. Pamaquine had moderate but useful activity against the blood-stage infections of benign tertian (*P. vivax*) and quartan (*P. malariae*) malaria and was the first treatment that could prevent relapse of vivax malaria. Pamaquine also showed potent gametocytocidal action against *P. falciparum,* reducing infectivity rapidly and later reducing circulating gametocyte density; it had little activity against its asexual stages.

1.1.1 Use for malaria prophylaxis

James and collaborators in England reported in 1931 that pamaquine was effective as prophylaxis for vivax malaria in mosquito-transmitted infections. None of 10 healthy volunteers given pamaquine at 60 mg daily (divided into three 20-mg doses) the day before infection and then for 7 consecutive days developed symptoms of malaria, and no parasites

were detectable in their blood for 28 days after infection, whereas control individuals developed symptomatic malaria within 14 days of infection (*3*). In a report of a study on a rubber plantation in the Federated Malay States (now part of Malaysia) in 1931, pamaquine was given as a malaria prophylactic twice weekly (adult dose, 40 mg daily). The clinical malaria incidence dropped from 82.1% the year before prophylaxis to 16.6% afterwards (*4*; see Annex 3). In 1948, newly synthesized 8-aminoquinolines that had shown good antimalarial activity in birds and low toxicity in monkeys were tested at the same time as pamaquine for prevention of infections with the *P. vivax* Chesson strain in non-immune prison inmate volunteers. Pamaquine at 90 mg daily (close to the maximum tolerated dose) from the day before exposure to sporozoites from mosquitoes and for 7 consecutive days thereafter did not prevent primary malaria attacks in the volunteers (*5*; see Annex 3). All the 6-methoxy-8-aminoquinoline compounds tested in this study were effective but were administered at doses approaching the maximum tolerated doses, and all were toxic.

A recent report was published on almost 60-year-old observations in a study conducted at the Cairns (Australia) experimental station with a New Guinea strain of *P. vivax* in Australian Army volunteers, who received 80 mg of pamaquine daily (in three divided doses of 20, 30 and 30 mg) either for 7 days from the day before exposure to mosquito bites (7-day regimen) or from 6 days after exposure for 5 days (5-day regimen). All the men developed malaria requiring treatment, although the onset of illness was delayed. Pamaquine had greater potency in preventing relapse than it did in preventing the primary infection. Overt malaria was first observed 17–21 days after exposure in the 7-day group, at 19–26 days in the 5-day group and at 11–13 days in control individuals (*6*). All the volunteers in the 5-day group relapsed 43–118 days after the end of treatment, as did control volunteers at 39–82 days, but none of the volunteers in the 7-day group had any further attacks of *P. vivax* malaria during a 380-day period. These results suggest that 8-aminoquinolines exert their pre-erythrocytic activity predominantly in the 5 days after sporozoite inoculation.

1.1.2 Use to prevent relapses of vivax malaria

Pamaquine was evaluated clinically shortly after its development in 1924 in Germany. Sinton and colleagues in India soon provided evidence that pamaquine reduced the rate of recurrence when added to quinine treatment of vivax malaria (*7–9*). "Sinton's regimen" of 1 week of quinine plus pamaquine was endorsed by the League of Nations and generally replaced the 2-month regimens previously used. In an early report from India in 1927, when pamaquine was given to patients with *Plasmodium* parasites (*vivax*, *ovale*, *malariae* or *falciparum*), the blood was free of parasites within 5–6 days in all cases, and fever was controlled within 24–48 h (*10*). Mepacrine (quinacrine, atebrine), identified in 1932 and subsequently introduced, provided a simpler, somewhat better tolerated treatment, although it had an unpleasant pharmacokinetic interaction with pamaquine, which precluded simultaneous use of the two drugs.

In a study reported in 1946 on the effect on relapse of *P. vivax* (Pacific origin) malaria, combined quinine–pamaquine treatment for 14 days reduced the number of relapses better than quinine alone: 8/72 (11.1%) relapses were observed with quinine–pamaquine over 120 days a nd 62/75 (85%) in the control group that received quinine alone (*11*; see Annex 3). A study on the therapeutic action of pamaquine against the US Saint Elizabeth strain of vivax malaria (a long-latency strain similar to the "Madagascar" strain evaluated in Europe) was conducted in 1949 in 23 white volunteer prison inmates infected by mosquito bites and presenting with initial late attacks (i.e. during the first of the wave of late attacks, which characteristically appear in Saint Elizabeth strain infections 6–12 months after exposure). No relapses occurred among 19 men who received pamaquine (30, 60 or 90 mg) and quinine (2 g) daily for 12 days, and only one man relapsed in the group receiving 6 days of pamaquine given after quinine, in contrast with 76% of the controls (*12*).

1.1.3 Use as a gametocytocide

Between 1927 and 1929, a study was conducted in Panama that established that pamaquine at doses of 0.2–1.4 mg/kg given to individuals with *P. falciparum* gametocytaemia prevented transmission to anopheline mosquitoes (*13, 14*). Of the very few antimalarial drugs then available, pamaquine was the only one active against mature gametocytes of *P. falciparum* (stages 4 and 5), whereas all the other drugs with asexual-stage activity were active only against the immature (sequestered) gametocytes stages 1–3. All drugs with asexual-stage activity against the other human malarias were also effective against the gametocytes of these species. Subsequent detailed studies in India, Italy and the Federated Malay States (now Malaysia) confirmed these findings. Two studies in the Federated Malay States published by Green in 1929 (involving mostly Tamil men) showed that pamaquine was a powerful gametocytocide. In the first study, men received 40 mg pamaquine daily with 500 mg quinine for 10–14 days. Gametocytes disappeared from peripheral blood within an average of 7.9 days (range, 4–13), whereas quinine alone did not clear the gametocytaemia over 12–20 days (*15*; see Annex 3). In the second study, the action of pamaquine against *P. malariae* malaria was investigated in 40 Malays. The average time for disappearance of parasites, including gametocytes, from blood was 6 days (range, 4–9) with pamaquine and 10 (4–14) with quinine (*16*; see Annex 3). In a study in British Mandate Palestine in 1930, over 1000 patients in villages infected with *P. vivax*, *ovale*, *malariae* or *falciparum*, including children, received mass administration of a pamaquine–quinine combination (adults: 900 mg quinine, 30 mg pamaquine daily; children were treated with proportionally lower doses). The treatment resulted in a 75% reduction in parasite rate (*17*; see Annex 3). In a report of a study of intravenous heroin users in Egypt in 1930, pamaquine was administered for 6 weeks at various doses (range, 40–60 mg daily) with quinine to users presenting to the hospital with falciparum malaria and gametocytaemia. The treatment was effective in eliminating gametocytaemia in an average of 5.45 days, and recurrences occurred in only three cases (6%) (*18*; see Annex 3).

A report from Italy in 1933 described the effect of a 20-mg single dose of pamaquine in 31 falciparum malaria patients treated previously in the acute phase with quinine and/or mepacrine. Pamaquine was shown on peripheral blood examination to have a powerful gametocytocidal effect (gametocytaemia increased on day 1 after pamaquine, decreased markedly by the second day and almost disappeared by the third day), preceded by a reduction in infectivity to mosquitoes. The proportion of fed anopheline mosquitoes that developed oocysts was 35.2% immediately before pamaquine administration, 2.95% the day after and <0.1% the next day; no mosquitoes were infected after biting patients on the third day after pamaquine (*19*).

In a study in Kenya reported in 1935, in which mepacrine was compared with pamaquine (30 mg daily) for the treatment of falciparum malaria, gametocytaemia cleared in 87.5% of cases treated with pamaquine, 20% of those given mepacrine and 29% given quinine alone (*20*; see Annex 3).

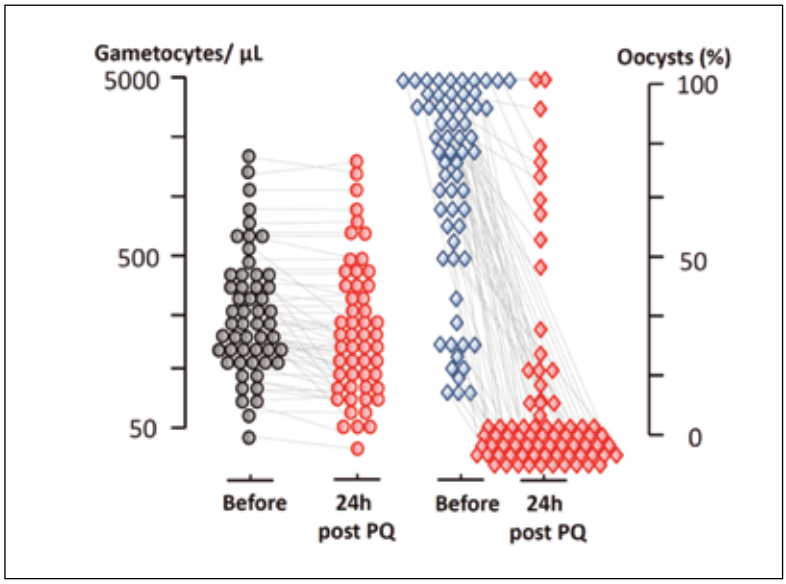
Immediately after the Second World War, a detailed investigation was conducted in Cairns, Australia, on the gametocytocidal activity of three antimalarial agents: quinine, mepacrine (quinacrine) and pamaquine. The study showed that mature gametocytes were not harmed by quinine or mepacrine but were extremely susceptible to a low dose of pamaquine (10 mg). Blood was not infectious to mosquitoes 15 h after pamaquine administration, and gametocytaemia cleared within 3–4 days. Effects on sporozoite formation were seen earlier than those on oocyst development and considerably earlier than effects on gametocytaemia (*21*). Indeed, gametocytaemia did not decline significantly in any of the studies for >24 h after drug administration (Figure 1). Another report from the same group, on the transmission of *P. vivax* gametocytes to mosquitoes after pamaquine treatment, showed that infectivity persisted for 30 h, although the observation was made in only one patient with relatively high-density gametocytaemia (*23*).

A report of a study in 1947 on gametocyte survival in people in British Somaliland (now Somalia) with malaria showed that pamaquine (30 mg daily) was an effective gametocytocide in falciparum malaria when used after routine quinine and mepacrine treatment. The mean average survival of gametocytes was >9 days for a control group, 3 days for 24 adult enlisted soldiers treated with pamaquine, >11 days for a control group and 2 days for 22 village people of all ages treated with pamaquine (*24*; see Annex 3).

1.1.4 Activity against blood stages

Shortly after the introduction of pamaquine, studies in several tropical centres showed that it had little useful activity against asexual stages of *P. falciparum* but had significant activity against the other human malarias. In a study in 1948 of pamaquine therapy (30 mg daily for 8 days) given with either quinine or proguanil for acute attacks of vivax or ovale malaria in soldiers who had returned to the United Kingdom from Burma (now Myanmar), India and the Federation of Malaysia (now Malaysia), both treatment groups showed rapid clinical cure and had a recurrence rate of

Figure 1. The rapid sterilizing effect of 8-aminoquinolines in falciparum malaria



Studies of mosquito infectivity after treatments of falciparum malaria with plasmoquine or primaquine in which oocysts were assessed in mosquitoes that fed 24 h after drug administration. Oocysts were usually assessed in 10–20 mosquitoes 6–7 days after feeding. Each pair of circles or diamonds represents a studied patient. Gametocytaemia changed little in 24 h, although it usually fell rapidly thereafter (left side), whereas oocyst numbers fell rapidly and none were found in the majority of mosquito batches fed on treated patients (right side). Sporozoites were correspondingly absent when evaluated later. Reproduced from reference 22
PQ – primaquine

just over 20% (*25*). The authors stated that the expected recurrence rate with proguanil given alone for 10–14 days was 35–40%. Another report in the same year on the antimalarial activity of pamaquine against various strains of *P. vivax* (McCoy) and *P. falciparum* (McClendon and Costa) given by intravenous injection or bites of mosquitoes infected with the *P. vivax* Chesson strain (*26*) challenged the previous findings. This study showed that, at maximum tolerated doses, pamaquine was unable to control these infections, although it did have a curative action against primary attacks of sporozoite-induced *P. vivax* Chesson strain malaria when used at 60–90 mg daily with 2 g of quinine for 14 days. The authors suggested that the curative effect seen by others might have been due to differences in strain susceptibility to the drug, later administration or lower infection density.

1.1.5 Toxicity

Pamaquine was a more toxic 8-aminoquinoline than primaquine. Severe adverse events were reviewed in 19 studies comprising approximately 24 000 patients in which the toxicity of pamaquine was reported and from case reports. At least 17 deaths and 79 cases of significant haemolysis and haemoglobinuria were attributed to ingestion of pamaquine. Three of the deaths and 58 cases of haemolysis or haemoglobinuria were reported in 21 studies of the safety of pamaquine, giving an estimated incidence of severe adverse effects of 2.4/1000 ($p = 0.24$; 95% CI; –0.06 to 0.54) and, in an era when renal replacement was not available, a mortality rate of 1/8000 ($p = 0.01$; 95% CI, 0.00–0.03).

After oral dosing with pamaquine, peak serum levels were achieved after approximately 3 h, after which they declined with a half-life of about 2 h (*27*). Pamaquine was poorly tolerated when given at doses >30 mg (base equivalent), and toxicity was worsened if it was given concomitantly with mepacrine. This was shown to be due to a pharmacokinetic interaction resulting in markedly elevated pamaquine levels. Abdominal pain was common when pamaquine was taken on an empty stomach but was prevented by taking it with food, as is the case with primaquine. Nausea, vomiting and cyanosis due to methaemoglobinaemia were common, leukopenia was seen at high doses, and occasional neurotoxicity was observed, but the most serious adverse effect of pamaquine was haemolysis. This was more common in patients of African or Asian descent than in whites originating from northern Europe. In some patients, pamaquine resulted in haemoglobinuria (blackwater fever). Annex 3 summarizes the 21 studies of the safety of pamaquine; pamaquine-related deaths are discussed in section 3.4.1.

By 1931, four iatrogenic deaths had been reported (*28*): one of a black Haitian who had received 40 mg pamaquine daily and developed haemoglobinuria (*29*); two of black Jamaican patients treated in Cuba with 80 mg of pamaquine daily, who developed severe intravascular haemolysis on days 3–4 of therapy and went into deep coma (*30, 31*); and one of an Indian soldier treated with 40 mg pamaquine daily who passed dark urine on day 5, which prompted discontinuation of therapy, but was followed by "cyanosis", anaemia and haemoglobinuria, and the patient died on day 10 from respiratory failure (*32*). Cordes in 1926 described six cases of serious side-effects over 2 years in 250 malaria patients in Cuba and Haiti given 80 mg/day of pamaquine, including the two deaths cited above; the effects were not seen with quinine alone (*30*). Two of the cases occurred after a reduced 40-mg daily dose. The first occurred in Haiti: a 45-year-old man given pamaquine for 5 days felt dizzy, vomited, was pale and jaundiced and his Hb level fell to 35%; he recovered within 1 week. The second case was a 22-year-old Haitian in Cuba who also felt ill, was pale and jaundiced with headache, weakness and epigastric pain after 2–3 days of pamaquine at 40 mg daily. His Hb level fell to 35%, treatment was discontinued, and he recovered slowly. The author noted that jaundice was observed in all cases

of pamaquine intoxication; however, haemoglobinuria was not observed in his patients, who were all of African origin.

A fatal case of pamaquine poisoning was reported in Southern Rhodesia (now Zimbabwe) in 1935 in a man who had taken 30 mg pamaquine (three 10-mg single doses over 24 h). Post-mortem examination revealed cyanosis, haemorrhagic nephritis and acidic urine containing methaemoglobin. The man started feeling unwell but was convinced to continue therapy (mepacrine and pamaquine) for another day. After taking 60 mg on day 2, he complained of constriction in the chest, difficulty in breathing, swallowing and articulation, and severe pains in the abdomen; his temperature had risen to 102 °F (38.9 °C), and his lips were blue. Therapy was discontinued, but the next morning he was hurried to the hospital, where he died within half an hour (*33*). Two deaths among four cases of haemoglobinuria were reported in India in 1936, which were probably related to pamaquine given in the mass treatment of 5650 individuals with injectable mepacrine and 20 mg pamaquine daily for 3 days after the mepacrine injections. All four patients with haemoglobinuria were also taking medication for syphilis (*34*).

In 1943, 15 cases of haemoglobinuria were reported among military personnel after pamaquine administration. The first case was in a soldier in Nyasaland (now Malawi), who also had severe renal failure, received a transfusion and recovered. Of the other 14, nine occurred in Egypt, two each in Iraq and Sudan and one in British Mandate Palestine; seven men were Indian, and one each was Rhodesian (white), Greek, Polish Jew, Palestinian Jew, East African native, Basuto and Mauritian (*35*). Six of the patients died. The author reported that all the patients had lived for years in malaria-endemic areas, where many thousands of people had received pamaquine. The regimen given was 10 mg pamaquine thrice daily (total daily dose, 30 mg) for 5 days after 3 days of quinine, followed by 5 days of mepacrine and then 3 days of rest. The authors of the report stated that it was uncertain whether the alarming, dangerous complication was true blackwater fever occurring in cases of mixed *P. falciparum* and *P. vivax* infection (only the latter parasite was found in peripheral blood) or whether it was entirely due to the toxic action of pamaquine. These reports preceded the identification of genetic susceptibility and, ultimately, of G6PD deficiency.

A report from Panama in 1946 described 258 adult male patients admitted to hospital with suspected pamaquine poisoning after taking 30 mg thrice daily for 5 days after mepacrine; 61 patients completed only 3 or 4 days of pamaquine. All the patients showed some degree of toxicity: 136 mild, 63 moderate and 59 severe. The reported symptoms of pamaquine toxicity were: abdominal pain (69%), dark urine (58%), anorexia (45%), jaundice (45%), headache (39%), nausea and vomiting (34%), fever (25%), weakness and malaise (23%), backache (22%) and, less frequently, vertigo, chest pain, diarrhoea, chills, nasal congestion and cyanosis. The symptoms began on the fourth day of pamaquine treatment and usually lasted 3–4 days; 75% of the patients had red cell counts <4 million/μL, 50% had Hb <70% of the

baseline value, and 22% had erythrocyte counts <2 million/μL; 16% of patients had Hb levels as low as 40% of the baseline value. The cases of mild anaemia improved within 3–4 days, while those of severe anaemia lasted 10 days to 2 weeks. Sixty patients received blood transfusions, and all recovered with no complications after treatment (*36*; see Annex 3).

Various pamaquine regimens were tested in male volunteers infected with *P. vivax* Chesson strain malaria in the USA in a study reported in 1947. The *P. vivax* Chesson strain originated in New Guinea. The adverse events reported after a high dose (63 mg) were anorexia, nausea, vomiting and epigastric distress or pain; some patients had to discontinue treatment before completing the 14-day course. Methaemoglobinaemia was common; the methaemoglobin concentration was 3.0% of total Hb at 15 mg, with mild toxicity at 30 mg (4.9%), moderate toxicity at 45 mg (5.6%) and severe toxicity at 63 mg (12%). When methaemoglobinaemia exceeded 6–7%, cyanosis was clinically evident. Granulocytopenia was less common, and no cases of severe haemolytic anaemia occurred (*37*; see Annex 3).

In another study in the USA, reported in 1948, several 8-aminoquinolines were tested, including high-dose pamaquine (90 mg daily for 7 days), in five male volunteers infected with *P. vivax* Chesson strain. Significant toxicity was observed with all drugs. Common side-effects included abdominal epigastric discomfort or pain, anorexia, nausea, vomiting and cyanosis. Methaemoglobin represented >6–7% of total Hb; the highest value with pamaquine was 11.7%. Total Hb fell slowly by an average of 1.75 g/dL; the greatest reduction was usually seen between days 12 and 14 after the beginning of medication (*5*; see Annex 3).

In three studies in the USA, adult male volunteer prison inmates received pamaquine daily with quinine. Some patients could not finish the treatment course because of abdominal discomfort. In the first report, in 1949, one patient receiving 90 mg pamaquine daily for Saint Elizabeth vivax malaria experienced severe cramps, associated with a reduction in his total white blood cell count to 3000/μL; treatment was discontinued on the ninth day (*12*; see Annex 3). In the second report, in 1952, three of 76 men receiving 63 mg pamaquine daily could not complete the course because of severe abdominal pain (*38*; see Annex 3); and in the third study, reported in 1953, one man receiving 60 mg pamaquine daily had such severe nausea and vomiting that treatment was stopped on the ninth day (*39*; see Annex 3).

One death was reported in 1949 in the USA of a patient with vivax malaria who was given 0.66 g of mepacrine (quinacrine) daily for 4 days, followed by 0.66 g of quinine thrice daily on the fifth day and once daily on the sixth day. Then, by mistake, he received three doses of 0.4 g pamaquine (total, 1.2 g). The next day he was cyanotic and apprehensive and had nausea, pain in the abdomen, back, chest and jaws and vomited. He received 1 L of glucose–saline solution intravenously, followed by 500 mL of whole blood. The cyanosis became more marked, with slight abdominal rigidity

the next day, difficulty in swallowing and blurred vision with haemoglobinuria. The following day he complained of numbness of the face, had difficulty in speaking, was sweating profusely and received another transfusion of 500 mL of blood (Hb concentration not reported). He remained deeply cyanotic for the next 2 days with little change in his condition, and then stridor developed. The soft palate was paralysed and oedematous, his respiration became gasping, and death occurred 7 days after the overdose of pamaquine (*40*).

All these reports were published before 1955, and many do not provide detailed clinical or laboratory descriptions. Details of studies of the safety of pamaquine are listed in Annex 3. The results of the studies with regard to pamaquine-associated haemolytic anaemia and haemoglobinuria can be summarized as follows:

- total number of patients included: ~24 000
- total number of deaths reported: 3, all due to haemolysis (1.25/10 000, $p = 0.01$; 95% confidence interval (CI), 0–3.0)
- total number of cases of haemoglobinuria reported: 18 (7.5/10 000, $p = 0.075$; 95% CI, 4–11)
- total number of cases of severe haemolytic anaemia: 40, with decreases in Hb ≥ 40% from initial values (severe anaemia) (1.7/1000, $p = 0.167$; 95% CI, –0.12 to 0.22)
- total number of severe or fatal adverse events: 58 (2.4/1000, $p = 0.24$; 95% CI, –0.06 to 0.54)
- total number of case reports (in addition to the studies in Annex 3) of anaemia and haemoglobinuria: 19 of haemoglobinuria and two of severe anaemia (35% decrease in Hb), including 14 deaths
- total number of cases of haemolytic anaemia: 42 severe anaemia (35–40% decrease in Hb)
- total number of cases of haemoglobinuria: 37
- total number of cases of haemolytic anaemia and haemoglobinuria due to pamaquine intake: 79

In a screen for safer alternatives in the late 1940s, new 8-aminoquinolines were first evaluated in rhesus monkeys and later in men. These compounds included pentaquine, isopentaquine, and primaquine (see below).

1.2 Pentaquine

Pentaquine was an effective hypnozoiticidal drug, but side-effects similar to those of pamaquine were observed frequently in carefully controlled studies, and its development was not continued.

Several 8-aminoquinoline analogues, including pentaquine, were tested and evaluated in clinical trials by Alving and colleagues (*41*) in a search for safer alternatives to the more toxic pamaquine. A report in 1946 showed that pentaquine was rapidly and completely absorbed from the gastrointestinal tracts of experimental animals (80–90% complete within 2 h in dogs

and monkeys); peak plasma levels were attained 1.5–2 h after treatment and declined steadily thereafter, being barely detectable at 6–8 h, with insignificant urinary elimination. These findings were similar to those for pamaquine in both animals and humans (*42*). In humans, pentaquine was absorbed rapidly from the gastrointestinal tract. Peak plasma levels were attained quickly and declined to undetectable levels within 24 h after the drug was stopped, but the plasma levels in different people often varied widely. Concurrent administration of quinine resulted in a slight increase in the plasma concentration. In both monkeys and rats, the toxicity of pentaquine was increased by two to four times by concurrent administration of quinine (as with pamaquine and quinine).

When pentaquine was given to 171 white volunteers at doses of 15–180 mg daily in prophylactic and curative tests, the toxicity of the drug was qualitatively the same and quantitatively approximately one half to three quarters that of pamaquine. Thus, 60 mg with 2 g quinine daily in divided doses for 14 days was equivalent to 30–45 mg pamaquine administered similarly, with symptoms of occasional anorexia, abdominal discomfort or pain and slight methaemoglobinaemia (average for 44 patients, 4.5%). At a higher dose (120 mg daily), the toxicity was considerably greater, approximately equal to that of 90 mg pamaquine. One symptom seen in 2 of 20 men—acute syncope due to postural hypotension, persisting for several months—had not been observed with the maximum dose of pamaquine (90 mg daily) (*42*). In a study reported in 1948, groups of five prison inmate volunteers were given doses of pentaquine of 120 mg (therapeutic, with or without quinine for 14 days; or prophylactic, for 8 days with no quinine) or 180 mg (prophylactic, for 8 days). The observed methaemoglobinaemia, fall in Hb concentration, abdominal distress or pain, chest pain, anorexia, nausea and vomiting were similar to the effects of pamaquine (*43*). In a study reported in 1950, however, in which five volunteers were given 60 mg pentaquine and 2 g quinine daily for 1 day before and 6 days after artificial infection with *P. vivax* Chesson strain, one volunteer had a severe haemolytic reaction after the first dose on the fourth day of administration, and pentaquine was discontinued (*44*). During 112 courses of treatment with pentaquine alone or in combination in this study, many toxic reactions were noted, in addition to anorexia, nausea, abdominal discomfort and cyanosis. Drug fever occurred in 16 men (most noticeable 6–8 days after the first course), and in 15 men the Hb concentration fell by 10% or more during therapy, with two cases requiring cessation of treatment; one of these two volunteers, a white man, had severe acute intravascular haemolysis. No deaths were reported.

Pentaquine was effective in preventing relapse of vivax malaria when given with quinine for 14 days. A 60-mg daily dose reduced the relapse rate in heavily infected patients from 98% to 18% and the rate in patients with moderately heavy infections from 67% to 4% (*45*). In this study, conducted in 1948, epigastric pain and anorexia were reported; although no haemolytic anaemia was observed, the Hb concentration fell by 1 g/dL (6.7% of initial Hb) in 16 of 17 men receiving pentaquine and quinine, and many showed

methaemoglobinaemia (average, 0.66 g/dL). As this study indicated that doses of 45 mg or less were less toxic, a study was conducted to compare a daily dose of 30 mg (10 mg thrice daily, each dose with 600 mg quinine sulfate, for 14 days) with chloroquine (600 mg on day 1 followed by 300 mg each on days 2 and 3) given to Second World War veterans with vivax malaria (50 in each group) (*46*). This regimen was effective in preventing relapse (98% had no recurrences versus 65.4% of those treated with chloroquine) during monthly follow-up until 18 months, and the toxicity was insignificant, with no haemolysis observed, although six patients showed a fall of ≤1 million RBCs. Any advantage of pentaquine (or isopentaquine) over pamaquine was relatively small, and its toxicity was significant; therefore, the search for a better 8-aminoquinoline continued.

1.3 Primaquine

Primaquine was developed in 1945 and introduced in the early 1950s. It was found to have a better safety profile and therapeutic ratio than pamaquine or pentaquine. Clinical investigation of primaquine-induced haemolysis led to the identification of G6PD deficiency, the most common human genetic enzymatic disorder.

Primaquine was tested in a screen of 8-aminoquinolines in the late 1940s. It showed the best therapeutic ratio, indicating that it was safer than pamaquine. The monkey model of relapsing malaria (*P. cynomolgi* in rhesus monkeys developed by Schmidt and co-workers (*47*)) showed that the ratio of the maximum tolerated dose to the curative dose in 90% of animals was seven times better for primaquine than pamaquine. In clinical studies primaquine was three times more active than pamaquine against pre-erythrocytic stages, four to six times better for radical cure of vivax malaria and half as toxic. Primaquine is a racemic mixture, and studies in mice and rhesus monkeys have shown that the D and L isoforms have different biological activities (*47*) and different efficacy and toxicity (reviewed in references *48* and *49*). Nevertheless, racemic primaquine is the only form available. Although it is used mainly as an antimalarial drug, it is also prescribed in combination with clindamycin to treat pneumonia caused by *Pneumocystis jiroveci* in HIV-infected patients (*50*).

1.3.1 Pharmacology

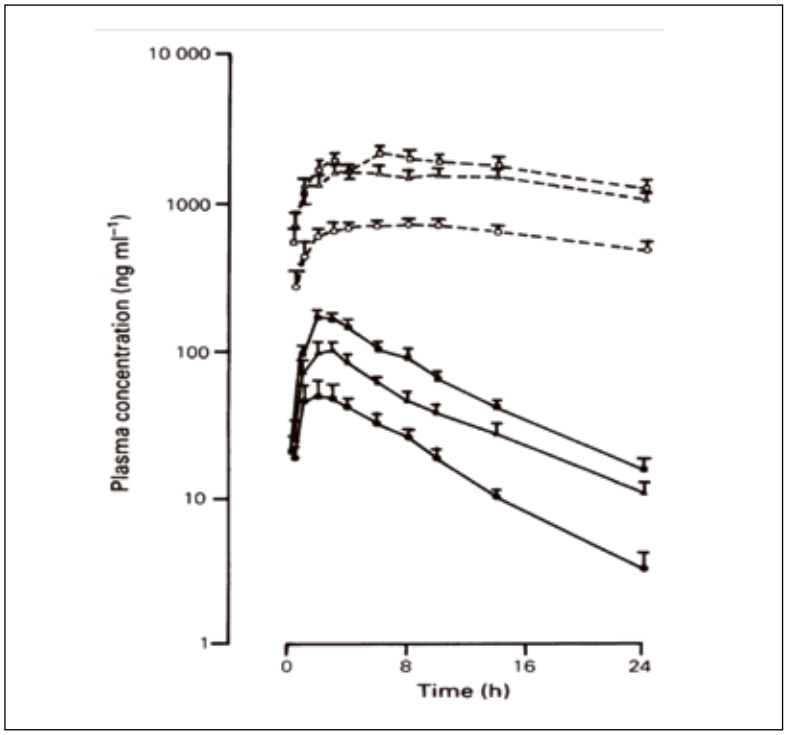
A report in 1981 on the pharmacokinetics of primaquine in Thai and white volunteers given various doses and regimens, and including Thais with G6PD deficiency, showed that the plasma concentrations of primaquine reached a peak about 1–2 h after oral administration, with a subsequent elimination half-life of about 4 h (*51*). Primaquine was not detectable in the plasma of any of the volunteers 24 h after each daily dose, and there was no difference in pharmacokinetics between Thais and whites or between G6PD-normal and G6PD-deficient volunteers. In a report from Thailand in 1985 on the pharmacokinetics of primaquine given as either repeated doses (15 mg daily for 14 days) or as a single dose (15 mg) to healthy volunteers, the mean (± standard

deviation) peak plasma concentration after the single dose was 65 ± 34 ng/mL and was achieved at 2 h; similar results were obtained after repeated dosing, although there was substantial variation among the volunteers in drug disposition. The mean elimination half-life was 4.4 ± 1.4 h for the single dose and 4.3 ± 1.5 h for repeated exposure (*52*). A more recent study confirmed the similarity of pharmacokinetics in G6PD-normal and -deficient individuals (*53*). A study in Korean patients suggested that pharmacogenetic factors might affect the disposition of primaquine in certain ethnic groups (*54*). Malaria itself does not substantially alter its disposition (*55*).

In a study of healthy volunteers in Viet Nam, administration of primaquine with food or grapefruit juice increased its oral bioavailability (*56*). No substantial difference was observed between males and females in the pharmacokinetics of primaquine, although recent steady-state studies in healthy Vietnamese volunteers indicated that women have approximately double the exposure to primaquine in terms of maximum concentration and area under the concentration–time curve (*57*). The clinical relevance of this finding is uncertain. In healthy Thai volunteers, concomitant administration of mefloquine had no effect on the pharmacokinetics of primaquine (*58*), whereas concomitant administration of quinine to patients with malaria resulted in a significantly higher median area under the curve (0.24) of the carboxylic acid metabolite than when primaquine was given alone. In a study of the pharmacokinetics of primaquine in Korean patients with vivax malaria, some pharmacokinetic parameters were different from those reported in other studies, raising the possibility that pharmacogenetic determinants cause variations in drug metabolism (*54*). Overall, there are surprisingly few data on the pharmacokinetics of primaquine.

Primaquine is the 8-aminoquinoline most widely used as an antimalarial agent, but its exact mechanism of action is still unknown. The biotransformation of all the 8-aminoquinolines via the cytochrome P_{450} (CYP) mixed function oxidases (particularly CYP2D6) to putative reactive intermediates (such as 5-OH primaquine) is considered to be crucial for both their antimalarial activity and their toxicity. Several possible modes of action have been proposed. One hypothesis is that the active metabolite impairs the mitochondrial metabolism of parasites, interfering with the function of ubiquinone as an electron carrier in the respiratory chain. Another theory is that the highly reactive metabolites generate intracellular reactive species, which cause oxidative damage. The biologically inert carboxylic acid metabolite, carboxyprimaquine, produced via MAO is the main primaquine metabolite in humans and can be detected in plasma within 30 min of administration; peak levels are reached within 3–12 h and remain 10-fold higher than those of the parent drug (*59*, *60*; Figure 2). Carboxyprimaquine has not been detected in urine, indicating that it undergoes additional metabolic transformation before excretion. Recent studies have implicated CYP 2D6 and MAO-A as the main enzymes associated with primaquine metabolism. The metabolites that mediate the efficacy of the drug and its haemolytic toxicity have been reported to be produced via CYP 2D6-mediated pathways (*61*).

Figure 2. Pharmacokinetics of primaquine and its metabolite carboxyprimaquine



Semi-logarithmic plot of plasma primaquine concentrations (measured by high-performance liquid chromatography) against time after oral primaquine administration in a cross-over study of five healthy adult volunteers receiving 15, 30 and 45 mg and of the corresponding plasma concentrations of the carboxylic acid metabolite. Reproduced from reference 59

The relative roles of each enzyme were determined quantitatively with recombinant metabolic enzymes of the CYP and MAO families; MAO-A was shown to constitute 76.1% of the activity, CYP 2C19 17%, CYP 3A4 5.2% and CYP 2D6 1.7%. CYP 2D6 was shown to produce at least six oxidative metabolites (i.e. the metabolites most probably responsible for the pharmacological effects) and demethylation products, while MAO-A products were derived from the primaquine aldehyde, a precursor to carboxyprimaquine. CYP 2C19 and CYP 3A4 produced only trace levels of hydroxylated species.

1.3.2 Antimalarial action

Primaquine differs structurally from pamaquine only in the terminal amino group. Until recently, all primaquine was contaminated with quinocide, which is a positional isomer (see section 1.4). Primaquine is active against pre-erythrocytic (liver) stages of all the parasites, the asexual and sexual stages of *P. vivax, ovale, malariae* and *knowlesi*, and the sexual stages of *P. falciparum* but is only very weakly active against the asexual blood stages

of *P. falciparum* (*62–64*). Primaquine is the only currently available antimalarial agent that kills the latent persistent stages of *P. vivax and P. ovale* (hypnozoites) in the liver (*65, 66*). As primaquine accelerates gametocyte clearance and reduces transmissibility (*67*), it is also used as a gametocytocidal drug against *P. falciparum* malaria (*68*).

ACTs are currently the first-line antimalarial treatment recommended in malaria-endemic regions by WHO (*68*). Artemisinin and its derivatives have a broader spectrum of anti-gametocyte activity than other antimalarial drugs. ACTs reduce the transmission potential of most treated infections (*67, 69–71)*, but not completely, as mature stage-5 *P. falciparum* gametocytes are relatively insensitive to artemisinins (*67*). Patients who present with transmissible densities of infectious gametocytes may remain infectious to mosquitoes for many days after receiving ACTs, and gametocyte carriage can persist for 7–21 days (*67, 70, 72–75*; see Annex 2). Addition of a single gametocytocidal dose of primaquine to an ACT kills mature gametocytes effectively and therefore reduces the transmission potential substantially (*67, 75*). In studies conducted in Kenya and the United Republic of Tanzania, addition of primaquine to ACT shortened the estimated time of gametocyte circulation from 4.6 and 5 days, respectively, to half a day (*76*). The recommendation of WHO in the Global Plan for Artemisinin Resistance Containment was to include a single oral dose of primaquine (0.75 mg base per kg) in the standard antimalarial drug regimen in order to reduce gametocyte carriage further in the treatment of uncomplicated falciparum malaria, when "the risk for G6PD deficiency is considered low or testing for deficiency is available" (*68*). Studies summarized in this review suggest that a lower dose (0.25 mg/kg) may be equally effective and safer. This lower single dose has therefore been recommended in the context of containment or elimination. It is considered safe even in patients with severe variants of G6PD deficiency, and so individual G6PD testing is not necessary for those who receive this dose (*77*).

1.4 Quinocide

Quinocide is a positional isomer of primaquine developed in the former USSR, which has similar properties. It was a significant contaminant of most preparations of primaquine but went undetected for decades. It was used in large quantities in mass drug administrations in Viet Nam and the republics of the former Soviet Union.

Quinocide is an 8-aminoquinoline drug with the same relative molecular mass as primaquine, as they are positional isomers: the methyl group on the aliphatic side-chain is at the 4 position in quinocide and at the 1 position in primaquine (see Table 1). Quinocide is the main contaminant of unprocessed primaquine and of its medical form as tablets. Only recently have research groups been able to separate and quantify the two drugs in primaquine preparations (*78*). The amount of quinocide in pharmaceutical preparations of primaquine from two manufacturers was 2.12% and 2.71%

(*79*). The concentration of quinocide in human plasma is maximal 3 h after ingestion of the drug, with little excreted unchanged in urine. The elimination half-life of quinocide is similar to that of primaquine: 4–6 h. After a single dose of 23 mg was given to a volunteer, the plasma concentration was 37 µg/L at 3 h, 24 µg/L at 8 h and 16 µg/L at 10 h (*80*).

Elderfield and colleagues synthesized quinocide (CN-1115) in 1949, but it was not tested in humans initially because its toxicity in rhesus monkeys was similar to that of pamaquine. The same compound was synthesized in Russia in 1952 by Braude and Stavrovskay and investigated as an antimalarial agent against *P. vivax*. Research showed that quinocide was much better tolerated if taken alone than with other antimalarials (from a review of studies published in Russian (*80*), including several by the author of the review). Mass treatment to prevent relapse and prophylaxis with quinocide were carried out in 1955–1957 in Tajikistan. Quinocide was also used as a mass prophylactic in Viet Nam, where it was taken daily (at either 23 mg for 10 days or 15 mg for 14 days) by more than 11 000 people, with no reported side-effects (*80*). The haemolytic effects of quinocide were reported to be more pronounced in healthy people (as much as 4–5% in some rare cases) than in ill patients. In Russia, quinocide was used in regimens of either 30 mg daily for 10 days or 20 mg for 14 days. Side-effects were reported in about 5% of patients and included nausea, cyanosis, urinary frequency and "microscopic haematuria" (which may have been haemoglobinuria). Quinocide was effective in preventing vivax relapse when used in Azerbaijan, Georgia and Tajikistan, with an average relapse rate never higher than 1% (from a review of studies published in Russian (*81*)).

In a randomized trial reported in 1962, Coatney and Getz compared the curative and relapse-preventing activities of quinocide and primaquine in young white prison inmates who were infected by mosquito bites with *P. vivax* Chesson strain (*82*). Once parasitaemia and fever were confirmed by day 3–4 after infection, each man received a single oral dose of 600 mg chloroquine and the next day a single oral dose of either 15 mg primaquine (n = 10) or 15 mg quinocide (n = 8) for 14 days. Neither primaquine nor chloroquine was significantly toxic, although the volunteers given quinocide reported abdominal discomfort, including cramps and nervousness; no other toxic manifestations were seen. The relapse rates were 30% with primaquine and 50% with quinocide, and more repeated relapses occurred with quinocide (three second and two third relapses) than with primaquine (one second relapse) during the average 2-year period of observation. Primaquine was therefore considered preferable to quinocide.

As the elimination half-life of quinocide after administration to humans and its toxicity are similar to those of primaquine, the presence of a small concentration of quinocide in primaquine preparations should not add any extra risk.

1.5 Bulaquine (elubaquine)

Bulaquine was developed in India in 1980. It has no clear advantages over primaquine, although one small study reported less haematological toxicity in malaria patients with G6PD deficiency. Primaquine is a major metabolite of bulaquine, suggesting that the difference in its toxicity might have been a function of the dose administered. Its development has been discontinued.

Bulaquine (also called elubaquine, initially CDRI 80/53) was developed in 1980 by the Central Drug Research Institute in India, where it was marketed for use in the prevention of relapse of vivax malaria (at 25 mg/day for 5 days) in a combination kit consisting of chloroquine and bulaquine and sold as Aablaquine (five tablets of 25 mg bulaquine, five tablets of 500 mg chloroquine). The pharmacokinetics of bulaquine was assessed in rats, rabbits and monkeys in 2007 (*83*). Bulaquine was absorbed rapidly and extensively converted to primaquine, although in monkeys a variable, irregular absorption profile was observed. The elimination half-life in rats and rabbits was comparable, approximately 1.2 h, while that in monkeys was 3.7 h. In monkeys given a 10 mg/kg oral dose, a double-peak phenomenon was observed, with mean maximum concentrations (C_{max}) reached at 0.25 h (159 ± 4 ng/mL) and 2 h (183 ± 46 ng/mL). The C_{max} of bulaquine after oral administration was 383 ± 132 ng/mL at 12 h. While bulaquine was detected up to 24 h, primaquine was found up to 48 h; in all three species, primaquine remained in the body for longer than bulaquine. As bulaquine is unstable under acidic conditions, in which it is converted to primaquine, primaquine is the major active metabolite of bulaquine. Primaquine and its metabolites are therefore probably responsible for its therapeutic effect.

In a study in Thailand reported in 2006, bulaquine was less toxic than 30 mg/day primaquine when administered at 25 mg/day for 7 days to G6PD-deficient patients. All patients first received a 3-day course of a total dose of 1500 mg chloroquine (*84*; see Annex 2). In this study, the four patients given primaquine had a clinically significant fall in haematocrit (erythrocyte volume fraction; from 35% to about 20% at day 8), while that of the three patients given bulaquine never fell below 30%. Thus, the main advantage of bulaquine as an anti-relapse agent in this very small study was considered to be lower oxidative toxicity than primaquine. The radical curative properties of the two drugs could not be compared satisfactorily in such small groups. In a study in India in 2006, the gametocytocidal activities of a single dose of 45 mg primaquine were compared with those of a single dose of 75 mg bulaquine given on the fourth day of therapy (quinine and doxycycline for 7 days) to patients with falciparum malaria and similar gametocytaemia on admission (gametocyte count, >55/μL within 72 h of diagnosis). Clearance of gametocytaemia was slow in both groups, but bulaquine cleared gametocytes faster: on day 8, 20/31 (65%) patients given primaquine and 19/59 (32%) given bulaquine had gametocytes on blood smears (p = 0.002). On days 15, 22 and 29, however, none of the patients had gametocytaemia (*85*).

In a study in India in 2001 of the prevention of vivax relapse, no difference was found between primaquine (15 mg daily, *n* = 220) and bulaquine (25 mg daily, *n* = 219) given for 5 days after 1500 mg chloroquine over 3 days (*86*). The two regimens were partially effective in preventing long-term relapse (>6 months): 23/220 (10.5%) relapses occurred after primaquine and 31/219 (14.2%) after bulaquine, whereas the incidences of relapse within 6 months were similar with the two drugs to that of a group given a placebo. Other studies suggest that a longer regimen and/or higher dose are needed to prevent relapse of vivax malaria (see section 2.2).

1.6 Tafenoquine

Tafenoquine is more active against blood stages than primaquine and may have greater transmission-blocking activity. It causes oxidant haemolysis in individuals with G6PD deficiency. Tafenoquine is eliminated slowly, allowing short-course radical curative treatment, but this results in potentially protracted haemolytic toxicity in G6PD-deficient individuals. Phase-IIb studies are under way to determine risks and optimize dosing.

Tafenoquine (WR238605) is an 8-aminoquinoline identified in 1978 at the Walter Reed Army Institute of Research, USA. Because of the rapid elimination of primaquine, which necessitates daily dosing for 2 weeks for radical cure, and its toxicity in G6PD-deficient individuals, other 8-aminoquinolines with the same properties as primaquine but with a longer elimination half-life and a safer profile have been sought. The most promising drug in this category is tafenoquine, an 8-aminoquinoline analogue of primaquine. It has a long absorption phase and is slowly metabolized, reaching a peak blood concentration of 417–489 ng/mL 13.8 h after administration of a dose of 600 mg to healthy volunteers. Its terminal elimination half-life of about 14 days (*87*) makes it an attractive candidate for short-course regimens. Tafenoquine is being developed by GlaxoSmithKline and the Medicines for Malaria Venture for treatment of *P. falciparum* and *P. vivax* malaria. It presents similar or perhaps greater risks than primaquine for haemolytic anaemia in G6PD-deficient individuals.

A randomized, double-blinded, placebo-controlled field trial of tafenoquine for preventing falciparum malaria was conducted in a high-transmission area of Kenya in 2001. In 223 adult volunteers, a prophylactic regimen of 200 mg or 400 mg weekly for 13 weeks was highly effective in preventing falciparum malaria. The authors concluded that tafenoquine was well tolerated in G6PD-normal adults (*88*). Although the authors intended to exclude G6PD-deficient individuals by initial screening, they missed and inadvertently included two women with G6PD deficiency, in whom haemolytic events occurred. One was later found to be heterozygous for the G6PD A– variant and developed intravascular haemolysis, with black urine, which required a blood transfusion; haemolysis did not continue after the acute event, and there was no renal compromise. The second woman was homozygous for G6PD A–, and she remained asymptomatic despite an acute 3-g decrease in Hb concentration,

which was noticed only in routine blood tests. Her Hb concentration returned to normal without intervention. Both women had received 400 mg tafenoquine daily for 3 days before the haemolytic events. Dose-finding studies in G6PD-deficient heterozygous women are under way in Thailand (personal communication, J.A. Green, GlaxoSmithKline, April 2012).

Like other 8-aminoquinolines, tafenoquine affects sporogonic development in mosquitoes, although there is some evidence that it has greater transmission-blocking activity than primaquine. The gametocytocidal and sporontocidal modes of action can be differentiated by exposing the parasite to a particular drug during different stages of parasite development. Gametocytocidal activity is assessed by administering the drug at the same time as the infectious blood meal and evaluating subsequent mosquito infection rates, whereas sporontocidal activity is assessed by exposing previously infected mosquitoes to the drug at a subsequent feed. In 2003, a study was reported from Thailand on the transmission-blocking activity of tafenoquine against strains of *P. vivax*, in which primaquine was used as a negative control for sporontocidal activity (*89*). Laboratory-reared *Anopheles dirus* mosquitoes were infected with *P. vivax* in blood collected from gametocytaemic volunteers reporting to local malaria clinics. Four days later, the mosquitoes were fed on uninfected mice treated 90 min earlier with a drug, and activity was determined by assessing oocyst and sporozoite development. Primaquine did not affect oocyst or sporozoite development at a dose of 100 mg/kg, while tafenoquine affected sporogonic development at doses as low as 25 and 0.39 mg/kg. In an earlier study in the USA, similar experiments were performed with chloroquine-sensitive *P. berghei* and chloroquine-resistant *P. falciparum* and *An. stephensi* mosquitoes fed on uninfected mice treated previously with a drug. Tafenoquine at 100 mg/kg effectively interrupted sporogonic development and prevented sporozoite invasion of the salivary glands of the mosquitoes, whereas primaquine at the same dose did not inhibit sporogonic development of *P. berghei* (*90*).

A small randomized, double-blinded study was conducted in Gabon in 2000 to assess the efficacy and safety of doses of tafenoquine of 250 mg (n = 17), 125 mg (n = 9), 62.5 mg (n = 26) and 31.25 mg (n = 6), each given for 3 days to people aged 12–20 years living in an area endemic for falciparum malaria. No significant difference in adverse events was found between the groups given tafenoquine and placebo; on day 28, the Hb concentrations were slightly (0.4 g/dL) but significantly lower than at screening in the group receiving 250 mg tafenoquine. All the doses above 31.25 mg protected against infection up to day 56 and conferred significant protection up to day 77; however, the greater protective efficacy of the higher doses was not statistically significant because of the small numbers (*91*).

Walsh and colleagues reported in 2004 on an evaluation of various doses of tafenoquine in Thailand. The first study addressed the use of tafenoquine for preventing *P. vivax* and multidrug-resistant *P. falciparum* malaria in 205 Thai soldiers, who received either a loading dose of 400 mg daily for 3 days

followed by single monthly 400-mg dose (n = 104) or placebo (n = 101) for up to 5 months (*92*). All but one of the 22 infections with *P. vivax*, 8 with *P. falciparum* and 1 mixed infection occurred in soldiers who received placebo. Thus, the protective efficacy of tafenoquine was 97% for all malaria (95% CI, 82–99), 96% for *P. vivax* malaria (76–99) and 100% for *P. falciparum* malaria (60–100). In this study, monthly tafenoquine was safe and well tolerated. The second study was a comparison of tafenoquine with primaquine for preventing vivax relapse (*93*; see Annex 2). Patients with *P. vivax* infection received tafenoquine at 300 mg/day for 7 days (n = 18), 600 mg/day for 3 days (n = 19) or 600 mg as a single dose (n = 18) or primaquine at 15 mg/day for 14 days (n = 12) after 1500 mg chloroquine for 3 days. The protective efficacy of chloroquine + tafenoquine over 8–24 weeks was 92.6% (7.3–99.9), while that of chloroquine + primaquine was 79.5% (14.5–96.5). Even though in all these studies tafenoquine was administered after a meal, gastrointestinal adverse events were observed more frequently with tafenoquine than with primaquine, and the levels of methaemoglobin were higher with tafenoquine (up to 12% versus 3.3% with primaquine) but were not associated with symptoms. It should be noted that the comparison was with low-dose primaquine (15 mg/day).

The Australian Defence Force has conducted trials during the past decade on the use of tafenoquine regimens for vivax malaria acquired in the south-west Pacific region in adults returning from areas holo-endemic for malaria. In a comparison of primaquine (22.5 mg daily for 14 days to 214 volunteers) and tafenoquine (292 volunteers received 400 mg daily for 3 days, and 86 received 200 mg base twice daily for 3 days) given as post-exposure prophylaxis for soldiers returning from Papua New Guinea in 2002, vivax malaria developed in 6 of 214 soldiers given primaquine and 7 of 378 given tafenoquine within 12 months, with a later onset after tafenoquine. The authors suggested that, at the doses used, tafenoquine is no more effective than primaquine in preventing vivax malaria, as failures occurred with both medications, indicating that Chesson-type parasites are also refractory to tafenoquine (*94*; see Annex 2). A report in 2008 of a trial in Australian Defence Force personnel in Bougainville and Timor-Leste showed that regimens of tafenoquine for 3 days (400 mg daily, 200 mg twice daily or 200 mg daily) or primaquine (22.5 mg plus 100 mg doxycycline over 14 days) were effective and well tolerated for post-exposure prophylaxis (*95*; see Annex 2). The relapse rate among personnel treated in Bougainville with tafenoquine (n = 173) was 1.2–2.3%, while that after the primaquine regimen (n = 175) was 3.4%. The rate among personnel treated in Timor-Leste was 4.9–11.0% with tafenoquine (n = 636) and 10.0% with primaquine (n = 289). The most frequent adverse events reported in all groups were nausea, abdominal pain and diarrhoea. A dose-dependent reduction in adverse events was seen with tafenoquine, the lowest dose (total, 600 mg over 3 days) resulting in rates equivalent to that with primaquine.

2. Efficacy of primaquine as an antimalarial drug

This section covers studies in which primaquine was administered either individually or during mass drug administration and in which its efficacy for malaria prophylaxis, prevention of vivax malaria relapse or as a gametocytocide in *P. falciparum* infections was evaluated.

Primaquine is given:

- for causal prophylaxis (mostly for travellers) to prevent malaria infections by all species that infect humans: usually an adult daily dose of 30 mg from the day before exposure and continuing for 1 week after return from a malaria-endemic region;
- for terminal prophylaxis: presumptive therapy to prevent relapse of *P. vivax* or *P. ovale* parasite stages in the liver (hypnozoites) that can occur weeks to years after the initial infection and were acquired under chemoprophylaxis;
- for radical cure after *P. vivax* or *P. ovale* malaria, used in conjunction with a blood-stage schizonticide such as chloroquine or ACT; and
- as a gametocytocidal agent to prevent *P. falciparum* transmission.

Studies of terminal prophylaxis began in the USA in the late 1940s (*41*) and eventually resulted in a standard radical curative treatment of 15 mg primaquine daily (0.25 mg/kg) given for 14 days (total dose, 210 mg), coinciding with the last 2 weeks of chloroquine, mefloquine or doxycycline prophylaxis or initiated during the final week of atovaquone–proguanil prophylaxis. This regimen, approved in the early 1950s, was developed in an attempt to provide a safe dose for African American soldiers returning from the Korean War, who had a higher likelihood of haemolysis. As shown for pamaquine, primaquine-induced haemolytic anaemia was usually dose-dependent, and 15 mg/day was chosen as the adult dose that could be given to all service personnel with a safety that was considered acceptable at the time.

For radical cure, the primaquine regimen recommended initially, 15 mg/day for 14 days, was found to be less effective against so-called "tolerant" strains of *P. vivax* (mostly in South-East Asia and Oceania) (*96–102*). The guidelines of various organizations for doses and regimens of primaquine have differed by region and country. WHO now recommends a higher dose of 0.5 mg/kg primaquine (i.e. 30 mg) daily for 14 days (total dose, 420 mg) in regions with primaquine-resistant *P. vivax* malaria (Oceania and South-East Asia) (*68*). The recommendation required G6PD testing and stipulated that primaquine should not be given to G6PD-deficient individuals. The United States Centers for Disease Control and Prevention currently recommends a daily dose of 0.5 mg/kg primaquine for 14 days, not exceeding 30 mg/day, for radical cure of vivax malaria (*102*). India and several other countries once recommended a 5-day regimen of primaquine

(instead of 14 days), although there is no evidence that this is effective; their policy was changed recently to 14 days of primaquine (Kamini Mendis, personal communication).

2.1 Malaria prophylaxis

Primaquine is an effective causal prophylactic drug for both vivax and falciparum malaria when given at 0.5 mg/kg (adult dose, 30 mg) daily in endemic areas. It is sometimes used for prevention by travellers, starting 1 day before departure and continued for 1 week after returning from an endemic area.

In 1955, the effect of primaquine on blood stages of *P. falciparum* strain PF6 from Panama was evaluated. PF6 was inoculated by mosquito bites into non-immune white prison inmate volunteers in the USA. Primaquine was effective as a causal prophylactic agent only when given on day 1 or 3 after inoculation but not when given on day 5 (*62*). Asexual blood stages that developed from the initial tissue stages 144 h after infection (trophozoites) were no longer susceptible to the action of primaquine given at non-toxic doses. In a study in Kenya in 1995 on falciparum malaria prophylaxis in 32 children aged 9–14 years after curative therapy with quinine and doxycycline, primaquine was compared with multivitamin intake. Daily primaquine at 15 mg/day was more effective in preventing falciparum malaria than a thrice weekly regimen of 15 mg on Monday, Wednesday and Friday. The preventive efficacy of daily primaquine was 83% (95% CI, 50–94), whereas all children given thrice weekly primaquine were parasitaemic by week 12. The group given primaquine developed parasitaemia significantly more slowly than those given vitamins, and the proportions in whom clinical malaria developed were 11% (95% CI, 3–31) with primaquine and 41% (95% CI, 23–59) with vitamins (*103*; see Annex 2).

Mass drug administration in Panama between 1965 and 1968 with pyrimethamine and primaquine administered every 2 weeks (adult dose of primaquine, 40 mg) decreased the malaria incidence from 17.4% at the beginning to 2.4% after 8 weeks and to 1% after another 8 weeks, and it remained at this level for the rest of the mass administration. There were no clinical cases of malaria. Vivax malaria was eliminated from the region for 32 weeks in the second year, but falciparum malaria was never eradicated, although the frequency decreased considerably (*104*). The authors noted that some of the cases of malaria occurred among certain religious groups that refused to take the drugs and in visitors from malaria-endemic regions. In contrast, mass drug administration for malaria elimination on the Vanuatu island of Aneityum in 1991 (N = 718) resulted in rapid disappearance of falciparum malaria. A few *P. vivax* infections were detected up to 5 years later, mainly in children, probably representing relapses rather than re-infections, suggesting that the primaquine regimen had incomplete radical curative efficacy (*105*; see Annex 2). Except for one reintroduction, Aneityum has remained malaria-free ever since.

In a study conducted in Indonesia in 1995, primaquine was given for prophylaxis to 42 volunteer Javanese men at an adult dose of 30 mg daily after a 1-year radical cure regimen. Primaquine had 94.5% protective efficacy against falciparum malaria, 90.4% against vivax and 92.3% against both (*106*; see Annex 2). In a report on the use of primaquine for malaria prophylaxis in non-immune transmigrants from Java and Bali conducted in 1995 in Irian Jaya, Indonesia, 30 mg primaquine given every other day had better prophylactic efficacy and tolerance than a single weekly dose of 300 mg chloroquine. The minimum protective efficacy of primaquine was 74% for falciparum and 90% for vivax malaria in a hyperendemic malaria setting (*107*; see Annex 2). In two studies conducted by the same group in Colombia in 1998 and 1999 (*108*, *109*; see Annex 2) in the same population, 122 and 100 male volunteer soldiers, respectively, received primaquine at 30 mg daily for 16–17 weeks for vivax and falciparum malaria prophylaxis. Primaquine was effective both when given alone (89% protective efficacy: 94% for falciparum, 85% for vivax malaria) and with chloroquine at 300 mg (88% protective efficacy: 89% for falciparum, 88% for vivax malaria). These two studies indicated that addition of chloroquine to primaquine for prophylaxis did not change either its protective efficacy or its toxicity.

In a report from Papua Indonesia in 2001 on use of primaquine in malaria prophylaxis, primaquine was given at 30 mg daily for 20 weeks after curative therapy with atovaquone–proguanil. Primaquine was effective, with a protective efficacy of 88% against falciparum malaria, >92% against vivax malaria and 93% against both (*110*; see Annex 2).

2.2 Prevention of relapse of vivax malaria

Assessments of the radical curative efficacy of primaquine have been confounded by variations in relapse patterns and study design, e.g. duration of follow-up. Radical curative efficacy appears to depend on the total dose of primaquine more than on the duration of therapy. A daily dose of 0.5 mg/kg is optimal for tropical strains, while 0.25 mg/kg is sufficient for temperate strains. A short-course regimen of 0.25 mg/kg per day for 5 days, used extensively in some countries, is substantially less effective than 14-day regimens for preventing vivax relapse. Poor adherence to radical treatment is a major issue. Short-course (7–10 days) high-dose regimens (>0.5 mg/kg per day) may be as effective as standard 14-day courses, but more evidence is needed from trials.

In this section, we review studies of the use of primaquine in preventing relapse of vivax malaria and variations in the regimens used in different regions in terms of the duration and dose of primaquine used. It is difficult to evaluate the efficacy of any antimalarial agent in preventing vivax relapse, as in endemic areas relapses cannot be distinguished reliably from re-infections. Relapse rates vary considerably by area, as do relapse intervals. Age is also a major confounder, as adults in endemic areas may have acquired sufficient immunity to suppress relapses whereas children have not, so studies in adults may show significantly lower relapse rates

than studies in children. The follow-up times in some studies were not long enough to rule out late relapse (particularly for temperate strains, for which the relapse interval is typically 8–10 months). We have included studies in endemic areas, where relapses were not specifically assessed but where primaquine was used and only parasite recurrence was reported. Most of the data are from South-East Asia and Oceania, with few recent data from India, the Middle East, the horn of Africa and South and Central America.

P. vivax infections relapse as a result of activation of persistent liver stages, hypnozoites. The incidence of relapses and the intervals between them depend on several factors, including the parasite strain (geographical origin), the size of the sporozoite inoculum and the immunity and age of the human host. Three general patterns of infection can be distinguished: tropical (*P. vivax* Chesson strain), with frequent relapses at approximately 3-week intervals after administration of a rapidly eliminated antimalarial agent; temperate (Saint Elizabeth or Madagascar strain), in which a primary infection is usually followed by an interval of 8–9 months before relapse; and long or "hibernans", in which there is no primary infection and the first illness occurs 8–9 months after sporozoite inoculation (*111*). In endemic areas, relapses are the major form of malaria in young children and an important source of malaria transmission. Both the proportion of infections that relapse and the number of symptomatic relapses per mosquito sporozoite inoculation decline with age. It is likely that immunity and therefore age is a significant confounder in epidemiological assessments based on passive case reporting in many areas endemic for *P. vivax*. Often, adults are more likely to present to malaria clinics than children: in India, which harbours most of the world's *P. vivax* infections, most presentations for malaria are by young men. Yet, in indigenous populations in transmission areas, a significant degree of immunity should have been gained by people of each sex by early adulthood, which should reduce the number of relapses. The paucity of data on children may contribute to the low apparent relapse rates reported from the Indian subcontinent, so that data from malaria clinics may not necessarily be representative of the epidemiology of the disease in some endemic areas.

The number of sporozoites inoculated by an anopheline mosquito is an important determinant of both the timing and the number of relapses. The sporozoite dose determines the clinical phenotype of long-latency vivax malaria in northern latitudes (*112*, *113*). In long-term observations of infections with the Saint Elizabeth strain, there was a clear bimodal pattern, in which a long pre-patent period (~300 days) occurred only after small sporozoite inocula, which reflects natural infection (*113*). A similar observation was reported with a strain from the Democratic People's Republic of Korea used for malaria therapy in a Moscow hospital between 1953 and 1968 (*112*). When the inoculum of sporozoites was small (10–100), initial parasitaemic illness occurred only after ≥ 9 months; when >1000 sporozoites were inoculated, illness occurred after a "normal" incubation period of 2 weeks. When

increasing sporozoite doses of the tropical *P. vivax* Chesson strain were inoculated, the incubation period shortened, and there was no evidence of a long pre-patent period (*114*, *115*).

Relapse of vivax malaria was well documented in all the tropical theatres of the Second World War. Immediately after the War, a huge research effort to find new antimalarial drugs resulted in chloroquine (identified in 1934 and initially overlooked), proguanil and the more effective 8-aminoquinolines. Chloroquine was clearly the best antimalarial agent, but, like quinine and mepacrine, it acted only on blood-stage parasites. It became the treatment of choice for all malaria throughout the world. As the 8-aminoquinolines also have significant blood-stage activity against *P. vivax* (and *P. knowlesi*), the widely used chloroquine–primaquine regimen should be considered a combination treatment.

The dose of primaquine recommended globally was chosen largely on the basis of studies on the sensitive Korean *P. vivax* (*116–118*). After a very high rate of relapse was observed in soldiers returning to the USA from the Korean War in 1950, all soldiers were given a radical curative regimen of 15 mg/day for 2 weeks during their return by sea, which was highly effective. The *P. vivax* Chesson strain was found to be more "resistant" to 8-aminoquinolines (*39*), but the recommendation for use of a higher primaquine dose (22.5 mg/day) was applied initially only in Oceania, although it might have been better to recommend higher doses for all areas in which frequent-relapse parasites were found, including South-East Asia. It is possible that all "temperate strains" are equally sensitive to primaquine and that all "tropical strains" are equivalent but have higher "activatable" hypnozoite burdens and so require a higher dose (i.e. 0.5 mg/kg for 14 days). Sinton's work in India suggested clearly that quinine and pamaquine were synergistic in preventing relapse (*7*, *8*), and studies during and after the Second World War provided further support for this notion. In the 1950s, Alving and colleagues conducted a formal interaction study, which provided evidence of marked synergy between quinine and chloroquine–primaquine (*119*). The mid-1950s saw a decline in clinical research on vivax malaria, and most countries adopted the 15-mg/day primaquine regimen.

It has been suggested that resistance to the radical curative activity of primaquine has emerged (*120*). A report in 2003 from India, where primaquine was given to patients with vivax malaria after 3 days of chloroquine, suggested that some resistance to the 15-mg primaquine regimen used in the study might be developing, as 13 recurrences were seen in 142 controls and 6 in 131 patients receiving primaquine, giving recurrence rates of 9.15% and 4.6%, respectively (*97*; see Annex 2). There are, however, other explanations for the incomplete efficacy seen in this and other studies. Resistance to primaquine in the blood stage has been induced experimentally in vivax malaria (*121*), whereas the evidence for acquired primaquine resistance in liver stage activity and resistance in gametocytocidal activity is weak.

2.2.1 14-day regimens

The 14-day primaquine regimen for preventing relapse of vivax malaria has proved in general to be efficacious. Relapse rates depend on the total dose and the parasite strain. For strains found in some areas of South-East Asia, in Oceania and possibly in the Americas (Colombia), higher daily doses (≥0.5 mg/kg) are needed, as there is evidence of reduced susceptibility in the local vivax strains. For example, in a recent report from Taiwan (China), two imported cases of vivax malaria acquired in Indonesia and the Solomon Islands relapsed after 3–4 months, despite having completed standard-dose primaquine therapy of 30 mg/day for 14 days for the first episode (*122*). Treatment with a higher dose of primaquine (60 mg/day for 14 days) prevented further relapse in both patients. A comparison in 2006 in Colombia of primaquine (15 mg/day with chloroquine) given for 3 (n = 71), 7 (n = 71) or 14 days (n = 68) for cure of uncomplicated vivax malaria showed 100% cure in a follow-up of 28 days in 210 adult patients with vivax malaria. Parasitaemia recurred due to re-infection, relapse or recrudescence in all three groups during 6 months of follow-up (45% for 3 days' primaquine, 36.6% for 7 days and 17.6% for the standard 14-day regimen), suggesting incomplete radical cure with low-dose primaquine in this setting. A higher daily or total dose might be necessary to prevent vivax relapse in this region, e.g. 30 mg/day for 14 days (*123*, *124*; see Annex 2).

In a study of 294 US soldiers exposed to *P. vivax* (long-latency malaria) during the Korean War in the summer of 1951, there were no relapses during a 6-month follow-up among men who took 15 mg primaquine in the morning for 14 days, while a placebo control group of 331 men had a 17.5% relapse rate (*116*). This study did not include African Americans. In another study in the same series, chloroquine alone was compared with chloroquine + primaquine (15 mg daily for 7 days). During a follow-up of a minimum of 3 months, 23 of 46 patients treated with chloroquine alone relapsed at least once, whereas only one of 31 patients (3%) receiving primaquine relapsed 49 days after treatment (*118*; see Annex 2), suggesting that a shorter (7 days rather than the standard 14 days) regimen was effective in preventing relapse in this case. In a comparison of chloroquine alone or with primaquine or pamaquine, both 8-aminoquinolines were effective in preventing relapse, primaquine being slightly more effective than pamaquine: after 4–11 months, 27.6% of the patients treated with chloroquine had relapsed at least once, and after 2 years the relapse rate was 39%; relapses occurred in only two patients given chloroquine plus pamaquine (0.7%) and in none of the patients given chloroquine plus primaquine (*117*, *125*).

In 1953, the efficacy of four 8-aminoquinolines against the *P. vivax* Chesson strain was compared in prison volunteers infected by mosquito bites. A dose of 20 mg primaquine for 14 days with quinine was more effective in preventing relapse for at least 1 year (15% relapse) than 10 mg primaquine with quinine for 14 days (65% relapse) (*39*; see Annex 2). In a study of 321 indigenous Miskito people in Nicaragua, reported in 1953, primaquine at 10, 15 or 20 mg daily for 14 days with chloroquine for 2 days were all 100%

effective against vivax relapse for 1 year, while the control group receiving only chloroquine had 7% relapses in the same period (*126*; see Annex 2). In a report in 1953 from the USA, 510 patients with late acute attacks of vivax and fever were given chloroquine alone or with daily doses of 10, 15, 20 or 30 mg primaquine. During 4 months' follow-up, the relapse rate was 43.8% with chloroquine alone and <3% when primaquine was added (*127*).

In a study in 1969 on 21 soldiers returning to the USA from Viet Nam, recurrence of malaria occurred within 6 months in six men given chloroquine–primaquine tablets (300 mg chloroquine, 45 mg primaquine) weekly for 8 weeks and in three given 15 mg primaquine daily for 14 days (*128*). In another report on vivax malaria in soldiers returning from Viet Nam, an unsupervised 8-week chloroquine–primaquine regimen (tablets containing 300 mg chloroquine and 45 mg primaquine taken weekly) given to 94 patients (84 white and 10 African American) was compared with a supervised regimen of 15 mg primaquine daily for 14 days given to 133 patients (116 white and 17 African American) (*129*). During the 6-month follow-up, 21 men (22.3%) treated for 8 weeks and 10 (7.5%) treated for 14 days experienced a relapse ($p < 0.0005$). The authors noted that many men in the unsupervised group did not complete the regimen, which might have contributed to the high relapse rate. A report from Thailand in 1994 described the effect of primaquine on vivax malaria relapse rates within 6 months in 167 adults; the authors considered episodes after that time to be re-infections. G6PD-deficient individuals were excluded. The relapse rate after primaquine given for 14 days after chloroquine on day 1 was significantly higher in people given 15 mg primaquine (18.4%) than in those given 22.5 mg (2.4%) ($p < 0.05$) (*98*; see Annex 2). In a study in Pakistan in 2008 on the use of primaquine to prevent relapse of malaria, primaquine was given for either 14 days ($n = 54$) or weekly for 8 weeks ($n = 68$). Relapses occurred in 22/71 (31.0%) patients given placebo, 1/55 (1.8%) on the 14-day regimen and 4/75 (5.1%) on the 8-week regimen during a 9-month follow-up (*130*). This is one of the few evaluations of the weekly regimen recommended for patients with mild G6PD deficiency variants.

2.2.2 Shorter regimens

As adherence to the 14-day regimen may be poor, shorter regimens have been tested. In 1960, the health records of 2314 migrant workers in a malaria-free region of India were reviewed. The workers had been treated with primaquine (15 mg daily for 5 days with a single dose of amodiaquine) and followed for about 18 months. Of the 678 cases of parasite-positive malaria before treatment (*P. falciparum*, *P. vivax* or *P. malariae*), 39 had recurrences (5.75%), whereas there were 19/47 recurrences (40.4%) among workers treated with amodiaquine only (*131*). In 25 patients with vivax malaria in Romania in 1962, two primaquine regimens for radical cure were compared: 15 mg daily for 15 days and 45 mg daily for 5 days with 3 days of chloroquine. The 15-day treatment was more effective in preventing relapse (no relapses observed during 55–668 days of follow-up) than the 5-day regimen (four relapses within 44–80 days) (*132*).

In a study in the USA in 1973, five non-immune prison inmate volunteers infected via mosquito bites with a West Pakistan vivax strain were given a 5-day regimen of 15 mg primaquine (*133*). Primary malaria attacks occurred on days 10–15 after infection, which were treated on day 3–4 of patent parasitaemia with 1500 mg chloroquine (600 mg, then 300 mg at 6, 24 and 48 h), followed by primaquine at 15 mg daily for 5 days. All the volunteers experienced relapse 121–181 days after exposure to infection (101–157 days after the last primaquine dose), representing a 100% failure rate of radical cure. Each volunteer was then treated with 1500 mg chloroquine over 48 h, followed by 15 mg primaquine base daily for 14 days. No subsequent relapses were observed during a formal observation period of 200 days. In 1974, a report presented the relapse rates among 57 prison inmate volunteers in the USA who were infected by mosquito bites with various *P. vivax* strains from Central America with long latent periods (> 5 months). The volunteers were then treated with a blood schizonticidal drug (not specified) and 15 mg primaquine daily for 14 days, with a follow up of 9–36 months (*134*). Only two relapses occurred, 40 and 160 days after primaquine. One volunteer was treated with 15 mg primaquine for 5 days after a primary attack of the vivax Salvador II strain to test the efficacy of a shorter regimen, but he experienced a relapse 223 days after treatment.

In field tests in El Salvador reported in 1978 of treatment of *P. vivax* infections, amodiaquine alone (single dose of 600 mg) was compared with either the same dose of amodiaquine and 5 days of 15 mg/day primaquine ($n = 90$; children <5 years received 7.5 mg/day) or amodiaquine with a single dose of 45 mg primaquine ($n = 67$; children aged 7–12 years received 30 mg, 3–7 years 22.5 mg and 1–3 years 15 mg), with a follow-up of 3–9 months and a visit every 2 weeks (*135*; see Annex 2). Although it was difficult to differentiate relapses from new infections, both primaquine treatments reduced the incidence of parasite-positive recurrences to a greater extent than amodiaquine alone: 46.3% with amodiaquine alone and 28.1% with amodiaquine and 5 days of primaquine; 68% with amodiaquine alone and 37% with amodiaquine and a single dose of primaquine. A greater impact was seen during the first 3 months of therapy, which was more pronounced for patients under 15 years of age with the 5-day regimen.

In India, a 5-day primaquine regimen was used for decades as radical cure, anti-relapse therapy for vivax malaria, children receiving proportionally lower doses. A report in 1954 of a comparison of 5 days of 15 mg primaquine daily ($n = 50$) with a pyrimethamine regimen showed no relapses with primaquine but 6% with pyrimethamine after a 6–7.5-month follow-up (*136*; see Annex 2). One third of the patients in each group presented with gametocytaemia at the start of treatment; the clearance rate was 100% with primaquine and 79% with pyrimethamine within 48 h. With pyrimethamine, 100% clearance was achieved at 96 h. In a study of vivax malaria in 1979, 15 mg/day primaquine was administered for 5 days after a single dose of 600 mg chloroquine (presumptive treatment for fever). If vivax parasitaemia was found within 1–2 weeks, the same treatment was given. Follow-up was monthly

up to 1 year. The treatment was effective, with a recurrence rate of only 0.7% (10 recurrences in 2016 vivax cases treated over 1 year) (*137*). A study reported in 1984 was conducted in 1203 vivax malaria patients to evaluate a 3-day regimen of primaquine–chloroquine at the same total adult dose of 75 mg but given as 30 mg on days 0 and 1 and at 15 mg on day 2. The recurrence rate over 1 year was 3.76%, which is comparable with that associated with the 5-day regimen (*138*; see Annex 2). In another report from India, the 5-day primaquine regimen was given after an antipyretic and chloroquine (600 mg on day 0 and 300 mg on day 1) to 726 vivax malaria patients. A first relapse occurred in 50/726 patients (6.9%) on days 27–395 of follow-up (*139*). In this study, a 19-year-old woman had four consecutive relapses in spite of repeated primaquine therapy. A report in 1990 described presumptive treatment followed by radical cure treatment with chloroquine and primaquine for 5 days in 995 vivax malaria patients. After 8 months of follow-up, the incidence of first relapse was 10.3% (104/995) (*140*). In a study in 1999, a 5-day regimen ($n = 80$) was compared with 14 days ($n = 81$) of primaquine at 15 mg/day after 3 days of chloroquine. The 5-day regimen was less effective in preventing vivax relapse (*141*; see Annex 2). All patients had cleared parasites in blood by day 4 and remained free of infection until day 29; however, the incidence of relapse was 26.7% with the 5-day regimen and 0% with the 14-day treatment over 6 months (60–63 patients in each group were followed up for the whole period); the recurrence rate with chloroquine alone was 11.7%.

In an investigation of relapse patterns in four major industrial complexes in India in 2001, 511 of 5541 cases of vivax malaria (9.2%) treated with a 5-day regimen of 15 mg primaquine relapsed, most within 1 year of treatment (*142*). A study was conducted in 2002 to evaluate the effectiveness of this regimen in a malaria-endemic region in Orissa, where *P. falciparum* predominates. Addition of primaquine to chloroquine treatment did not affect the incidence of vivax recurrence within 1 year (*143*). The prevalence of recurrence of parasitaemia with fever was 8.6% in 723 cases of *P. vivax* infection treated with chloroquine alone and 6.5% (49/759) in patients treated with chloroquine and 5 days of 15-mg/day primaquine. In a pilot study in Sri Lanka reported in 2003, the 5-day regimen was ineffective, as five of six patients given 15 mg primaquine daily by directly observed therapy had a relapse within 6 months (*144*).

In Peru in 2002, the standard primaquine regimen (15 mg daily for 14 days) was compared with a shorter regimen (30 mg daily for 7 days) after 3 days of standard chloroquine therapy for vivax malaria. No significant difference in efficacy was found: three patients on the shorter regimen and two patients on the 14-day regimen were parasite-positive (indicating relapse or re-infection) in the second month of follow-up. No parasites were seen on blood slides from patients in either group during the first month (*145*).

A report from Thailand in 2003 described a clinical trial in which 394 patients received artesunate alone or artesunate and primaquine for preventing relapse of vivax malaria for either 5 or 7 days. Half the patients receiving

artesunate alone had relapsed by day 28. Addition of primaquine at 0.6 mg/kg per day for 14 days prevented the reappearance of malaria in all patients by day 28; however, the follow-up was too short to detect later relapses (*146*). A preliminary study on the safety and efficacy of a short artesunate–primaquine regimen for vivax malaria in adult patients in Viet Nam reported in 2007 showed that 22.5 mg primaquine daily for 7 days given 0, 12, 24 and 36 h after 200 mg artesunate was effective during a follow-up of 28 days. Only one of 28 patients (3.6%) had a recurrence on day 28 (*147*; see Annex 2). In 2008, the efficacy of five primaquine regimens (30 mg given for 5, 7, 9, 11 or 14 days) combined with artesunate was assessed in patients with acute, symptomatic vivax malaria. The 5- and 7-day regimens were significantly less effective (<90%) against early relapse than the 9-, 11- and 14-day regimens (>90%) ($p < 0.05$). The efficacy in preventing early relapse of twice-daily 30-mg doses given for 7 days was 94% (*148*; see Annex 2).

The efficacy of various primaquine regimens given concomitantly with chloroquine, with 122 days of follow-up, was reported in Colombia in 2009 (*149*; see Annex 2). Patients were given the standard total dose for 14 days ($n = 68$) or 3 days of treatment with either the standard dose ($n = 65$) or 71% ($n = 28$) or 50% ($n = 27$) of the standard dose. The mean latency before the first recurrence was 166 days after 14 days of the standard dose, 97 days after 3 days of the standard dose, 91 days after 3 days of 71% of the dose and 108 days after 3 days of 50% of the dose. One-third fewer recurrences were observed within 120 days of follow-up with the 14-day regimen. The monthly recurrence incidence up to day 60 was 1.5% with the 14-day regimen and 35.4% with the same dose given over 3 days. The recurrence rate between days 61 and 90 was also low (3.0%) with the 14-day regimen but was 26.8% when the same dose was given over 3 days. Between days 91 and 120, the recurrence rate was similar in the two groups (14 days, 10.9%; 3 days, 5.5%). The authors suggested that the standard regimen of 14 days be maintained for relapse prevention and that chloroquine and primaquine be given together, whereas the guidelines in Colombia were for primaquine to be given after chloroquine. In a second study in Colombia, reported in 2010, two primaquine regimens with the same total dose were compared in children aged 10–17 years: 0.50 mg/kg daily for 7 days ($n = 41$) or 1.17 mg/kg daily for 3 days ($n = 38$), both given with 3 days of chloroquine. The 3-day regimen was followed by significantly more recurrences (68.4%) than the 7-day regimen (34.2%) (*150*; see Annex 2).

In a study reported in 2010, Thai men were given primaquine at either 30 mg ($n = 43$) or 60 mg ($n = 42$) daily for 7 days, with 28 days' follow-up. The extrapolated dose–response relation suggested that the 30-mg regimen provided 72% of maximum relapse prevention, while that with the 60-mg regimen was 92% (*151*). In another study in 2010 on the effectiveness of directly observed therapy with chloroquine–primaquine (15 mg daily for 14 days) with a 90-day follow-up, the recurrence rates on days 28, 60 and 90 were significantly lower with directly observed therapy (0, 3.18 and 3.37) than with self-administered therapy (3.35, 6.39 and 13.49) (*152*). A second

study in Thailand, published in 2011, also showed that directly observed therapy results in better adherence and prevention of vivax malaria recurrence during a 90-day follow-up, with recurrences in 5 of 46 patients on self-administered therapy (days 21–87) and in none of 46 patients on directly observed therapy (*153*). All the patients with recurrences were children aged 2–15 years. These findings underline the importance of adherence and indicate why the effectiveness of primaquine is often poor.

When the results of these studies are taken together, there is little evidence that the once widely recommended 5-day primaquine regimen is as effective as "standard" 14-day treatment. Whether shorter higher-dose regimens will be as effective or more effective remains to be determined.

2.2.3 Total dose versus length of regimen

Two studies reported in the USA in 1977 demonstrated that the curative effect of primaquine against relapsing malaria depends on the total dose administered rather than the duration of treatment. In the first study, rhesus monkeys were infected with sporozoites of the M and B strains of *P. cynomolgi*. At the same total dose, a 7-day course of primaquine (with chloroquine as the accompanying blood schizonticidal) was at least as effective as a 14-day regimen in radical cure of infections with the B strain, as were single doses on days 3 and 7 (*154*). The doses administered per day (for either 7 or 14 days) were 2.625, 5.25, 10.1 and 21 mg/kg for the M strain and 1.31, 1.75, 2.625, 3.5, 5.25, 7 and 10.5 mg/kg for the B strain. A similar dependence on dose rather than duration was demonstrated (with quinine as asexual stage treatment) for 7-day and 14-day regimens of other 8-aminoquinolines, including pamaquine and pentaquine, which resulted in radical cure of infections with the M strain. In the second report, 11 white volunteers infected with *P. vivax* Chesson strain by mosquito bites were cured by a regimen consisting of 7 days of 60 mg daily of primaquine, instead of the equivalent total dose given over 14 days at 30 mg/day after 1500 mg chloroquine over 3 days, showing no relapses after follow-up periods of 33–427 days (*155*).

2.3 Efficacy as a gametocytocidal drug for *P. falciparum* malaria

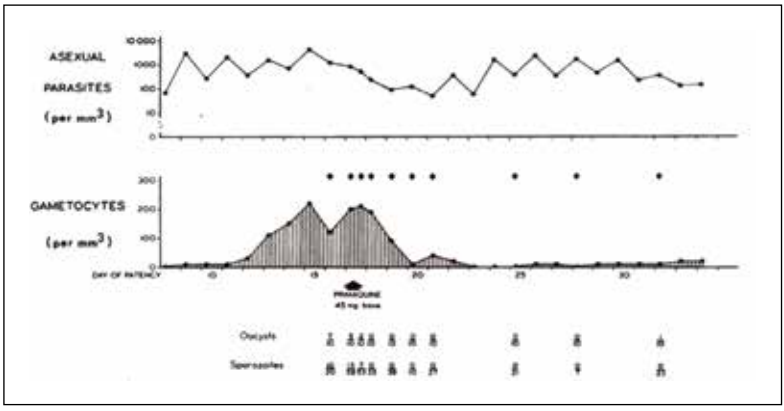
A single dose of primaquine rapidly sterilizes gametocytes of all malaria species that infect humans. The optimum dose of primaquine for use as a gametocytocide in falciparum malaria is unknown, but pooled data from several studies of mosquito infectivity suggest that adult doses as low as 7.5 mg/kg in combination with an artemisinin derivative have maximum transmission-blocking effects. Artemisinin derivatives augment the transmission-blocking activity of primaquine. The timing of a single primaquine dose (on day 0 or day 2 of a 3-day ACT regimen) does not appear to affect its efficacy as a gametocytocide.

The reported efficacy of primaquine as a gametocytocide for *P. falciparum* depends on dose, timing and the accompanying antimalarial agents given to the same patients. Generally, primaquine has proved to be effective in

eliminating gametocyte carriage within 5–7 days after administration and in preventing transmission to mosquitoes when given as a 45-mg single adult dose (0.75 mg/kg). In interpreting the results of these studies, it should be recalled that primaquine has no useful asexual-stage activity against *P. falciparum* and therefore does not stop the input of young gametocytes from asexual stages. Thus, the asexual-stage activity of curative drugs also affects their overall transmission-blocking effect. The gametocytocidal effect of primaquine is greater when given with ACT, as artemisinin derivatives themselves have gametocytocidal activity. Mathematical modelling indicates that mass administration of ACT is an effective means of reducing transmission, and some models suggest that the addition of a single dose of primaquine will accelerate a reduction in transmission as part of an elimination strategy (*156*, *157*). The additional benefit of primaquine in mass drug administration is the rapidity with which infections are sterilized. Most of the effect of mass drug administration is derived from treatment of asymptomatic infections and from mass prophylaxis provided by the slowly eliminated antimalarial drug, which prevents reinfection. Increased use of primaquine could counter the transmission advantage of artemisinin-resistant *P. falciparum* and thus play a critical role in containing this major threat.

The gametocytocidal activity of primaquine has been investigated in penitentiary volunteers and in patients with neurosyphilis (*158–164*) and in an endemic setting in Liberia (*165*, *166*). The majority of patients received relatively large doses (>0.5 mg/kg). Rieckmann and colleagues made detailed assessments of adult doses of 15, 30 and 45 mg in a University of Chicago–Army Medical research project in the Stateville penitentiary (*161*, *162*). Those were the only early studies in which transmission-blocking activity was truly measured. Oocysts in mosquito mid-guts and sporozoites in salivary glands were counted, and the infectivity of mosquitoes that had bitten a volunteer to other volunteers was evaluated (Figure 3). Mosquitoes that had fed on an infected volunteer before primaquine administration had numerous oocysts (average, 46.2 per mosquito), which developed into normal sporozoites and successfully infected a naive volunteer. The number of oocytes was markedly reduced 12 h after administration of primaquine. When oocysts and sporozoites were present, they were morphologically abnormal, and the mosquitoes failed to infect another naive volunteer. Batches that fed at 24 h and on days 3, 4 and 8 were also not infectious. By day 11, oocysts and sporozoites were again detected in small numbers. The slow, delayed rise in gametocytaemia that followed the primaquine-induced decline suggested gametocytocidal action with relatively broad sexual stage specificity. Recent studies to assess the dose–response relation for transmission-blocking activity were conducted in Cambodia and Viet Nam (*167;* Figure 4). These indicate that, when combined with ACTs, a dose of 0.25 mg base/kg provides maximum transmission-blocking activity (see below).

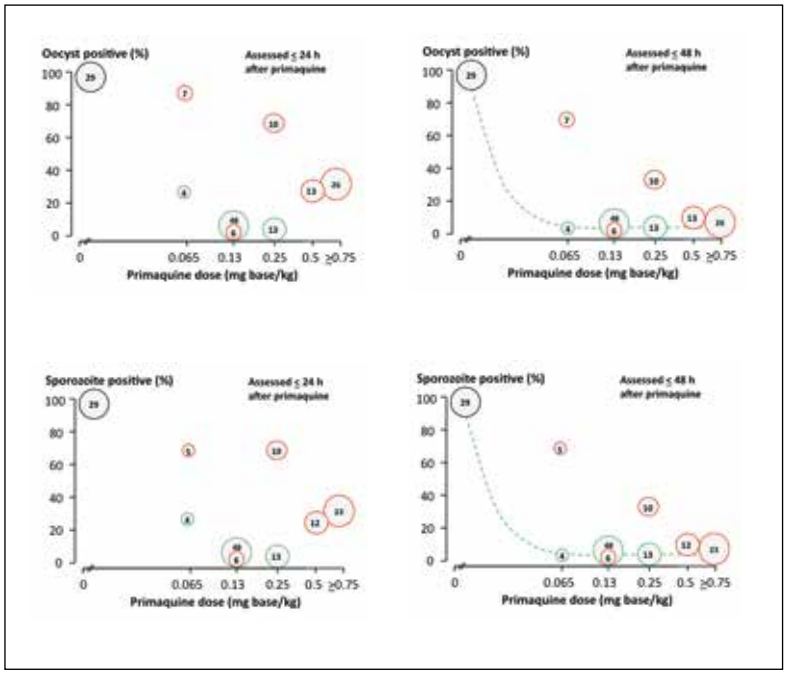
Figure 3. Primaquine, although not effective as a blood schizontocide, exerted a marked gametocytocidal and sporontocidal effect against a *P. falciparum* strain.



Studies in a volunteer of the effects of a single dose of 45 mg primaquine on mature gametocytes of the chloroquine-resistant *P. falciparum* Malayan (Camp.) strain. Primaquine was administered on day 17 of patency of the second infection, 5–6 days after the onset of a wave of gametocytaemia. *A. stephensi* mosquitoes were allowed to bite the volunteer 24 h before, immediately before, 6 and 12 h after and 1, 2, 3, 4, 8, 11 and 14 days after primaquine administration (times indicated by downward-pointing arrows) and were examined for oocysts from day 6 and for sporozoites from day 10. Between days 12 and 14, the mosquitoes (75 per batch) were allowed to feed on uninfected volunteers. Reproduced from reference 161

Studies of the gametocytocidal properties of 8-aminoquinolines have consistently shown that their transmission-blocking effect precedes the reduction in gametocytes. Thus, the number and viability of oocysts that developed in mosquitoes fed on volunteers who had received primaquine were reduced well before a significant reduction in gametocyte density. Mosquitoes that fed during the first 24 h usually (but not always) showed marked reductions in oocyst numbers. In the pooled results of all published studies in which oocysts were quantified 24 h after a single dose of primaquine or pamaquine, no oocysts were seen in 70% (57/80) of batches of mosquitoes that fed on volunteers the day after drug administration. Often, the few oocysts formed were abnormal morphologically, and either no salivary gland infection ensued or the sporozoite numbers and viability were reduced. By 48 h after primaquine administration, nearly all infections had been sterilized. In a few volunteers, however, particularly those receiving low doses, there was little or no immediate effect. Most of the early studies were conducted in patients with chronic gametocytaemia, and no effective asexual stage treatment was given. These early evaluations of pamaquine and primaquine given alone to people with falciparum malaria also indicated that gametocyte density was suppressed for many days after a single dose, despite lack of activity against asexual stages. This suggests a broad specificity of action against sexual stages of the parasite, which would overlap with the effects of other antimalarial drugs on stages 1–3 of *P. falciparum* gametocytes. When no effective

Figure 4. Dose–response relation for primaquine in reducing infectivity to anopheline mosquitoes



Pooled individual data showing doses administered and proportions of fed mosquitoes that were positive. Top left: oocyst formation (proportion of patients who were still infectious to mosquitoes) from blood sampled 24 h after primaquine dosing. Top right: oocyst formation from blood sampled 48 h after primaquine dosing. Bottom left: sporozoite formation assessed from blood sampled 24 h after dosing. Bottom right: sporozoite formation assessed from blood sampled 48 h after dosing. The results after primaquine given with an artemisinin derivative are shown in green, and those after administration with a non-artemisinin derivative or no antimalarial agent are shown in red. The black circle indicates the control groups in the same studies, which did not receive primaquine. The size of the circle is proportional to the number of people in each group (shown within). A dose of 0.25 mg/kg corresponds to an adult dose of 15 mg, and 0.75 mg/kg to an adult dose of 45 mg. Reproduced from reference 167

treatment against the asexual stages was given, the numbers and viability of gametocyte increased again, indicating the importance of effective treatment in limiting transmission (*161, 162*).

Gametocyte clearance rates tend to underestimate immediate transmission-blocking effects. Most studies conducted in vivo show that pamaquine or primaquine markedly accelerates gametocyte clearance (*24, 76, 168–171*), although one study gave disappointing results (*172*). Infectivity can also be assessed ex vivo by feeding gametocytes to anopheline mosquitoes (*72, 173*), but this method of assessment is compromised for 8-aminoquinolines because they are biotransformed in vivo to active metabolites, which mediate most of their gametocytocidal and hypnozoitocidal effects.

The studies of infectivity in vivo indicate that gametocytes are damaged and killed rapidly, within hours of exposure to peak concentrations of the presumed active metabolites of primaquine, and that there is a lag between damage (which prevents infectivity) and clearance. Thus, although drug-induced acceleration of gametocyte clearance results in underestimates of transmission-blocking activity, gametocyte clearance rates are still a valuable pharmacodynamic end-point in studies of dose–response relations. Such studies require accurate, frequent gametocyte counts, either directly by microscopy or automated cell counting or indirectly by quantitative PCR for gametocyte-specific mRNA. Measuring gametocyte clearance is easier than conducting mosquito feeding studies, and it can therefore be done on a larger scale, in different locations and in patients of a wider age range.

The assessments of transmission-blocking in *P. falciparum* infections have been conducted in patients with relatively high gametocyte densities, representing the upper tail of the distribution of gametocyte carriage. Effects in this group will result in underestimates of the probable transmission-blocking effect of a single gametocytocidal dose of primaquine given to all patients (most of whom do not have gametocytaemia detectable by microscopy) for the following reasons. Gametocyte density, like asexual parasite density, tends to have a logarithmic distribution, so the majority of patients with transmissible levels of gametocytaemia have low densities, close to or just below the limit of detection. In contrast to mosquitoes fed artificially in the laboratory, wild-caught anopheline vectors have low oocyst numbers, with a typical median of two oocysts per gut. As with all anti-infective drugs, gametocytocidal killing is likely to be fractional. A drug-induced 99% reduction in gametocyte viability for counts of less than 100/μL will usually result in no transmission, whereas the same effect for counts over this value may not prevent transmission. One successful oocyst is all that is necessary to confer infectivity on that anopheline mosquito. Studying patients with high gametocyte densities and using the binary outcome of the presence or absence of sporozoites in mosquito salivary glands therefore results in underestimates of the probable transmission-blocking benefit at population level. The efficacy of an asexual-stage drug is of paramount importance, as continued input of gametocytes at transmissible densities beyond the action of the single-dose gametocytocide clearly negates its benefit. Thus, ACT is the treatment of choice for asexual stages, in order to maximize the gametocytocidal effect. The greatest benefit of adding a single dose of primaquine is probably achieved in patients with low gametocyte densities. Early treatment therefore has the twin benefits of reducing the proportion of patients who are infectious and maximizing the success of gametocytocidal treatment.

In a study in Colombia in 2012, patients were given amodiaquine–sulfadoxine–pyrimethamine (SP) or mefloquine–artesunate with or without a single dose of 0.75 mg/kg primaquine on day 2 as a *P. falciparum* gametocytocide. None of the treatments resulted in clearance of gametocytaemia by day 8, although a marked decline was seen on days 4 and 8 when primaquine was added; without primaquine, gametocytaemia increased steadily until

day 8. Treatment with mefloquine–artesunate (ACT) resulted in reduced gametocytaemia on days 4 and 8, and the addition of primaquine reduced it even further (*174*). Another report from Colombia, in 2008, described the therapeutic efficacy of amodiaquine–SP, artesunate–SP and amodiaquine–artesunate given without primaquine. Amodiaquine–SP was less effective than the artesunate treatments in reducing gametocyte carriage (*175*). None of the treatments had completely eliminated gametocytaemia by day 7; similar results were obtained with the three regimens at day 21, including four therapeutic failures (1.7%).

In a study in the Federation of Malaya (now part of Malaysia) in 1953, 114 children aged 6 months to 6 years received one to three treatments of 100–200 mg amodiaquine with 15–30 mg primaquine monthly at the beginning of the malaria season. At the peak of the malaria season, one *P. falciparum* gametocyte was seen in the thick blood film of a child who had received only one dose of primaquine; 19.3% had *P. vivax* parasitaemia. On the same day in the same area, 19.7% of 31 untreated children had *P. falciparum* and 41.9% had *P. vivax* parasitaemia (*176*). In the same study, only one *P. falciparum* gametocyte carrier and six *P. vivax* carriers were found among 99 children after one to three doses, whereas four of 17 who did not receive primaquine had *P. falciparum* infections and none had *P. vivax*. In Thailand in 1980, 10 naturally infected *P. falciparum* malaria patients received a single dose of 45 mg primaquine on day 0, and 10 received no treatment; their blood was fed to mosquitoes on days 0, 1, 3 and 7. Primaquine was effective in eliminating gametocytes, blood counts being markedly decreased at 3.5 days in the primaquine group and 11.4 days in the controls. No sporozoite formation was observed in the mosquitoes fed on blood from treated patients, although a few oocysts were found, whereas mosquitoes fed on the control group had sporozoites (*168*).

In 1961, mass drug administration was reported with combined amodiaquine and primaquine (30 mg weekly, fortnightly or monthly) in three malaria-holoendemic locations in Tanganyika (now part of the United Republic of Tanzania), each of which had a population of 5000–7000, consisting mainly of highly immune Bantu adults. Weekly and fortnightly treatments were effective, the only infected people being those who had missed some doses; the sporozoite rate dropped from 9% to 1%. With the monthly regimen, parasitaemia recurred before the next treatment dose. Although the sporozoite rate was reduced by half, the gametocyte rate increased (*177*; see Annex 2). A report in 1961 of a study in Liberia described treatment of *P. falciparum* gametocytaemia and subsequent sporogony in naturally acquired infections with a single dose of 40 mg primaquine and 50 mg pyrimethamine, which is also known to have sporontocidal activity; 19 of the 22 people in this study were children (average age, 3.5 years) who received a minimum dose of 10 mg primaquine with 12.5 mg pyrimethamine. The drug combination was effective in preventing transmission to mosquitoes fed 1–3 days after treatment. The average clearance time for gametocytaemia in the human host was 5 days (range, 3–11) (*178*; see Annex 2).

In a study reported in 1968, three volunteers were infected with the chloroquine-resistant *P. falciparum* Malayan (Camp.) strain. A single dose of 45 mg primaquine given to two volunteers 5–6 days after the onset of the first wave of gametocytaemia had strong gametocytocidal activity. The third volunteer was treated with sulfadiazine (500 mg every 6 h for 5 days) and pyrimethamine (50 mg daily for 3 days) and showed no reduction in gametocytaemia. Mosquitoes were allowed to bite one of the primaquine-treated volunteers and later to bite healthy volunteers; mosquitoes that bit 12, 24 or 48 h after primaquine treatment were not infective, whereas those that bit before primaquine administration were highly infective (*161*). A similar study reported a year later showed that the strong gametocytocidal effect of primaquine extended to a chloroquine-sensitive *P. falciparum* strain (Uganda I) (*162*). In this study, 16 prison inmate volunteers were mosquito-infected with falciparum malaria and treated with 15, 30 or 45 mg primaquine as a single dose or at 1- or 2-week intervals. All doses (but 30 and 45 mg more consistently) had both gametocytocidal and sporontocidal effects against both *P. falciparum* strains tested (Uganda I and Malayan (Camp.)). The 45-mg dose was most consistently effective, followed by the 30-mg dose; the effects of these two doses were complete within 3 days.

In a study in Indonesia reported in 1989, primaquine was given to 489 patients with falciparum malaria as a 45-mg single dose in addition to SP. Gametocytaemia was still present at week 5 in 11.5% of patients given SP alone; in 87 patients who were followed up, there was no change between day 0 and week 2, followed by a slow decline. In patients given primaquine–SP, of whom 131 were followed up, the gametocyte rate decreased from 77% on day 0 to 30% on day 7 and was 7.1% at week 3 (*179*). A retrospective study conducted in 2004 in Mumbai, India, involved administration of a single 45-mg dose of primaquine to gametocytaemic patients with uncomplicated falciparum malaria the day after treatment with 10 mg/kg chloroquine on days 1 and 2 and 5 mg/kg on day 3, with or without a single dose of 1500 mg sulfadoxine with 75 mg pyrimethamine. Weekly follow-up of gametocytaemia and gametocyte viability (assessed by exflagellation) over 29 days showed gametocytaemia at all follow-up times in 13/22 (59.1%) patients who did not receive primaquine and only 1/24 (4.2%) patients given primaquine by day 29 (*180*). In a trial in 53 children aged 3–15 with fever and *P. falciparum* infection in an area of high malaria endemicity in the United Republic of Tanzania reported in 2007, a 45-mg single dose of primaquine given on day 3 of treatment significantly increased *P. falciparum* gametocyte clearance. Only 3.9% of children given artesunate–primaquine–SP and 62.7% of those given artesunate–SP were gametocytaemic on day 14 (*75*; see Annex 2). As an increase in gametocyte carriage is commonly observed after treatment with SP, any difference between primaquine- and non-primaquine-containing regimens will tend to be magnified when SP is used for treatment.

A short report from Thailand in 1980 on patients with uncomplicated falciparum malaria presented the results of three gametocytocidal primaquine regimens: 15 mg daily for 5 days, 30 mg single dose or 45 mg

single dose, all administered with a single dose of SP (1 g sulfadoxine, 50 mg pyrimethamine) on day 0, followed-up with blood examinations on days 0, 7, 14, 21 and 28. Gametocytaemia had disappeared by day 21 with all three regimens (*172*). In a study in India reported in 2006, the gametocytocidal activity of primaquine was compared with that of its analogue bulaquine, which was licensed for use in India for radical cure of vivax malaria at 25 mg/day for 5 days but not as a gametocytocidal agent for *P. falciparum*. Patients with gametocyte counts > 55/μL within 72 h of diagnosis, regardless of asexual parasite counts, were treated with quinine and doxycycline for 7 days, with primaquine or bulaquine given on day 4. Of the patients who received 45 mg single-dose primaquine, 20/31 (65%) were gametocytaemic on day 8; by days 15, 22 and 29, all patients in both treatment groups had cleared gametocytaemia (*85*; see Annex 2).

In a study in Thailand reported in 2008, the efficacy, safety and tolerability of 3 days of Artequick®, a fixed-dosed combination of 3.2 mg/kg artemisinin, 16 mg/kg piperaquine and 0.16 mg/kg primaquine, given once a day orally for 3 days, were compared with those of artesunate–mefloquine for 3 days. The regimens were equally effective and well tolerated: parasite clearance in each treatment group was equally rapid ($p = 0.098$), with medians of 35 h (range, 24–82 h) and 33.0 h (22–84 h), respectively; fever clearance times also did not differ significantly ($p = 0.580$), and parasites were cleared from peripheral blood smears by 84 h in all patients. The cure rates at the 28-day follow-up were 98.5% with Artequick® and 100% with artesunate-mefloquine ($p = 0.496$) (*181*; see Annex 2). A report on mass administration of artemisinin–piperaquine followed by primaquine (9 mg adult dose every 10 days for 6 months) in two locations in Cambodia in 2010 to 6040 individuals described the treatment as effective. The parasite rate in the first location (17 villages) was dramatically reduced, from 52.3% to 2.6%, after 3 years, and the falciparum rates in children decreased from 37.0% to 1.4%, reaching 0% in 8 of the 17 villages; in the second location, the falciparum rate in children was reduced from 20.8% to 0% within 6 months (*182*; see Annex 2).

In 2009–2010, 1372 eligible patients were recruited at 21 sites in India for a study of the effectiveness of primaquine as a falciparum gametocytocide. Patients received 4 mg/kg artesunate for 3 days plus 25 sulfadoxine and 1.25 mg pyrimethamine with ($n = 541$) or without ($n = 794$) primaquine on the third day of treatment (0.75 mg/kg, adult dose of 45 mg). The pretreatment prevalence of gametocytaemia was similar in the two groups (18% ($n = 141$) without and 20% ($n = 107$) with primaquine). The prevalence of gametocytaemia in the two arms was similar on days 0, 1 and 2, and decreased from day 3 in the group receiving primaquine. In 248 patients with pretreatment gametocytaemia, the median time to gametocyte clearance was 7 days with primaquine and 14 days without primaquine ($p < 0.001$). Primaquine–artesunate–SP cleared gametocytes faster than artesunate–SP alone, the persistence of gametocytaemia at the end of follow-up being 3% and 23%, respectively. Primaquine accelerated the rate of gametocyte clearance by a factor of 2.2 (95% CI, 1.2–4.2) over 28 days. After adjustment for region, addition of

primaquine increased the rate of gametocyte clearance by 1.9 times (95% CI, 1.1–3.3) over that with artesunate–SP alone over 28 days (*183*).

The relation between the dose of 8-aminoquinoline and the response in terms of transmission-blocking activity was re-examined recently. In some studies, pamaquine blocked transmission at doses as low as 10 mg. The effectiveness of a small dose of primaquine (3 mg/day) was studied in patients with neurosyphilis who were also receiving malaria therapy (*160*). Two patients with falciparum malaria who were infectious before starting the daily treatment and were exposed daily to mosquito feeding no longer had oocysts by days 3 and 5. Two others (one of whom had a maximum gametocyte density of 1862/μL) remained uninfectious for 1 month. In all the published studies of mosquito infectivity, only 10 volunteers received a dose of primaquine <0.25 mg/kg, of whom eight were still infectious 24 h later (as assessed by the presence of oocysts), whereas 12 of 37 volunteers who received a larger dose remained infectious. Although this finding suggests that primaquine acts less rapidly at doses <0.25 mg/kg, three of the eight volunteers given the low dose were uninfectious the following day; therefore, the difference in maximum effect may be small.

Although the activities of pamaquine and primaquine were not compared directly, this finding suggests that doses much lower than those currently recommended might be effective. We pooled all the available data, including unpublished studies conducted in China (kindly provided by Li Guo Qaio, Chen Pei Quan and Gao Qi), on 158 individual gametocytaemic patients in studies conducted in various locations with different vectors and different drugs, spanning approximately 80 years. Infectivity was assessed from both oocyst and sporozoite production 24 and 48 h after drug treatment. Of the 158 patients, 31 received pamaquine (before 1950) and 127 received primaquine, 69 of these with an artemisinin derivative. The analysis clearly shows that both pamaquine and primaquine rapidly and potently reduce the infectivity of *P. falciparum*. Primaquine increases the rate of gametocyte clearance, but there is a lag before clearance accelerates. As in previous studies, the effect on transmissibility, assessed from mosquito oocyst numbers and consequent sporozoite numbers and viability, was more rapid than the effect on gametocyte density, suggesting that most or all the gametocytes counted in blood films in the days after primaquine administration are dying or dead. The gametocyte density is generally much lower under natural conditions than in volunteer studies, in which the density is usually below the limit of microscopic detection. Infected wild anopheline mosquito vectors (when examined) have correspondingly less intense infections (median, two oocysts per gut). Thus, artificial infection studies tend to underestimate transmission-blocking drug effects at population level. Nevertheless, in the individual assessments of transmission-blocking activity, nearly all the adults (102/108; 94.4%) who received primaquine at a dose ≥ 0.13 mg/kg (adult dose, ≥ 7.5 mg) had a sterilized infection within 48 h of taking the drug. Furthermore, artemisinin derivatives augmented the transmission-blocking effect significantly. The

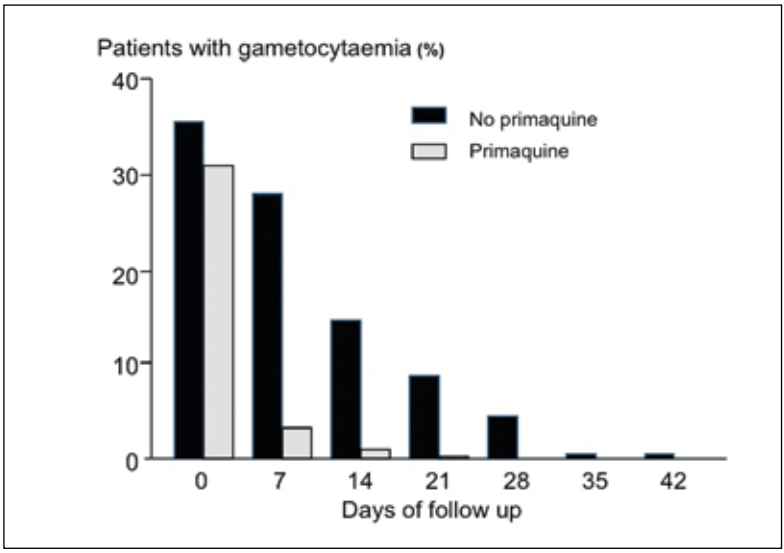
dose–response assessments suggest that the dose of primaquine that sterilizes 90% of *P. falciparum* gametocytes (ED90)) is approximately 0.06 mg/kg when it is given with an artemisinin derivative and 0.09 mg/kg when it is given alone (Figure 4).

When given with chloroquine or ACT (each administered on 3 consecutive days), primaquine may be added on day 0, 1 or 2 of the antimalarial drug. There is no conclusive evidence that the timing of primaquine administration over this 3-day period affects its gametocytocidal activity in falciparum malaria infections, although later administration (i.e. on day 1 or 2) provides an extra one or two days of potential malaria transmission.

In a study in Sri Lanka reported in 2002, primaquine was given to 30 patients as a single 45-mg gametocytocidal dose on day 0 with the standard 3-day ACT regimen. Gametocytaemia had cleared in three of five patients by day 7 and in all patients by day 14; there was no recrudescence of parasitaemia during the 28-day follow-up (*184*; see Annex 2). In a study in central Java, Indonesia, in 2006, 45 mg of primaquine were given on day 0 (n = 28) or day 2 (n = 28) as a single-dose gametocytocide added to a chloroquine–SP regimen for uncomplicated falciparum malaria. The gametocyte rates declined steadily after primaquine, to 0% by day 11, and more slowly with chloroquine–SP. A single dose of primaquine greatly accelerated the gametocyte clearance rate, and administering the dose on day 2 rather than day 0 accelerated the clearance time (0% versus 7% on day 7); however, this difference was not statistically significant (*185*; see Annex 2). In a comparison of the effectiveness of four ACT regimens administered to 397 adults and children in Myanmar with acute uncomplicated falciparum malaria or mixed infection and reported in 2010, a single 45-mg adult dose of primaquine was given on day 0 after an evening meal as a gametocytocide. The gametocyte carriage rates with all ACT regimens fell by a factor of 11.9 (95% CI, 7.4–20.5; Figure 5). New gametocytaemia on day 7 was also reduced by primaquine (1/272 versus 10/268) (*171*; see Annex 2). The advantage of giving primaquine on day 0 is that the treatment can be supervised.

Two recent reports from Colombia provide evidence that addition of primaquine to a 3-day artesunate–mefloquine regimen accelerates gametocyte clearance in patients presenting with falciparum infection. The first report, in 2009 (*186*; see Annex 2), showed that the efficacy of both treatments (artesunate–mefloquine with (n = 25) and without (n = 25) a single dose of 45 mg primaquine at the end of the third day) was 100% (95% CI, 86.3–100), and fever and parasitaemia were completely eliminated by day 3 in all patients. By day 3, gametocytes had disappeared in 92% of patients who received primaquine (95% CI, 74–99) and 78.3% (59–93) of those who did not. The second report, in 2010, showed that gametocyte clearance was complete in symptomatic patients treated with artesunate–mefloquine by day 21 (n = 25) but was more rapid after the addition of 45 mg primaquine on the first day of treatment (n = 25), with elimination from all patients 1 week earlier (*141*, *187*).

Figure 5. A single gametocytocidal 45-mg dose of primaquine reduces gametocyte carriage when given with ACT



Effects of a single dose of primaquine on *P. falciparum* gametocyte carriage after ACT in Myanmar. Pooled results for four ACT regimens (artesunate–amodiaquine, artemether–lumefantrine, artesunate–mefloquine, dihydroartemisinin–piperaquine).No gametocytaemia was detected after day 42. Reproduced with permission from reference 171

Methylene blue is being evaluated as an alternative transmission-blocking drug. This compound has been in medical use for over a century and should be less toxic in G6PD-deficient individuals. The avermectins may also reduce malaria transmission by reducing mosquito survival, and a number of studies have been conducted to evaluate the ef fect of endectocides on anopheline vectors. To date, however, mass administration of these drugs has been used to control onchocerciasis and lymphatic filariasis, but not malaria (*188*).

3. Tolerability and safety of primaquine

This section covers adverse events that were associated with primaquine administration and documented in studies of its safety (see Annex 2) and in published and unpublished case reports (the latter from the WHO historical archives). Non-life-threatening adverse events reported in people receiving primaquine were gastric distress and methaemoglobinaemia (with or without cyanosis), whereas the more serious adverse events were acute haemolytic anaemia and haemoglobinuria, which are potentially life-threatening conditions in G6PD-deficient individuals after primaquine administration, sometimes requiring discontinuation of treatment and blood transfusion; renal failure was reported occasionally, dialysis being required in some cases. The prevalence of G6PD deficiency, diagnostic tests for this condition and G6PD variants are also discussed. Tables show the results of analyses of all the severe adverse events reported, in studies of mass drug administration, in all studies of smaller cohorts and by G6PD category (normal versus deficient) to estimate the risk for severe adverse events in these populations.

3.1 Tolerability and minor adverse effects

Gastrointestinal discomfort is the main non-life-threatening adverse event caused by primaquine, especially when it is not taken with food or at daily doses ≥ 30 mg. Methaemoglobinaemia with or without cyanosisis is not uncommon, usually not requiring discontinuation of treatment and with values returning to normal soon after completion of primaquine treatment.

Minor adverse events reported by individuals receiving primaquine as an antimalarial drug include headache, abdominal pain, nausea and diarrhoea. In a study in Colombia in 2009 in which primaquine was administered to 25 patients as a gametocytocide at a single dose of 45 mg on the third day after treatment with artesunate–mefloquine, common adverse events were vertigo (42%) and motor imbalance, such as unstable walking (36%), although these are more likely to have been due to mefloquine (*154*).

Ingestion of primaquine sometimes results in gastrointestinal discomfort, the severity and frequency being related to the amount received (*38, 189*). At an adult daily dose of 30 mg, mild-to-moderate abdominal cramps can occur, especially when primaquine is taken on an empty stomach. As primaquine is often given with food and/or at a 15-mg daily dose, moderate-to-severe abdominal pain is rarely observed. Table 2 lists the studies in which severe gastrointestinal adverse events were reported after primaquine, in some instances requiring discontinuation of treatment. Although doses up to 240 mg daily can cause severe abdominal cramps, nausea and vomiting, such amounts are not currently recommended. Toxicity was reported mainly in prison inmate volunteers in studies in the USA in the 1950s. In the first study, in 1950, primaquine was given for vivax malaria to volunteers. A 240-mg daily dose caused severe

Table 2. Reports of abdominal discomfort due to primaquine

Reference and population	Primaquine dose	Abdominal adverse event
Edgcomb et al., 1950 (190), USA Intravenously vivax-infected prison volunteers	Doses ≤240 mg daily (each divided into six daily doses)	240 mg: severe abdominal cramps and 9–10% methaemoglobin, but no irreversible damage observed 120 mg: methaemoglobinaemia observed when given with quinine (mean, 10%) was half that without quinine (20%)
Clayman et al., 1952 (38), USA Uninfected or latently infected prison volunteers	Various doses daily or weekly	15, 22.5 and 30 mg: mild-to-moderate abdominal cramps 60 mg: mild-to-severe abdominal cramps, nausea, anorexia, vomiting, mild epigastric distress, cyanosis due to methaemoglobinaemia (with primaquine alone, not with chloroquine) 120 mg: abdominal cramps and methaemoglobinaemia with cyanosis in all volunteers (only two of six given primaquine–quinine appeared cyanotic)
Soto et al., 1998 (108), Colombia Prophylaxis for volunteer soldiers	30 mg daily for 16 weeks taken with breakfast	Three primaquine recipients (2.5%) had epigastric pain, abdominal pain or vomiting severe enough to withdraw; six others (5.0%) had mild or moderate gastrointestinal symptoms. Only one placebo recipient (2.0%) had mild gastrointestinal symptoms.
Soto et al., 1999 (109), Colombia Prophylaxis for volunteer soldiers	30 mg daily for 17 weeks taken with breakfast and chloroquine once weekly	Two (2%) chloroquine–primaquine recipients (no placebo recipients) had to terminate prophylaxis prematurely because of gastrointestinal side-effects

More details are given in Annex 1.

abdominal cramps and 9–10% methaemoglobin but no irreversible damage; interestingly, when primaquine was given at 120 mg with quinine, the methaemoglobinaemia observed (mean, 10%) was half that without quinine (20%) (*190*; see Annex 2). In a study reported in 1952 on the effect of primaquine in volunteers who were either not infected or were treated in the latent interval between clinical malaria attacks, low doses (15, 22.5 and 30 mg daily) resulted in only mild-to-moderate abdominal cramps; 60 mg caused mild-to-severe abdominal cramps, nausea, anorexia, vomiting, mild epigastric distress, cyanosis due to methaemoglobinaemia (with primaquine alone and not with primaquine–chloroquine); and 120 mg caused abdominal cramps and methaemoglobinaemia with cyanosis (while only two of six men given primaquine–quinine appeared cyanotic). This study also showed that weekly and twice-weekly doses of 30 mg primaquine for up to 52 weeks did not cause dangerous toxicity when given with 300 mg chloroquine (*38*; see Annex 2). In studies of prophylactic primaquine use in Indonesia, a 30 mg daily dose taken with food or after a morning meal for 20–50 weeks was safer and better tolerated than a placebo (*106*; see Annex 2) or atovaquone–proguanil (*110*; see Annex 2). Primaquine appeared to be well tolerated by 1–5-year-old children, even when given without food (*191*; see Annex 2). In two studies of prophylactic primaquine by Soto and colleagues in Colombia in 1998 and 1999, however, three (2.5%) participants given 30 mg daily for 16 or 17 weeks with breakfast and two (2%) patients given chloroquine–primaquine had to withdraw because of epigastric pain, abdominal pain or vomiting. There were no withdrawals in the placebo groups (*108, 109*; see Annex 2).

In a study in the USA in 1953 to compare the toxicity of various 8-aminoquinolines given with quinine, there was no significant haemolysis. Primaquine given at 10 or 20 mg with quinine to prison inmate volunteers infected by mosquitoes with *P. vivax* Chesson strain was the best tolerated, especially in terms of abdominal discomfort; however, all the drugs caused varying degrees of methaemoglobinaemia (highest with pamaquine and lowest with primaquine), and cyanosis occurred in 9/34 receiving pamaquine and in 2/34 receiving primaquine at 20 mg (*39*; see Annex 2). Another report from the USA in 1953 described an evaluation of the toxicity and curative effects of primaquine for Korean vivax malaria. Mild abdominal pain and cyanosis due to methaemoglobinaemia occurred in ~25% of white patients given 30 mg primaquine; 20% were cyanotic, with methaemoglobin levels of 8–14% on the last day, but the cyanosis cleared promptly after treatment was completed (*127*; see Annex 2). In a report from Indonesia in 1995 on a trial of prophylaxis of falciparum and vivax malaria with primaquine at 30 mg daily adult dose for 1 year, the mean increase in methaemoglobin was 5.8%, while that in individuals given placebo was 1.2% and that for people given chloroquine was 0.8%. No clinical signs of methaemoglobinaemia were seen, and the methaemoglobin levels had decreased to 2.4% by 7 days after the last primaquine dose (*107*).

In a report of a study in Thailand in 2001, 15 mg primaquine were given daily for 14 days to both G6PD-normal and G6PD-deficient patients with vivax malaria. A 100% cure rate was reported in both groups during a short 28-day follow-up, and parasite clearance time was comparable (59.4 ± 17.5 and 59.8 ± 15.0 h, respectively; $p = 0.91$). The fever clearance time was, however, significantly longer in the G6PD-deficient group (45.2 ± 35.2 h versus 28.0 ± 22.2 h for G6PD-normal, $p < 0.01$) (*192*; see Annex 2). In a study in Myanmar in 2010, a single gametocytocidal dose of 45 mg primaquine was given on day 0 after a meal with one of four ACT regimens for acute uncomplicated falciparum malaria. Abdominal pain was reported more often with primaquine than without it. The mean increase in Hb was slightly reduced by primaquine (0.75 g/dL versus 1.04 g/dL; $p = 0{\cdot}036$; mean difference, 0.295 g/dL; 95% CI, 0.199–0.570) (*171*).

3.2 Glucose-6-phosphate dehydrogenase deficiency and haemolysis

G6PD deficiency affects more than 400 million people worldwide and is particularly common in malaria-endemic areas. It is an X-linked disorder that affects erythrocytes. Male hemizygotes and female homozygotes are at greatest risk for acute haemolytic anaemia after exposure to oxidative stress imposed by infections and certain chemicals and drugs, including primaquine.

The main concern with regard to the use of primaquine in malaria-endemic regions is acute haemolytic anaemia in G6PD-deficient individuals. This is the commonest RBC polymorphism and is frequent in tropical countries because it is associated with some degree of protection against malaria (*193*). G6PD deficiency was first identified after haemolysis was observed

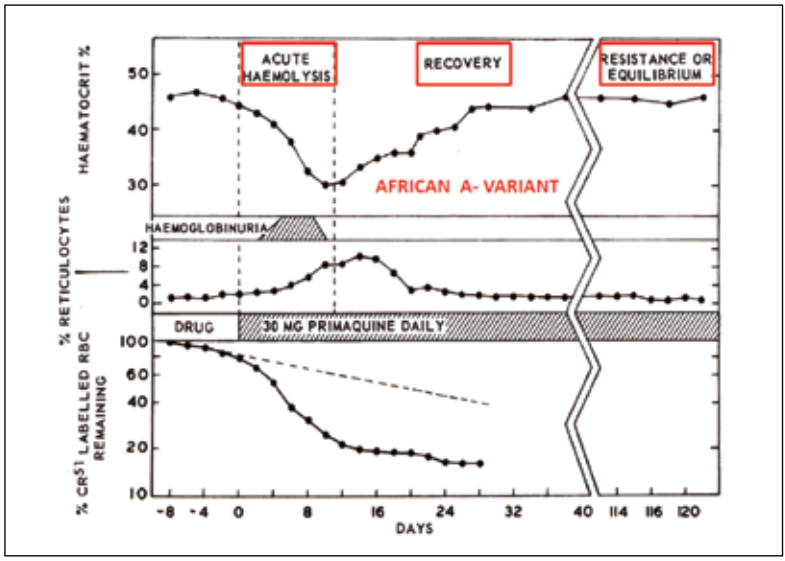
in malaria patients who had received primaquine (*194*). It is the most prevalent enzyme deficiency worldwide, with an estimated 400 million people affected, most of whom live in malaria-endemic regions. G6PD, the first enzyme in the pentose phosphate pathway, is involved in the production of NADPH, which is required for a supply of reduced glutathione in RBCs. It is also essential for the function of catalase. Both provide essential cellular protection from oxidative stress. The activity of G6PD decreases exponentially as red cells age, with a half-life of normal enzyme of about 50 days. Point mutations in the gene encoding G6PD result in a change in individual amino acids, which decreases the stability of G6PD, resulting in enzyme deficiency. G6PD deficiency is reviewed in detail in references *195–197*.

3.2.1 Pathogenesis of haemolysis in glucose-6-phosphate dehydrogenase-deficient patients after primaquine administration

Haemolysis is inevitable if primaquine is given to a G6PD-deficient individual, but many factors determine its severity. Studies by Alving and colleagues in the USA 50 years ago resulted in detailed characterization of the course of haemolysis in healthy African Americans (presumably with G6PDd variant A–) after receiving a 30-mg/day primaquine regimen. The Hb concentration began to fall on day 2 of drug administration. The haemolysis that occurred with continuous administration was arbitrarily divided into three phases: an acute phase (7–12 days), with the lowest Hb and dark urine; a recovery phase (days 10–40), with reticulocytosis and Hb returning to normal by week 4–5; and an equilibrium phase, which continued as long as primaquine was given, with only mild haemolysis. Haemolysis triggered by primaquine depends on the dose administered, the duration of administration and the severity of G6PD deficiency. Severe reactions have been described most frequently in individuals with the G6PD Mediterranean variant, but they occur with other variants. Other factors affect the risk of haemolysis, including fever and other oxidant drugs or foods. In G6PD-normal individuals, primaquine causes either no significant change or a small reduction in Hb concentration.

Reduced glutathione is important in the detoxification of free radicals and thus cellular defences against oxidative stresses. Once oxidized, glutathione can be reduced back by glutathione reductase, using NADPH as an electron donor. In normal cells, NADPH is regenerated by G6PD during oxidative stress. Impairment of this step prevents reduced glutathione recycling, exposing the cell to oxidative damage. Alternative pathways to G6PD-dependent NADPH production exist in most human cells but not in erythrocytes, in which the lack of protein synthesis prevents replacement of lost enzyme. Thus, G6PD-deficient cells are uniquely vulnerable to oxidative stress. As the protein variants arising from different mutations in the *G6PD* gene result in a range of G6PD activity phenotypes, the degree of drug-induced haemolytic anaemia in G6PD-deficient individuals depends on the genetic basis of the deficiency (although other co-factors are also

Figure 6. Characterization of haemolysis phases in "primaquine-sensitive" (probably G6PD A–) individuals



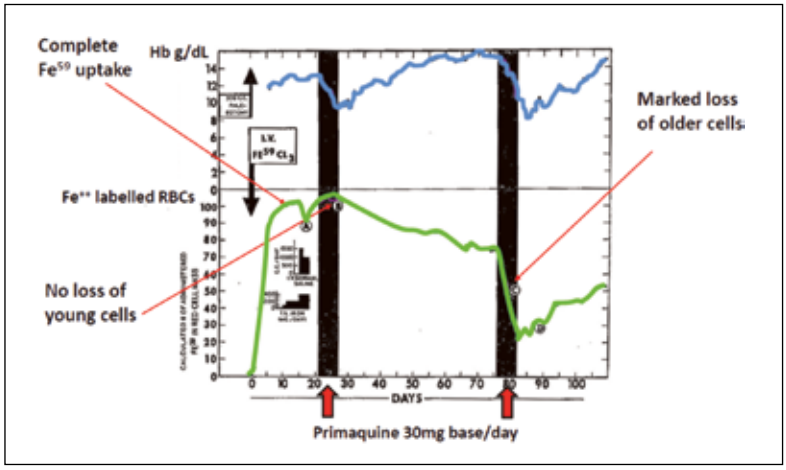
Haemolysis in healthy African Americans, probably with G6PD-deficient variant A–, given 30 mg primaquine daily (198). The erythrocyte volume fraction (EVF; haematocrit) usually started falling on the second day. Haemolysis can be divided into three phases. An acute phase lasts 7–12 days, in which the haematocrit falls to its lowest level (30%) and 30–50% of the red cell mass is destroyed, the urine is dark, sometimes black, bilirubin levels may increase to 3–5 mg/dL and jaundice may occur. If primaquine is stopped during the acute phase, erythrocyte destruction ceases within 48–96 h. The recovery phase occurs between days 10 and 40, when reticulocyte levels increase to a peak of 8–12%, and Hb and haematocrit slowly return to normal by week 4 or 5. The equilibrium phase begins when anaemia disappears, and it continues as long as primaquine is given; there may be mild haemolysis, which is detectable only by measuring erythrocyte survival. Adapted from references *198–201*.

important). For example, with the African variant G6PD A–, haemolysis is usually self-limiting and rarely life-threatening (Figure 6); after a dose of 30 mg/day primaquine, the Hb concentration is stable for 48–72 h, before decreasing from approximately 14 g/dL to 6–10 g/dL with continued drug administration, sometimes with black urine and general weakness, before recovery despite continued drug administration (*199*). With the more severe G6PD Mediterranean variant, severe haemolysis continues, which cannot be compensated and is therefore life-threatening (reviewed in *195*). Variations in phenotype among G6PD variants are attributable mainly to variations in diet and enzyme stability (*197*). The self-limiting nature of the haemolytic anaemia observed in most G6PD-deficient individuals after a standard drug challenge is due to destruction of older red cells; these are the most deficient in the enzyme, whereas newly produced erythrocytes have nearly normal levels of G6PD and are hence less susceptible to the oxidative stress induced by exposure to drugs (Figure 7).

The clinical course of drug-induced G6PD deficiency-related haemolysis is highly variable and may be influenced by the G6PD variant, the type and dose of drug, patient status and disease factors. The time course of haemolysis is different with different drugs and doses. For example, haemolysis caused by primaquine is detectable between 1 and 3 days after drug administration, whereas favism may take only hours to cause significant haemolysis. High fever (37–40 °C) appears to exacerbate the clinical severity of G6PD deficiency-related haemolysis (*203*).

Studies in the 1950s and 1960s by Alving's group (*199, 202, 204–206*), which were later reviewed and summarized by the same group (*198*), resulted in detailed characterization of the course of haemolysis in primaquine-sensitive individuals, all of whom were African American men who probably carried the G6PD A– variant. Primaquine-induced haemolysis in these individuals generally progressed in three distinct phases (acute, recovery and equilibrium) and was self-contained, with haemolytic recovery within 4–5 weeks. Figure 6 shows the sequence of haemolytic phases after a 30-mg daily dose in primaquine-sensitive individuals with anaemia and recovery, the presence of dark urine during the acute phase of haemolysis and simultaneous reticulocytosis. The young red cells that predominate in the recovery phase are "resistant" to the dose of primaquine used (Table 3 and Figure 8). A series of dose escalation studies was then designed to determine whether all erythrocytes in G6PD-deficient individuals were susceptible to haemolysis to some degree, susceptibility being a function of RBC age, or whether a subpopulation was completely resistant to haemolysis (*204*). An experimental increase in the

Figure 7. The haemolytic effect of primaquine depends on the age of red blood cells



Effect of primaquine on a Fe^{59}-labelled red cell population with a narrow age range. During the first (shaded) period, Fe^{59} was contained in erythrocytes 8–21 days of age. No perceptible destruction of these young cells took place. When another course of primaquine was given 55 days later, the same cells were rapidly destroyed. Reproduced from reference *202*

Table 3. Degree of haemolysis and anaemia in primaquine-sensitive African American men depends on daily primaquine dose

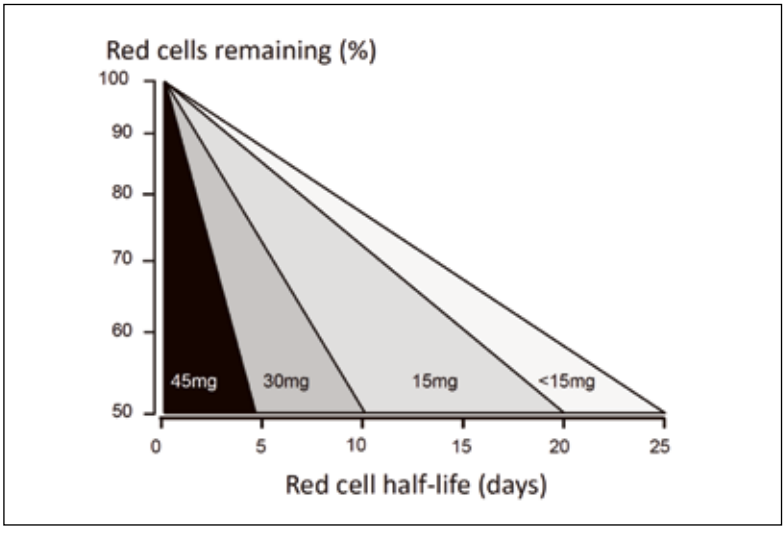
	45 mg/day	30 mg/day	15 mg/day	< 15 mg/day
Haemolysis	Dangerous haemolytic anaemia	Severe	Moderate	Mild
Anaemia	Dangerous haemolytic anaemia	Acute	Mild	None
Half-life of Cr51-labelled red blood cells (days)a	0–10	5–10	10–20	20–25

Data from reference 207

a Half-life without primaquine, > 25 days

primaquine dose to 240 mg after haemolysis had been induced by a lower dose provoked a second episode of clinical haemolysis during the "resistant" phase. Increasing the daily dose step-wise to 60 mg and 120 mg caused subclinical haemolysis of increasing severity, as demonstrated with Cr^{51}-labelled RBCs. From studies conducted before 1962, Alving's group classified the degree of haemolytic anaemia in G6PD-deficient hemizygous (primaquine-sensitive) African American men by the daily dose of primaquine (Table 3). Dangerous haemolytic anaemia was caused by a 45-mg daily dose, 30 mg resulted in acute anaemia (Figure 6), whereas lower doses resulted in mild-to-moderate

Figure 8. The severity of haemolysis in primaquine-sensitive African American men depends on the daily primaquine dose



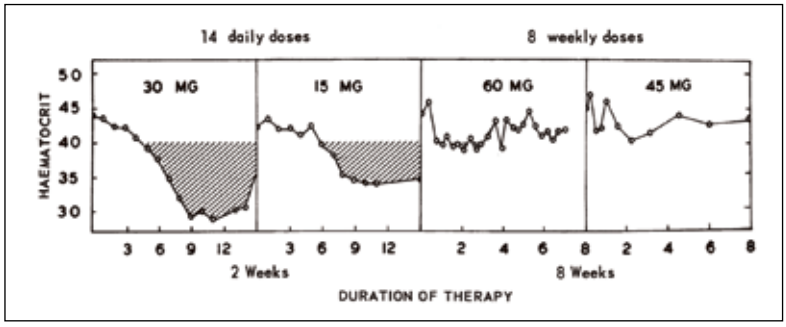
Different doses of primaquine were given daily to African American male volunteers with presumed A– variant G6PD deficiency. The red blood cell (RBC) survival is presented as percentage Cr^{51}-labelled RBCs remaining. Data from reference *198;* figure reproduced from reference *167*

haemolysis with mild or no anaemia. This suggested that all red cells could be haemolysed if the primaquine dose was high enough or the deficiency severe enough.

As a 45-mg weekly dose of primaquine usually does not cause clinically significant haemolysis in G6PD A– variant individuals (Figure 9), a regimen of 45 mg/week for 8 weeks to prevent relapse was evaluated and shown by Alving's group to be highly effective against severe *P. vivax* Chesson strain infections, resulting in cure of 90% of infections with no clinically demonstrable haemolysis in primaquine-sensitive men with major expression of the haemolytic trait (*208*). Eight men with the A– variant developed only mild, asymptomatic haemolysis (Hb decrease, 0.5–2.5 g/dL) (*209*; see Annex 2).

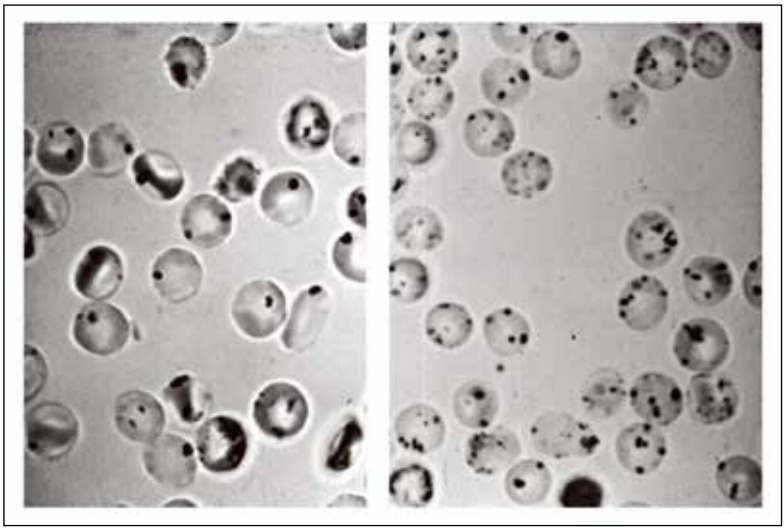
Haemolysis caused by primaquine can be diagnosed on the basis of clinical or laboratory criteria. Typical clinical manifestations are symptoms such as fever, general malaise, pallor, yellow sclerae and sometimes abdominal or loin pain. The urine turns dark, red–brown, grey or black. Laboratory evidence for haemolysis as the mechanism for primaquine-associated anaemia includes marked poikilocytosis, sometimes characteristic "bite cells", reticulocytosis (which may be suppressed in symptomatic malaria) and suitably staining Heinz bodies in peripheral blood films (Figure 10). Patients also have a decreased serum haptoglobin concentration (a common finding in malaria), increased unconjugated bilirubin and sometimes haemoglobinuria (without RBCs). Survival of RBCs can be assessed by labelling with ^{51}Cr. In the early studies of the pathophysiology of primaquine-associated anaemia, haemolysis was usually defined strictly by these laboratory criteria and by serial measurements of Hb or haematocrit, often supported

Figure 9. Mitigation of haemolysis by intermittent weekly primaquine administration



The lowest haematocrit (erythrocyte volume fraction) reading after each dose is shown for 14 daily doses of 15 or 30 mg primaquine and for 8 weekly doses of 60 or 45 mg primaquine, each administered to the same volunteer known to be sensitive to 30 mg/day primaquine (presumed A- variant). The trials were conducted at intervals of 6 months to allow erythrocytes to regain sensitivity to primaquine-induced haemolysis. Reproduced from reference *208*

Figure 10. Mitigation Heinz bodies



Results of the Heinz body test for "primaquine sensitivity" (G6PD deficiency) are shown. The red cells in the right-hand panel are from a G6PD-deficient donor and the cells in the left-hand panel from a G6PD-normal control. Reproduced with permission from reference *210*

by red cell survival studies. In more recent reports, the terms "anaemia" and "haemolysis" are often used interchangeably in the context of primaquine administration, which is misleading, particularly as malaria itself causes haemolytic anaemia, which may be sufficiently severe to result in haemoglobinuria (blackwater fever).

The haemolytic response to primaquine depends on the dose, the G6PD status of the recipient and the length of treatment. The classification of G6PD variants has changed in some cases as genotyping has become available; most mechanistic studies preceded the availability of genotyping. A study in the USA reported in 1967 (*211*) characterized haemolysis after a single dose of 45 mg primaquine in four G6PD-deficient individuals, possibly with different G6PD variants. The results underscore the complexity of the primaquine-induced G6PD deficiency haemolytic reaction in terms of intensity, accompanying symptoms and clinical measures. Table 4 shows the characteristics of the four people evaluated: one was an African American, one was a white Englishman, one a Sicilian and one an Ashkenazi Jew. After receiving 45 mg of primaquine, the African American had a mild haemolytic reaction (8% of RBCs destroyed), and the three whites showed more acute haemolysis, with ~20% RBC destruction. The G6PD activity of these three people before and after primaquine and certain symptoms accompanying haemolysis differed, however, despite equivalent RBC destruction. The G6PD activity of the Englishman, measured in RBC haemolysates, was similar to that of the African American: about 10% of normal activity (18.1 U/dL and 16.4 U/dL before primaquine and 27.5 U/dL and 24 U/dL after

Table 4. Reactions to primaquine in four glucose-6-phosphate dehydrogenase-deficient individuals, by ethnic group

Ethnic group	G6PD variant	G6PD activity in RBCs (before/ after primaquine, U/dL)	RBC destruction (%)	Jaundice	Bilirubin (peak serum level, mg/dL)
African American	A–	16.4/24	8	No	2.8
English	Similar to A– but abnormal pH optimum curve	18.1/27.5	19	Yes	7.9
Sicilian	B	0/0	23	No	2.4
Ashkenazi Jew	Mediterranean (B–)	0/0	21	Yes	6.1

Data from reference 211

primaquine, respectively), whereas no G6PD activity was detected for the Sicilian and the Ashkenazi Jew. In these two individuals, the Mediterranean variant was confirmed by assays with purified G6PD enzyme. The Sicilian reported having eaten fava beans with no ill effects, including no jaundice, whereas the other two whites reported adverse events after eating fava beans. The serum bilirubin peak of the Sicilian (reached in all individuals on days 2–4 after primaquine) was similar to that of the African American (2.4–2.8 mg/dL), while the other two white males showed distinct icterus of the skin and sclerae, with peak serum bilirubin values of 6.1–7.9 mg/dL. In all four cases, the Hb returned to initial levels within 4 weeks.

In a study in Italy in 1969, 45 mg primaquine were given as a single dose with 300 mg chloroquine on one or two occasions a week to 25 (24 men and one woman) G6PD-deficient Sardinians (undetectable G6PD activity in 18) and three G6PD-normal people. Nineteen of the 25 had significant haemolysis; the half-life of ^{51}Cr-labelled RBCs decreased from 26 days before to 9.6 days after primaquine. The extent of haemolysis ranged from moderate to severe. The Hb started to rise to normal levels after a few days. The ^{51}Cr-labelled RBCs from 14 G6PD-deficient subjects were transfused into 25 normal recipients who also took one or two doses of chloroquine plus primaquine (45 mg). All showed accelerated red cell destruction after the drugs (mean red cell survival shortened from 21 to 7 days). Interestingly, the red cells from G6PD-deficient individuals who did not show haemolysis after primaquine were all destroyed when injected into normal recipients who had received single doses of the drugs (*212*). This may be explained by reduced bioactivation in these G6PD-deficient donors. In 1970, ^{51}Cr-labelled RBCs from two severely G6PD-deficient men (one Burmese, one Indian) were injected into matched healthy Burmese adults who received 15 mg/day primaquine for 14 days. Primaquine caused haemolysis of 34–48% of the injected G6PD-deficient cells (*213*). In a study in Cambodia reported in 1977, 15 mg/day primaquine were administered for 14 days for radical cure to Khmer Air Force troops with vivax malaria. A significant fall in Hb was seen in 15 G6PD-deficient

patients, from 43% on day 1 to 34% on days 7 and 15, whereas the values in 31 G6PD-normal controls were 45% before primaquine and 44% afterwards. The haemolysis was not considered dangerous (*214*; see Annex 2). In a study in Azerbaijan of mass drug administration for post-eradication malaria therapy, "intermittent" treatment was administered in areas of high prevalence of G6PD deficiency: 4 days of 15 mg primaquine, no drug on days 5–7 when most primaquine-induced haemolytic reactions occur, and 15 mg on days 8–17. All treated people experienced a reduction of Hb (1–2 g/dL for G6PD-normal, 3–5 g/dL for G6PD-deficient, accompanied by reticulocytosis; Hb was never <9 g/dL), which was more pronounced in children under 11 years (*200*, *201*; see Annex 2).

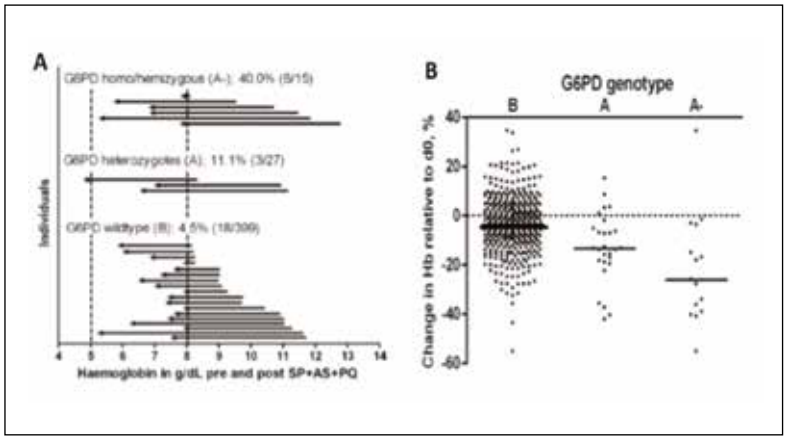
In a survey conducted in several Pathan (Pashtun) and other Afghan refugee populations in Pakistan, the prevalence of G6PD deficiency was found to range from 2.1% to 15.8%. The Mediterranean variant predominated. Severe haemolytic crises were reported to have been triggered by various agents, including antimalarials (*215*).

In a study in Myanmar in 1994, primaq uine was given at 45 mg weekly for 8 weeks to 31 patients infected with *P. vivax* and at a single dose of 45 mg to 32 patients with *P. falciparum* gametocytaemia after quinine, as a safer alternative for severely G6PD-deficient patients. No acute haemolysis was observed in 22 G6PD-deficient patients, two with the "B–" variant, resulting in mild deficiency (40–60% enzyme activity), and 20 with the severe "Myanmar" variant (enzyme activity <5% normal) (*216*; see Annex 2). G6PD Mahidol predominates in Myanmar, but several other variants are prevalent, and the genotypes of these patients were unknown. In contrast, treatment of 22 G6PD-deficient patients with vivax malaria in Thailand (where G6PD Mahidol and Viangjan predominate) with a 3-day course of chloroquine (total dose, 1500 mg) followed by primaquine at 15 mg/day for 14 days resulted in a significant decrease in Hb, although blood transfusion was not required (*53*; see Annex 2).

The four G6PD variants that are the best characterized with regard to oxidant haemolysis are G6PD A–, Mediterranean, Mahidol and Viangjan. Although G6PD A– is considered one of the mildest variants in terms of severity of deficiency, there is considerable overlap in the phenotypes associated with different genotypes. The severity of haemolysis depends more on the precipitating agent, the dose given and the length of exposure than on the G6PD variant (*197*). Favism is usually associated with the Mediterranean variant but has been documented in people with the G6PD A– variant (*217*, *218*).

The available evidence indicates a very low risk for substantial primaquine-induced haemolysis in people without G6PD deficiency given standard therapeutic doses. An early study in the USA showed that a dose of 240 mg primaquine given daily could eventually induce low-grade haemolysis in G6PD-normal volunteers (*204*). The findings of a recent

Figure 11. Haemoglobin concentrations before and after sulfadoxine–pyrimethamine + artesunate + primaquine in Tanzanian children



(A) Hb concentrations of Tanzanian children who became moderately anaemic (<8 g/dL) after receiving sulfadoxine–pyrimethamine (SP) + artesunate (AS) + primaquine (PQ). Individual observations are given for children with homo-/hemizygous (6/15 treated or 40%) A– variant, heterozygous (3/27 or 11.1%) or wild-type (18/399 or 4.5%) G6PD genotypes. Arrows represent individual Hb measurements at baseline (right) and after the intervention (left). Dashed lines show the values below which children were considered to have severe (5 g/dL) and moderate (8 g/dL) anaemia.

(B) Hb concentrations 7 days after SP + AS + PQ shown as percentages of baseline value in relation to G6PD genotype. Significant reductions in Hb concentration were seen in children with G6PD B ($p < 0.0001$), G6PD A ($p < 0.0001$) or G6PD A– ($p = 0.001$) on day 7 after treatment when compared with day 0 (d0).
Reproduced with permission from reference *219*

study in East Africa cast some doubt on the assumption that primaquine is entirely safe in G6PD-normal populations. In a cluster-randomized mass drug administration in 2008, 4601 Tanzanian children aged 1–12 years were given either SP–artesunate–primaquine (target dose, 0.75 mg/kg body weight, but age-based dosing used) or SP–artesunate–placebo (*219*). Children with an Hb concentration at baseline of <5 g/dL were excluded and referred to hospital, and all children with Hb <8 g/dL were allocated to the placebo arm. Haemolysis was assessed by measuring Hb on day 7 and from clinical signs and symptoms in a subset of 734 children. The Hb concentration was unchanged in the placebo arm (mean difference from day 0, –0.05 g/dL; 95% CI, –0.28 to 0.18) but reduced by day 7 (mean, 0.58 g/dL; 95% CI, –0.46 to –0.71 g/dL) in the primaquine arm (Figure 11). In one child, the Hb concentration dropped by 3.5 to 4.8 g/dL but recovered without transfusion. Genotyping results for 562 children showed 4% G6PD A– homozygotes, 8.4% G6PD A heterozygotes, 2.4% α-thalassaemia homozygotes (α/α) and 25.2% α-thalassaemia heterozygotes (αα/α). Eighteen (4.5%) of the children with G6PD wild-type (B) and paired Hb measurements developed moderate anaemia, with a drop of >6 g/dL Hb in one child. Anaemia was diagnosed as haemolytic on the

basis of a fall in Hb only, and G6PD deficiency was assessed by genotyping and not phenotyping. Age-based dosing would have resulted in variable doses of primaquine in milligrams per kilogram, including higher than target doses for G6PD-deficient children; this would not have been expected to affect G6PD-normal children.

The unpredictability of the haemolytic reactions following primaquine administration in G6PD-deficient individuals suggests that other factors are involved in determining haemolytic risk. Favism is more common in children than in adults. Age might act as a confounder of dose (i.e. resulting in relative overdosing). Variations in pharmacokinetics and pharmacodynamics could also play a role; e.g. concurrent administration with food has been shown to increase the bioavailability of primaquine in some studies. Concomitant disease, such as malaria and other infections, may increase the risk for haemolysis. G6PD deficiency is a risk factor for blackwater fever (massive haemolysis) independent of drugs. Co-existence with other conditions may also be relevant, such as the haemoglobinopathies that are commonly associated with increased intraerythrocytic oxidant stress; however, people with variant Hbs may have increased RBC turnover, a younger population of erythrocytes and hence higher G6PD activity, with a theoretically reduced risk for oxidant drug haemolysis. There are very few studies of G6PD deficiency and haemoglobinopathy, even though the two conditions commonly co-exist in the same populations.

3.2.2 Drugs that cause haemolysis in glucose-6-phosphate dehydrogenase-deficient patients

People with G6PD deficiency who are subjected to oxidative stress from infection, certain medication and foods such as fava beans are at risk for acute haemolytic anaemia. G6PD deficiency is also a common cause of neonatal jaundice. G6PD deficiency usually manifests as drug-induced or infection-induced acute haemolytic anaemia, favism, neonatal jaundice or chronic non-spherocytic haemolytic anaemia; however, most G6PD-deficient individuals are unaware of their status and are asymptomatic. The drugs and chemicals associated with haemolytic risk in G6PD-deficient individuals include antimalarial agents such as pamaquine and primaquine and also some sulfonamides, dapsone, nalidixic acid, nitrofurantoin, co-trimoxazole and possibly aspirin (Table 5). A report from the United Arab Emirates in 2001 described four cases of haemolytic crisis in G6PD-deficient children after topical application of henna. One was a female neonate (Hb, 5 g/dL), who recovered after exchange transfusion. A male infant (Hb, 2.8 g/dL) died despite transfusion, and two children aged 3–4 years (Hb, 4 and 4.1 g/dL, respectively) received transfusions and survived (*220*). In 2006, in a report from Hong Kong (China) on acute haemolytic crises in six G6PD-deficient children, one had been exposed to mothballs (naphthalene), and two had been treated with herbal medicines (*221*).

Table 5. Drugs that can trigger haemolysis in glucose-6-phosphate dehydrogenase-deficient individuals

Type of drug	Confirmed risk	Possible risk
Antimalarial agent	Primaquine, pamaquine, pentaquine, bulaquine, tafenoquine	
Analgesic	Acetanilid	Aspirin
Sulfonamide, sulfone	Sulfamethoxazole, co-trimoxazole, dapsone	Sulfasalazine, sulfadiazine
Quinolone	Nalidixic acid, ciprofloxacin, norfloxacin, oxifloxacin, ofloxacin	
Other antimicrobial agent	Nitrofurantoin, methylene blue	Chloramphenicol
Other	Niridazole	Vitamin K, rasburicase, ascorbic acid, glibenclamide, henna, naphthalene

Data from reference 211

3.2.3 Chlorproguanil–dapsone

Chlorproguanil–dapsone (Lapdap®) was marketed as a fixed-dose combination antimalarial agent and later combined with artesunate. Both drugs were withdrawn because of haematological toxicity observed in G6PD-deficient individuals in several studies conducted in Africa.

Chlorproguanil–dapsone (Lapdap®), licensed in the United Kingdom in October 2003, was a fixed-dose synergistic combination of two antifolate drugs, chlorproguanil and dapsone, which were relatively rapidly eliminated from the body. Dapsone, a sulfone, was well established as a treatment for leprosy and was already known to precipitate haemolysis in some G6PD-deficient individuals. Lapdap® was marketed and used in Africa and the United Kingdom. The risk for haemolytic effects was not initially evident in clinical trials and studies. After Lapdap® was launched in several African countries, Dodoo in Ghana pointed out in 2004 that these countries had poorly developed or inexistent monitoring systems and that Lapdap® posed a potential threat to public health, particularly for G6PD-deficient individuals (gene frequencies typically range from 5% to 30% in Africa); he also noted that the risk was not indicated on the product label for these countries (*222*). At a WHO expert meeting in June 2004 to discuss the safety of Lapdap® in the management of malaria, the group concluded that this antimalarial combination was contraindicated in patients with G6PD deficiency and recommended its use only after exclusion of both severe anaemia (Hb, <5 g/dL) and G6PD deficiency. They recommended the use of alternative drugs in regions where G6PD deficiency is prevalent, and commented that appropriate tests were not available. If alternative drugs were not available, they recommended that Lapdap® be used but with particular attention to the haematological risks. Lapdap® remained on the market in many African countries until 2008, when it was withdrawn by GlaxoSmithKline because of "significant reductions in Hb levels of patients with G6PD deficiency" (reviewed in reference *203*).

In contrast to 8-aminoquinolines, for which studies in some populations are scarce, extensive research was conducted on Lapdap®, including among pregnant women and children with uncomplicated malaria. In 2008, a report was published of an open-label clinical trial of treatment of uncomplicated falciparum malaria with Lapdap® alone or in combination with artesunate in The Gambia and Malawi in patients of unknown G6PD status. Drug-related adverse events were found in 35.3% (41/116) adults and 70.1% (75/107) children, most of which were haematological and gastroenterological. Two severe adverse events thought to be related to the drugs were observed. Severe haemolytic anaemia was diagnosed in one adult given Lapdap®–artesunate at 2 mg/kg, who presented on day 9 with an Hb level of 6.6 g/dL (baseline, 15.4 g/dL); he was treated with two units of blood and recovered, but on day 14 he experienced anaphylactic shock, which resulted in death. The death was considered to be related to herbal preparations he had taken. The other was a case of anaemia in a 10-year-old child given Lapdap®–artesunate at 4 mg/kg, who presented on day 7 with an Hb level of 3.8 g/dL (baseline, 12.0 g/dL), was treated with blood transfusion and antibiotics and recovered (*223*). In a study in 2009 to compare the efficacy of artemether–lumefantrine with that of Lapdap® (422 patients with uncomplicated falciparum malaria received Lapdap®) as possible alternatives to SP in Malawi, one child died from severe malaria a day after treatment with Lapdap®. It was not clear whether this death was due to poor treatment efficacy or was an inevitable consequence of the infection. Two patients developed severe anaemia within 1 week, with Hb falling by ~50% despite parasite clearance, but the G6PD status of the participants was unknown (*224*).

A phase III randomized trial conducted in Burkina Faso, Ghana, Mali and Nigeria to compare the efficacy and safety of chlorproguanil–dapsone with or without artesunate in the treatment of acute uncomplicated falciparum malaria was reported in 2009. Neither regimen was safe for G6PD-deficient individuals. In patients given chlorproguanil–dapsone–artesunate, haemolytic adverse events (fall in Hb of ≥ 4 g/dL or ≥ 40% from baseline, Hb <5 g/dL or blood transfusion) occurred in 13/44 (30%) G6PD-deficient individuals and 4/448 (<1%) G6PD-normal patients. Among the patients given Lapdap®, 7/24 (29%) G6PD-deficient and 6/221 (3%) G6PD-normal patients had such adverse events (*225*). Phase III clinical trials of chlorproguanil–dapsone–artesunate were reported in 2009 (*226*), which showed severe adverse events in 63/914 (7%) patients who received this combination, compared with 15/458 (3%) in the group given artemether–lumefantrine. Events probably related to oxidative haemolysis were observed in 46/914 (5%) patients given chlorproguanil–dapsone–artesunate and in 3/458 (0.1%) given artemether–lumefantrine. Fourteen patients given chlorproguanil–dapsone–artesunate, 12 G6PD-deficient patients, one heterozygous female and one G6PD-normal patient, required a blood transfusion for a significant decrease in Hb. The decreases in Hb were more marked for G6PD-deficient patients given chlorproguanil–dapsone–artesunate than for those given artemether–lumefantrine on days 3, 7 and 14. In G6PD-deficient individuals, the lowest mean Hb level was 7.5 g/dL (95% CI, 7.1–7.9) at day 7

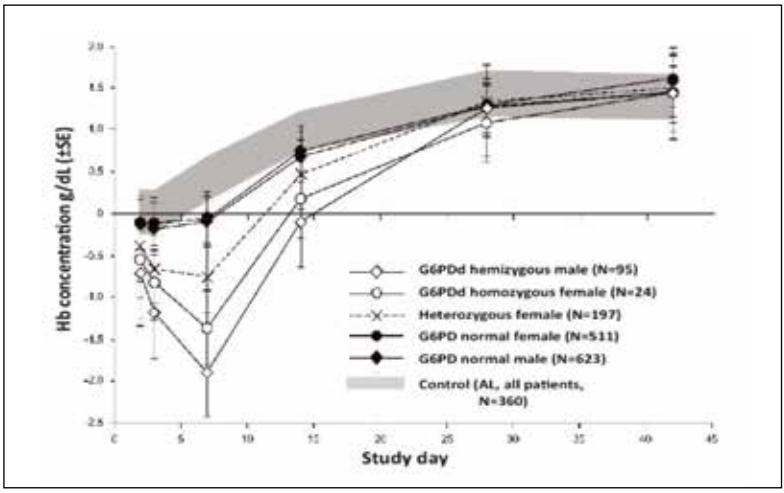
with chlorproguanil–dapsone–artesunate and 9.7 g/dL (95% CI, 9.1–1.02) with artemether–lumefantrine. In the group given artemether–lumefantrine, all but one of the severe adverse events were in G6PD-normal patients. The exception was a case of malaria in a heterozygous female. The commonest severe adverse event reported was malaria (7/458; 2%).

A trial was conducted with Lapdap® in 2004 to compare its safety and effectiveness with that of artemether–lumefantrine under conditions of routine use in G6PD-normal and G6PD-deficient children aged 6 months to 10 years with uncomplicated malaria in The Gambia. Severe anaemia developed in 23 children, 17 (2.9%) of whom had been treated with Lapdap® and 6 (1%) with artemether–lumefantrine (*227*). The Hb concentrations on day 3 were lower among children treated with Lapdap® than those given artemether–lumefantrine (difference, 0.43 g/dL; 95% CI, 0.24–0.62), and the levels in the Lapdap® group were lower in children with higher parasite densities at enrolment. Only 17 of the 1069 children typed were G6PD A–; of these, 2/9 treated with Lapdap® and 1/8 treated with artemether–lumefantrine developed severe anaemia, and 5/9 treated with Lapdap® had a fall of 2 g/dL or more in Hb concentration by day 3. Thus, there were more cases of severe malaria and anaemia after Lapdap® treatment, although G6PD deficiency was uncommon in this population.

In a clinical trial reported in 2008 to compare the safety of Lapdap® given with artesunate and amodiaquine + SP for the treatment of uncomplicated falciparum malaria, Lapdap® resulted in a mean decrease in haematocrit of 1.94% per day (95% CI, 1.54–2.33) in patients with G6PD deficiency and 1.05% per day (95% CI, 0.95-1.15) in G6PD-normal patients. A mean reduction of 1.3% per day was observed among patients who received amodiaquine–SP, regardless of G6PD status (95% CI, 1.25–1.45). G6PD-deficient Lapdap® recipients had significantly lower haematocrits than the other groups up to day 7 ($p = 0.04$). Patients with G6PD deficiency had a higher risk for severe anaemia after treatment with Lapdap® (relative risk, 10.2; 95% CI, 1.8–59.3) or amodiaquine–SP (5.6; 1–32.7) (*228*).

A recent analysis of data collected retrospectively on the clinical course of acute haemolytic events in 119 children under 15 years treated with dapsone as chlorproguanil–dapsone or chlorproguanil–dapsone–artesunate in phase-III trials, who were hemizygous or homozygous for G6PD A–, confirmed the haemolytic risk of G6PD-deficient populations for dapsone-containing formulations (*229*). Of 119 G6PD-deficient children, 26 (21.8%) showed a mean decrease in Hb concentration of ≤13% after artemether–lumefantrine, whereas the decrease in 24/119 (20.2%) G6PD-deficient children was ≥ 40%. The decrease in heterozygote females ($N = 200$) was approximately half way between that of G6PD-normal and G6PD-deficient children. Among the heterozygote children, isolated cases of severe haemolysis were seen (Figure 12). This study suggests that the G6PD A– variant, often associated with "mild" haemolysis, can result in severe acute haemolytic anaemia after administration of drugs that impose a haemolytic risk for G6PD-deficient individuals.

Figure 12. Haemoglobin concentrations in children receiving a chlorproguanil–dapsone combination or artemether–lumefantrine for the treatment of falciparum malaria



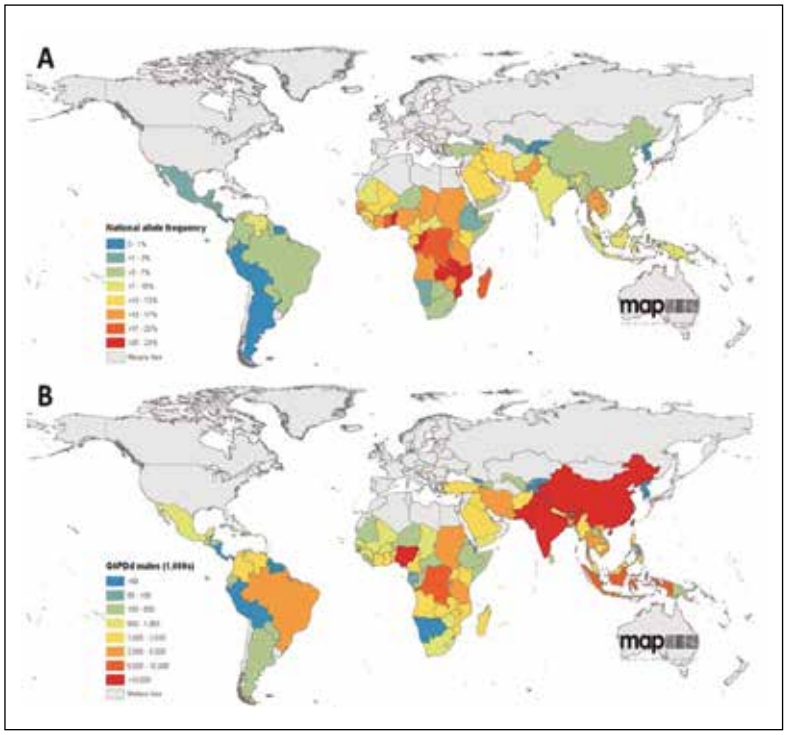
Hb, haemoglobin; SE, standard error; G6PDd, G6PD-deficient; AL, artemether–lumefantrine
Changes in Hb concentration are shown for the indicated G6PD (variant A–) genotypes relative to concentrations on day 1. Reproduced with permission from reference *229*

3.2.4 Regional prevalence of glucose-6-phosphate dehydrogenase deficiency

A recent world map of the distribution of G6PD deficiency, based on an analysis of 1734 studies, including 1289 in malaria-endemic countries, illustrates the contributions in numbers of G6PD-deficient individuals in China and India (an estimated 41.3% of all G6PD-deficient males), whereas the national allele frequencies are higher in sub-Saharan Africa (28% of all G6PD-deficient males). One study of 64 813 Army personnel in the USA suggests that the highest G6PD deficiency rates are for African American males (12.2%), followed by Asian males (4.3%), African American females (4.1%), Hispanic males (2.0%), Hispanic females (1.2%) and Asian females (0.9%), with a low prevalence in whites (males, 0.3%; females, 0%).

A map of the prevalence of G6PD deficiency and estimates of the affected populations worldwide, based on a flexible Bayesian model developed for the analysis of evidence from 1734 studies, 1289 (74%) of which were in malaria-endemic countries (*230*), are shown in Figure 13. The geographical distribution of the published surveys was not even: some areas were examined by micro-mapping (Sri Lanka) and universal screening (Philippines), while others have not been studied in detail (regions in Indonesia, Madagascar and central Africa). Within the malaria-endemic regions, 85% of the surveys (n = 1101) were in 23 Asian countries, 10% (n = 132) in 23 African countries and 4% (n = 56) in nine countries in the

Figure 13. World map of the prevalence of glucose-6-phosphate dehydrogenase deficiency



Population-weighted area estimates of national prevalence of G6PD deficiency. (A) National-level allele frequencies. (B) National-level population estimates for males. Reproduced from reference *230*

Americas. Of the people sampled, 2.4 million were males and 2.0 million were females; 99% of the surveys reported data for males and 62% for females.

The overall estimated frequency of the G6PD deficiency allele in malaria-endemic countries was 8.0% (interquartile range, 7.4–8.8). This estimate is based on population data for 2010 and corresponds to 220 million males (interquartile range, 203–241) and 133 million females (122–148), including 17 million homozygous females. The prevalence in malaria-endemic countries was lower (5.3%; 4.4–6.7), with 61 million G6PD-deficient males (51–77) and an expected 35 million G6PD-deficient females (29–46), including 3 million homozygous females. The estimates of national allele frequency were generally lowest in the Americas and highest in Africa. G6PD-deficient populations are greatest in highly populated Asian countries, notably China and India, where an estimated 41.3% of all G6PD-deficient males in the world live. Overall, the Americas contributed only 4.5% of the malaria-endemic G6PD-deficient male population, sub-Saharan Africa 28.0% and Asia 67.5%.

Large areas of the American malaria-endemic countries were predicted to have median G6PD deficiency frequencies ≤1% (40.8% land area), the condition being almost absent in much of Argentina, Bolivia, Costa Rica, northern Mexico and Peru (Figure 13). The prevalence increases towards the coastal regions, reflecting the genetic origins of the population, peaking in Venezuela (with most predictions > 5%). Model uncertainty was relatively low: interquartile range, <5%, increasing to 5–10% across the Amazon region, for which data were extremely scarce, and peaking at 15–20% across Venezuela. In sub-Saharan Africa, 65.9% of the land area was predicted to have a median G6PD deficiency prevalence ≥ 5%, and 37.5% a median prevalence ≥ 10%, ranging from <1% at the continental extremities to >20% in isolated pockets of Sudan, coastal West Africa and the mouth of the Congo River. Striking variation was predicted within countries, with G6PD deficiency hotspots, including Nigeria (range, 2% (interquartile range, 1–6) to 31% (22–42)), the Sudan (1% (0–2) to 29% (19–41)) and the Democratic Republic of the Congo (4% (1–11) to 32% (23–41)). In these areas, however, the highest uncertainty was recorded because of subnational heterogeneity and the scarcity of data. Data were also lacking for large areas of northern China, so that the prevalence in this region is probably overestimated. The highest median predicted prevalence of G6PD deficiency in all malaria-endemic countries was 32.5% in the Eastern Province of Saudi Arabia, although the rates were heterogeneous, dropping to 3% (interquartile range, 2–4) in central areas of the country. The predicted prevalence was also high in southern Pakistan, but the greatest uncertainty was found for this region (interquartile range >30%). Southern parts of the Islamic Republic of Iran, Oman and western India had predicted prevalences >20%. The prevalence in central and southeast Asia remained <10%, with three notable hotspots peaking to >20%: among the tribal groups of Orissa province in eastern India, a patch along the northern border between the Lao People's Democratic Republic and Thailand and much of the Solomon Islands archipelago. Some areas were predicted to have highly heterogeneous subnational prevalences of G6PD deficiency, such as the Lao People's Democratic Republic, with a predicted frequency ranging from 1% (interquartile range, 0–2) to 23% (16–32); Indonesia, from 0% (0–1) to 15% (10–21); and Papua New Guinea, from 1% (0–2) along the southern coast to 15% (10–22) along the northern coast.

The prevalence of G6PD deficiency in US Army personnel was reported in 2007 (*231*). Data were derived from a retrospective examination of 64 813 G6PD test results, matched to the person's sex and self-reported ethnicity and analysed according to the WHO classification (Table 6). Complete data were available for 63 302 people (54 874 men, 8428 women); 67% were white, 18% were African American, 8% were Hispanic, 3% were Asian, 1% were American Indian or Alaskan, and 3% were other or unknown. G6PD deficiency was found in 2.5% men and 1.6% women. The highest rates were among African American men (12.2%), followed by Asian men (4.3%), African American women (4.1%), Hispanic men (2.0%), Hispanic women (1.2%) and Asian women (0.9%). The rates in the 4018 whites were low

Table 6. WHO classification of glucose-6-phosphate dehydrogenase deficiency by severity

Class	Severity of G6PD deficiency	Percentage of normal G6PD activity
I	Very severe, associated with chronic non-spherocytic haemolytic anaemia	< 1
II	Severe	1–10
III	Moderate	10–60
IV	Normal activity	60–150
V	Increased activity	> 150

From reference 232

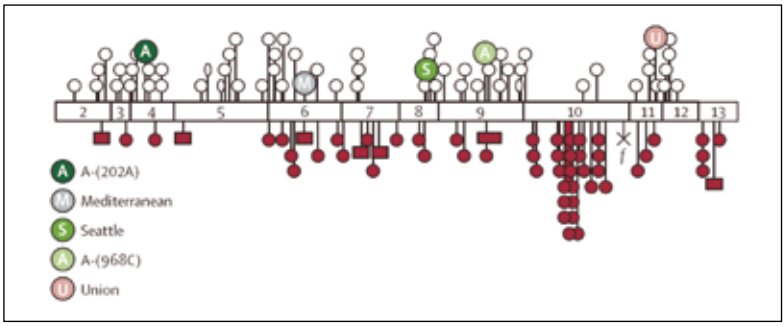
(men, 0.3%; women, 0%). None had class I deficiency. Of the deficient men, 46 were in class II (23 Asians, 9 whites, 9 African Americans, 2 Hispanics, and 3 other or unknowns), but most (1265) were in class III. Most of the deficient women (79) were in class IV, but 51 (of 2763) African Americans were in class III and one in class II. Therefore, Asian males had the highest risk (1.4%) for severe haemolysis, whereas the risks of other groups were 1/1000 for African American men, 0.4/1000 for African American women and Hispanic men and 0.2/1000 for white men.

3.2.5 Glucose-6-phosphate dehydrogenase variants

The gene encoding G6PD is located on the X chromosome. It is 18.5 kilobases long and contains 13 exons encoding 515 amino acids. It exhibits considerable polymorphism in human populations, and over 400 variants of the G6PD enzyme have been described on the basis of different electrophoretic and biochemical characteristics. G6PD is an essential enzyme: gene knockouts in mice are embryolethal. Some variants do not show reduced enzyme activity (*233*), but numerous mutations in the *G6PD* gene have been described that result in enzyme instability and thus deficiency in erythrocytes. Almost all the G6PD-deficiency variants are caused by point mutations of the genomic DNA resulting in amino acid substitutions (*234*). In a recent review, the G6PD mutation database was updated to 186 mutations (*235*). Of these, 159 (85.4%) are single-nucleotide substitutions (missense variants), 15 (8.0%) are multiple mutations (two or more substitutions), 10 (5.3%) are deletions, and 2 (1.0%) are mutations that affect introns. As *G6PD* is X-linked, males are hemizygous and can, therefore, have normal expression or be G6PD deficient, depending on which allele they inherit on their X chromosome. Homozygote females are as deficient as hemizygous males. Heterozygous females are genetic mosaics as a result of early embryonic random X-chromosome inactivation (lyonization), and so the abnormal red cells of a heterozygous female can sometimes be as deficient for G6PD as those of a G6PD-deficient male. On average, however, heterozygotes are half as deficient as homozygotes or hemizygotes. Phenotypically, G6PD deficiency is therefore most commonly seen in hemizygous males.

G6PD variants (WHO classes II and III; for WHO G6PD deficiency classification, see Table 6) have reached high gene frequencies in some populations. The commonest *G6PD* gene mutations are shown in Figure 14. The G6PD

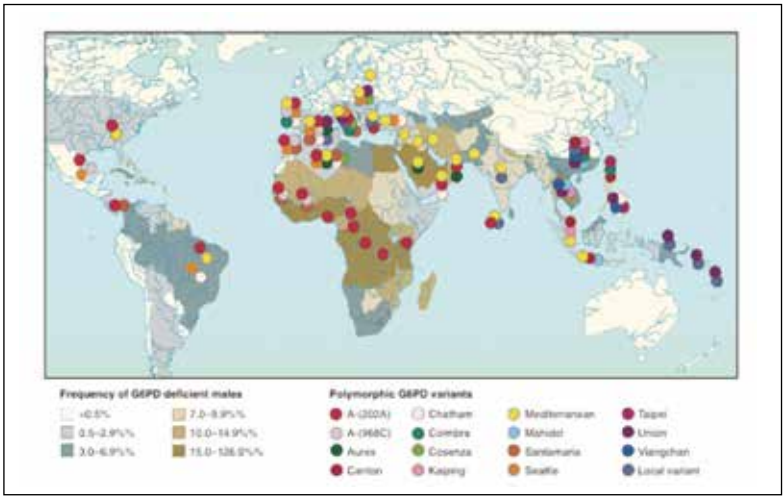
Figure 14. Commonest mutations along the G6PD gene coding sequence



Exons are shown as open numbered boxes. Open circles are mutations that cause classes II and III variants. Filled circles represent sporadic mutations that give rise to severe variants (class I). Open ellipses are mutations that cause class IV variants. Filled squares are small deletions. X indicates a nonsense mutation and F a splice site mutation. 202A and 968C are the two sites of base substitution in the African G6PD A– variant. Reproduced with permission from reference *196*

Mediterranean variant is widespread in Greece, Italy, Spain, the Middle East and the Indian subcontinent and parts of South-East Asia. The G6PD A– allele containing the mutations *A376G* and *G202A* is the commonest G6PD deficiency allele in Africa and has been imported to the Americas (Figure 15). A study in Burkina Faso, Ghana, Kenya, Mali, Nigeria and the United Republic of Tanzania comprised 2045 patients with uncomplicated *P. falciparum* malaria for whom the G6PD genotype was available (1018 males, 1027 females; all the G6PD deficiency A– genotypes were

Figure 15. World map of glucose-6-phosphate dehydrogenase deficiency variants



Overall prevalence of G6PD deficiency in males per country is shown by shading, and the distribution of specific variants is indicated by coloured circles. Reproduced with permission from reference *197*

A376G/G202A). The prevalences of G6PD deficiency were 9.0% male hemizygous, 1.8% female homozygous, 13.3% female heterozygous and 77.7% G6PD normal (*236*). A study of the prevalence of G6PD deficiency in 200 males in the Brazilian Amazon showed that six (3%) were G6PD deficient, of whom five had the G6PD A– African variant (*237*). The *A376G* mutation results in the G6PD deficiency A allele, which has 80% of the wild-type enzyme activity. When *A376G* and *G202A* are both present, the resultant G6PD A– allele produces an enzyme with approximately 12% of the wild-type activity (*238*, *239*). Two other G6PD A– alleles, which account for 5% of this genotype, are due to mutations at nucleotide position 680 or 968 with *A376G* (*238*).

Among Kurdish Jews, who have the highest known incidence of G6PD deficiency in the world (about 70% of males), the deficiency is due almost entirely to the Mediterranean allele (*C563T*) (*240*). The main G6PD variants reported in South-East Asian countries are Viangchan (*G871A*) and Mahidol (*G487A*). A recent report on G6PD alleles in eastern Indonesian populations showed that the G6PD Vanua Lava variant (*T383C*) is the most common, whereas Viangchan, Mahidol, Mediterranean and Canton (*G1376T*, of Chinese origin), the commonest variants in continental South-East Asian populations, were rare or absent (*241*). G6PD Kaiping (*G1388A*), another common variant of Chinese origin in South-East Asia, is widely distributed among native Flores and Palue islanders.

Modelling of the distribution of G6PD deficiency from the available data (R.E. Howes et al., unpublished data) resulted in the prediction that sub-Saharan Africa had predominantly mild (A–) variants, although some class II variants were reported in The Gambia and Senegal in West Africa and in South Africa and Sudan. Relatively few data were available for the Americas, but they indicated a wider diversity of variants, with a minority of class II. Variants were more heterogeneous across Asia; most were class II (most commonly Mediterranean, then Canton and Kaiping), although certain class III variants were also widely reported (Mahidol, then Chinese-5 and Gaohe).

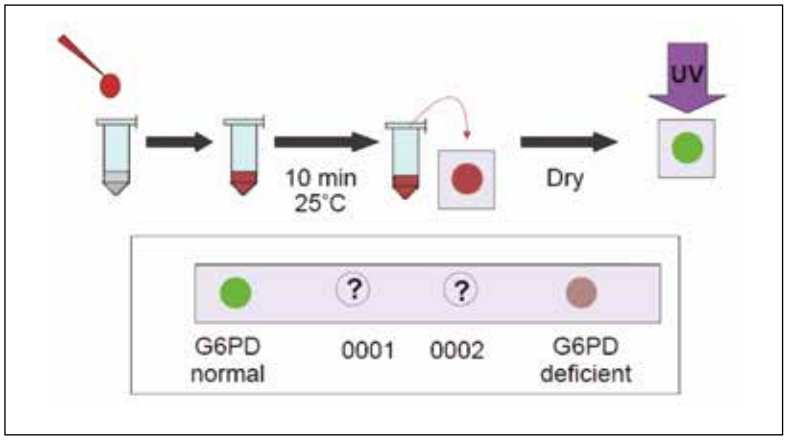
A correlation was found between the frequency of *G6PD* alleles in a population and the presence of malaria (*242*), and it is generally accepted that G6PD deficiency confers protection against malaria (*242–244*). This assumption is not conclusively supported by evidence, however, probably because of the complexity of G6PD deficiency, with different variants present in different regions and possible interaction among malaria species. For example, in a study of the effect of the G6PD Mahidol 487A variant on vivax and falciparum malaria, the density of vivax but not falciparum parasites in humans was reduced (*245*). A study of Afghan refugees in Pakistan, with predominantly the Mediterranean G6PD deficiency variant, showed that G6PD deficiency, measured by phenotype or genotype, confers substantial protection against vivax malaria infection (*246*). Studies in vitro have shown that the growth of parasites in RBCs is reduced significantly when the cells are G6PD deficient.

3.2.6 Diagnosis of glucose-6-phosphate dehydrogenase deficiency

The conversion of NADP+ to NADPH in erythrocytes is measured to determine their G6PD activity. The assay used most commonly is the qualitative ultraviolet "spot test", which is relatively specific in identifying red cells with G6PD activity <30% of normal. Individuals undergoing acute haemolytic reactions can be misclassified as normal because the remaining red cells are young and have higher G6PD activity than the cells that have been haemolysed. These patients should be retested when they are no longer anaemic. In quantitative tests for G6PD activity, it is important to determine the threshold value below which individuals are classified as G6PD-deficient. Rapid tests for G6PD deficiency have been developed for use at points of care, but more information is needed on their performance, stability and costs.

The tests used to assess G6PD activity are based on conversion of the coenzyme NADP to its reduced form (NADPH) in erythrocytes. In the fluorescence "spot test" used for screening, the generation of NADPH is detected visually under ultraviolet light or quantitatively in a spectrophotometric assay. A 5-µL volume of fresh blood is incubated with a special reagent for 10 min, and 15 µL of the solution are transferred onto filter paper and left to dry for 15–20 min before examination under an ultraviolet lamp. If the sample is red, the sample is G6PD deficient; if it is green, it is G6PD normal (Figure 16). Dye reduction tests, first introduced as the brilliant cresyl blue decolorization test, have been widely used. Other receptors for the electrons from NADPH include methylene blue, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) and methaemoglobin. New tests, often adaptations of older methods, are being developed and tested in field locations in which a rapid method is highly desirable for diagnosis before primaquine administration. In patient screening for risk assessment, such as excluding G6PD-deficient individuals for the treatment of malaria with primaquine, qualitative tests reliably detect G6PD deficiency only in males and homozygous females. Heterozygous females may be recorded as normal in screening tests, depending on the proportions of normal and deficient cells, because of unequal X chromosome inactivation. Genotyping studies usually target only the specific mutations commonly found in the population (such as those in the G6PD A– African variant), and other polymorphisms or mutations in the *G6PD* gene are not detected. All the available assays are PCR-based and require molecular reagents such as primers, enzymes and PCR machines in a laboratory setting; they are therefore not currently realistic options for testing at points of care. Both the screening fluorescence test and the quantitative test could indicate normal G6PD activity during an acute haemolytic episode in G6PD-deficient individuals, because they detect and measure the activity of the remaining (non-haemolysed), younger red cell population, which has higher levels of G6PD activity, after the G6PD-deficient cells have been destroyed. If G6PD deficiency-related acute intravascular haemolytic anaemia is suspected, any potentially dangerous drug, such as primaquine, should be discontinued and the G6PD test repeated after 10–30 days, or

Figure 16. The fluorescence spot assay for glucose-6-phosphate dehydrogenase deficiency



Courtesy of G. Bancone, Shoklo Malaria Research Unit, Thailand, 2012

later if the patient has been transfused. In these cases, genotyping for G6PD deficiency or family studies can help in the diagnosis. In regions with a high prevalence of anaemia, standardization of the tests to low Hb levels is necessary in order to obtain reliable results.

In 2005, in a phase-II clinical trial of methylene blue for antimalarial therapy in 80 men and 222 children in Burkina Faso, a qualitative point-of-care fluorescence test on paper was compared with PCR genotyping of blood samples on filter paper for detecting G6PD deficiency. There was good agreement between the two tests, although the fluorescence test can result in classification of heterozygous females as normal, as was the case for seven girls in this study. The estimated sensitivity of the test was 98.2% (95.8–99.6%), and the specificity was 97.1% (94.2–99.2%), suggesting that it is a reliable method for large-scale G6PD deficiency screening in rural West Africa. The cost per test (labour and equipment) was <0.40 euros, while that for PCR genotyping was 8 euros; however, the fluorescence test on paper requires an ultraviolet light source costing 200 euros (*247*).

No threshold of G6PD activity has been defined experimentally that predicts risk for haemolysis reliably, although experts have recommended that 30% of normal levels be used as a cut-off point, above which clinically significant haemolytic reactions are unlikely to occur (*197*). It is relevant that the widely used modified fluorescence spot test for G6PD screening usually detects levels <30% of normal. In a study of 13 G6PD-deficient and 13 non-deficient Thai patients with vivax malaria treated with chloroquine followed by 15 mg primaquine daily for 14 days, no correlation was found between G6PD activity and degree of haemolysis in the G6PD-deficient patients. The activity levels were, however, all <30% normal: mean (standard deviation), 0.25 (0.40) U/g Hb, as compared with 6.35 (0.70) U/g Hb in G6PD-normal patients (*248*).

Recent tests

A recently described enzymatic method (*249*) involves use of the terazolium salt WST8 and a less light-sensitive form of the phenazinium methyl sulfate hydrogen carrier, resulting in a colorimetric assay that can be used with dried blood spots and which is suitable for field use, both qualitatively by visual examination and quantitatively by reading absorbance. When this test was used in malaria-endemic villages in Indonesia and Myanmar, 30 cases of severe (25 males, five females) and 23 of mild G6PD deficiency (6 males, 17 females) were found among 1286 participants in Indonesia and 57 cases of severe (45 males, 12 females) and 34 of mild G6PD deficiency (5 males, 29 females) were detected among 855 people in Myanmar (*250*). A comparison with another method (Hirono) indicated that the new enzymatic method can detect both severely deficient individuals and heterozygous females in the field with 100% specificity and sensitivity. No estimates of cost were provided.

A method for mass screening for G6PD deficiency in the field in Isabel Province, Solomon Islands, was developed by optimizing the WST8 method for use with dried blood spots. The method was tested during a malaria survey, which already required sampling of finger-prick blood for microscopy and filter paper for PCR. Fresh controls (normal, moderately deficient, severely deficient and no enzyme) were also spotted onto filter paper, dried at ambient temperature, stored individually in re-sealable plastic bags containing silica desiccant beads and refrigerated (4–8 °C) when possible. All samples and calibrating control dried spots were assayed in 96-well plates, which were examined visually and the absorbance quantified in a microplate reader at $OD_{450–595}$. Normal G6PD activity was indicated by a dark yellow-to-orange strip and severe and moderate deficiency by almost colourless and faintly yellow strips, respectively. Comparison of biochemical and rapid diagnostic tests indicated that the results were accurate if blood spots were assayed within 5 days when stored at ambient temperature and 10 days when stored at 4 °C. During screening of 8541 people in 41 villages, the prevalence of G6PD deficiency (defined as enzyme activity <30% of normal) was 20.3% and that of severe deficiency (WHO classes I–II) was 6.9% (*251*).

BinaxNOW®, a qualitative chromatographic rapid diagnostic test for G6PD activity, was recently tested in the USA and showed high sensitivity (98%) and specificity (97–98%), accurately detecting samples with <4.0 U/g Hb and those with higher enzyme activity (*252*). The device consists of a lateral flow test strip with reagents for G6PD reaction and reduction of a nitro blue tetrazolium dye into its concomitant blue formazan product. A change in colour from red to brown or black indicates G6PD activity, and no change indicates G6PD deficiency. Significant limitations of this test are that it must be performed at 18–25 °C (so most tropical environments are unsuitable), will apparently cost up to US$ 25 per test and has not been validated with samples of blood containing malaria parasites. False-normal results were obtained for a sample with a G6PD level close to the threshold value (3.72 U/g Hb). At 4.0 U/g Hb, it included patients with moderate enzyme

deficiency and classified more patients as deficient than the fluorescence spot method. Most of the G6PD-deficient individuals evaluated were African Americans, i.e. likely to have the A– variant.

An experimental qualitative, rapid diagnostic test for G6PD deficiency called CareStart™ is based on a chromatographic change from the colourless nitro blue tetrazolium dye to dark-purple formazan in the presence of G6PD-normal blood. It was first tested in the field in a comparison of its performance with that of quantitative spectrophotometry for estimating G6PD enzyme activity in 903 individuals enrolled in four villages in Pailin Province, Cambodia (*253*). The test had relatively low sensitivity (68%; 95% CI, 58–77%) and high specificity (100%; 95% CI, 99–100%), as it detected G6PD deficiency at an activity threshold of <2.7 U/g Hb (22% of G6PD-normal activity threshold) in individuals with severe enzyme deficiency (WHO classes I and II and some class III variants). This result is similar to that with the fluorescence spot method, which has been used since 1979. Individuals with intermediate or mild G6PD deficiency (3.6–2.7 U/g Hb, 22–30% normal activity) were not identified as deficient, and only 40% of G6PD Viangchan heterozygote females were identified as deficient. Whether this is "good" or "bad" in terms of primaquine therapy depends on the sensitivity of these patients to the drug. False-negative results were obtained for 12 males and 1 female with enzyme activity <2 U/g Hb; these individuals would be classified as G6PD-normal, receive primaquine and be at risk for haemolysis. The authors discussed the possibility of classifying any purple colour, even if pale (as opposed to dark purple), as positive in order to avoid false-negative results. One advantage of this test for field use is its thermal stability: similar performance was found when it was stored at 35 °C or 45 °C for 90 days and at 55 °C for 72 h.

A modified, hand-held CareStart™ G6PD biosensor system has been developed, which is composed of an electrochemical photometric biosensor for simultaneous, quantitative detection of G6PD activity and Hb concentrations in whole blood within 4 min from a 10-µL finger-prick whole-blood sample (Young Choi, Access Bio, personal communication). It involves electrochemical measurement of G6PD activity and photometric measurement by the azide methaemoglobin method for Hb concentration. This system can avoid interference from the haematocrit by simultaneously measuring the amount of Hb in one strip, analysing G6PD activity per gram of Hb and adjusting for the temperature (37 °C), which changes automatically from the ambient temperature by use of a temperature sensor. The device is small and light, weighing only 124 g, and it can store up to 1000 results. Its price is yet to be announced. Clearly, more information on the costs and performance of the rapid point-of-care tests is needed before they can be recommended widely.

3.2.7 Haemolytic anaemia and malaria

Evaluating the risk for haemolytic anaemia of G6PD-deficient individuals who receive primaquine as an antimalarial drug is not straightforward, as many patients presenting with falciparum or vivax malaria are already anaemic, sometimes severely (Hb <5 g/dL). Most malaria-associated anaemia is found in children. Thus, when evaluating the haemolytic risk posed by primaquine, it is critical to know Hb levels before treatment. Anaemia might confer some protection from the haemolytic effects of primaquine because a younger circulating RBC population is more resistant to drug-induced haemolysis and, in chronic anaemia, there is time for an adaptive right shift in the oxygen dissociation curve.

The terms "anaemia" and "haemolysis" are often used interchangeably in the context of primaquine administration, which is misleading, particularly as malaria, especially in children, is strongly associated with anaemia. In 2008, it was estimated that anaemia affects approximately 24.8% of the world's population, or 1.62 billion people (*254*). A list of mean Hb concentrations was given by country, based on national and subnational surveys of variable quality. Threshold levels of Hb were used to define anaemia in various age and gender groups living at sea level: 11 g/dL for children <5 years and pregnant women, 11.5 g/dL for children aged 5–12 years, 12 g/dL for children aged 5–15 years, 12 g/dL for non-pregnant women and 13 g/dL for men (> 15 years). It was estimated that the highest prevalence was in preschool-age children (47.4%; 95% CI, 45.7–49.1) and the largest number of individuals affected were non-pregnant women (468.4 million; 95% CI, 446.2–490.6). The proportion of people with anaemia is highest in Africa, but the largest numbers of people with anaemia live in Asia.

Patients with malaria are frequently anaemic, for various reasons. Anaemia may also precede acute infection and be related to nutritional deficiency in iron or other micronutrients, intestinal helminthiasis, pregnancy, previous recurrent malaria infections and other co-morbid conditions including HIV infection, all of which are common in tropical countries. Haemoglobinopathies (thalassaemias, sickle-cell anaemia, ovalocytosis, Hb C and Hb E) are common in malaria-endemic regions. Thus, the haemolytic effects of primaquine in malaria may occur against a background of disease-associated haemolysis, which in some cases is severe. Haemolysis in malaria patients with G6PD deficiency may be triggered by the malaria infection itself, another infection or drugs. Studies of patients with malaria who were screened for G6PD deficiency have not shown that deficient patients are more anaemic at presentation than G6PD-normal individuals, which does not suggest preferential haemolysis of the more G6PD-deficient red cells associated with malaria (*236*, *255*). In acute malaria, the severity of anaemia can range from mild to severe and life-threatening. Development of anaemia in malaria is secondary to haemolysis (of both parasitized and unparasitized red cells), compounded by dyserythropoiesis. Haemolysis may be massive, as in the poorly understood "blackwater fever" often associated with quinine therapy in the

past (some but not all cases are associated with G6PD deficiency). In low-transmission settings, it is common for Hb levels to fall after treatment of acute falciparum malaria, typically reaching a nadir 7 days after the start of treatment and then gradually returning to pretreatment levels over 2–4 weeks. In higher-transmission settings, an increase in Hb levels is immediate after effective treatment (*256*). In vivax malaria, similar falls in Hb concentration are observed, despite the fact that the parasite biomass is typically lower than in symptomatic falciparum infections. This has been attributed to reticulocyte invasion by *P. vivax*, which has a predilection for younger erythrocytes and proportionally greater removal of uninfected RBCs than *P. falciparum* (*257*). Malaria-associated anaemia is attenuated by effective treatment. This benefit is offset slightly by treatment regimens containing artemisinin derivatives, which are associated with a slightly greater early fall in Hb because they temporarily suppress erythropoiesis. Sporadic cases of haemolysis after ACT administration have also been reported, e.g. with artemether–lumefantrine (*258*, *259*). In studies of severe malaria, blackwater fever, reflecting substantial intravascular haemolysis, was slightly commoner after artesunate and artemether than after quinine (*260–262*). In patients with high parasite densities treated with artesunate, delayed haemolysis may be evident, probably resulting from accelerated destruction of once-infected "pitted" erythrocytes (*263*).

Extensive information has been compiled for more than a century on the relation between malaria and anaemia. The prevalence of anaemia in children is directly proportional to the intensity of malaria transmission. A review published in 1992 on anaemia in falciparum malaria patients, including children, in Africa included unpublished studies by the authors on severe malaria in children on the coast of Kenya, where 109 (25%) of 452 paediatric patients admitted with a primary diagnosis of falciparum malaria had an Hb concentration <5 g/dL (*264*). In Uganda, 16% of deaths in infants were attributed to malarial anaemia (*265*). In a retrospective review of the clinical records of 1116 children <5 years of age admitted to a hospital in western Kenya, reported in 2007, 86% of admissions were children <3 years; 83% had malaria parasitaemia, 86% were anaemic (Hb <11 g/dL), 21% were severely anaemic (Hb ≤5 g/dL), and 20% required transfusion. Severe anaemia was associated with parasitaemia in 85% of the admissions and contributed to 53% of malaria-related deaths. A total of 191 of 1067 children (18%) had severe malarial anaemia; 171 of these (89.5%) were <3 years of age. Children aged 1–5 months had the highest prevalence (59/227, 26.0%) and children 48–59 months of age the lowest (5/52, 9.6%) (*266*).

A similar strong association of anaemia with malaria-endemic populations has been reported in South America. A report from Venezuela in 2008 on the incidence of anaemia and malaria in semi-nomadic indigenous Yanomami populations in the Amazon region showed that malaria and low Hb concentration were strongly associated. The mean Hb concentration was 2 g/dL lower in people with malaria than in those not infected (8.97 g/dL and 10.98 g/dL, respectively). The malaria was mainly *P. vivax*

(60.9%), with some *P. falciparum* (30.4%), and 8.7% were mixed infections (*267*). As reported in several other studies in malaria-endemic regions, the prevalence of malaria was greatest in children <5 years (20%) and declined with age: 14.3% in children aged 5–14 years and 5.9% in adults. Recent reports from Colombia on symptoms observed in endemic populations suffering from vivax malaria, including children, further underscore the importance of measuring initial Hb concentrations and anaemia before administering antimalarial drugs such as primaquine that pose a haemolytic risk for G6PD-deficient individuals. One such report, from 2008, showed that 1% of 4–10-year-old children, many with malnutrition, had severe anaemia (Hb <5 g/dL) (*268*). A report in 2009 showed that 85% of 4–10-year-old children with confirmed malaria had pathogenic intestinal parasites (*269*).

A report in 2010 of a study on the effect of adding primaquine to four different ACT regimens for falciparum malaria in Myanmar showed that patients had low Hb levels at presentation: 246 patients (30%) had <10 g/dL (mild anaemia) and 70 (9%) <8 g/dL (moderate anaemia). Young children were more likely to be anaemic at presentation (28/87, 32%) than older children (26/298, 9%) and adults (16/423, 4%). On day 63, eight of 693 patients (1%) were moderately anaemic and 72 (10%) were mildly anaemic. The mean increase in Hb concentration was similar in all treatment groups. It was unaffected by intercurrent *P. vivax* infection, but was slightly reduced by primaquine (0.75 g/dL versus 1.04 g/dL; $p = 0{\cdot}036$; mean difference, 0.295 g/dL; 95% CI, 0.199–0.570) (*171*).

The uncertainties over the risks associated with primaquine administration are related to unresolved questions about the risks for anaemia itself. The exact risks for death from anaemia in the context of haemolysis associated with malaria treatment are not well defined. In African children with strictly defined severe malaria, the hospital mortality rate rises steeply at an Hb concentration <3 g/dL (*242*). Perceived risks define transfusion thresholds. The transfusion threshold is often set at 4 g/dL for severe anaemia not associated with malaria when the availability of blood is limited. The threshold for severe malaria-associated anaemia in malaria-endemic areas is usually set at higher, at 5 g/dL, yet it has been suggested that this threshold should be raised, as children with severe anaemia have significant risks for both immediate and delayed mortality (*243–245*). In low-transmission areas, the threshold for transfusion is usually 7 g/dL. How this risk is translated for primaquine use is uncertain. Our review of detailed descriptions of severe and fatal haemolytic reactions after administration of 8-aminoquinolines suggests that two discrete lethal processes result from haemolysis. The first is severe anaemia and thus reduced oxygen delivery to vital organs. This depends on both the Hb concentration and the oxygen dissociation curve, which is shifted to the right in chronic anaemia, hence the relevance of the preceding degree and duration of anaemia. The second is renal failure after massive haemoglobinuria, which is more common in adults and is related more to the amount of red cells haemolysed than the absolute remaining Hb concentration.

An additional important unanswered question is whether G6PD-deficient patients with malaria are at additional risk for haemolysis when given primaquine or, conversely, whether malaria attenuates the haemolytic risk associated with primaquine by haemolysing older cells. For example, haematological recovery may be observed after an initial episode of haemolysis despite continuation of primaquine treatment in patients with mild variants of G6PD deficiency (such as the A– variant). In a study presented in two reports from the USA in 1952 and 1953 (*117*, *125*) on use of primaquine (15 mg daily for 14 days) or pamaquine, both given with chloroquine, in US soldiers returning to the USA after the Korean War with vivax malaria, no significant toxicity was observed. The 15% of patients who had mild-to-moderate anaemia at the beginning of treatment (Hb, 6.4–12 g/dL) all showed increasing Hb levels as the acute attack was controlled. A report from 1969 showed that in 176 US soldiers returning from Viet Nam, 93% with vivax and 3% with falciparum malaria, anaemia was common (15%) and was due mainly to haemolysis. Of the 27 patients presenting with anaemia, 9 had haemolytic anaemia due to malaria, with a rapidly falling haematocrit, reticulocytosis and hyperbilirubinaemia, in the absence of blood loss. Two patients were G6PD deficient, and the anaemia was related to unspecified antimalarial drugs; 16 had anaemia of undetermined etiology. Splenomegaly was found in 70% of anaemic patients and only 30% of non-anaemic patients (*128*).

A weekly 0.75-mg base/kg primaquine dose for radical cure of vivax malaria in patients with "mild" G6PD deficiency is one approach to mitigating haemolytic risk, by allowing haematological recovery while dosing. Another approach was developed by investigators in the former Soviet Union. A report on mass drug administration in Azerbaijan for post-eradication malaria therapy described "intermittent" treatment (4 days of 15 mg primaquine, no drug on days 5–7 when most primaquine-induced haemolytic reactions present, and 15 mg on days 8–17) that had been administered in areas with a high prevalence of G6PD deficiency. It was noted that G6PD-deficient malaria patients "tolerated" primaquine much better than uninfected G6PD-deficient patients. The authors hypothesized that malarial anaemia in infected patients decreases the number of old erythrocytes, and the new reticulocytes decrease the haemolytic action of primaquine in general (*200*, *201*).

Nine reports have been published on the effects of primaquine in a total of 126 G6PD-deficient people without malaria. Most of the participants were male (94%), including both adults and children. The participants were African American (presumably mainly G6PD A–), Iranian (common G6PD variants: Mediterranean, Chatham, Cosenza), Khmer (G6PD Mahidol) and white (English, Sicilian, Ashkenazi Jewish, Sardinian, Turkish, presumably mainly with the G6PD Mediterranean variant). The doses of primaquine varied from 0.3 mg/kg to 0.75 mg/kg in most studies, and the frequency of administration from daily to twice weekly to weekly. The quality and completeness of reporting of clinical and laboratory data were variable.

The initial Hb levels (if reported) were in the normal range. A haemolytic reaction was evident clinically in 36 cases (29%), and one required a blood transfusion. A drop in Hb by > 2 g/dL after treatment was recorded in 67 people (53%; 95% CI, 45–62%), and a drop of ≥ 5 g/dL was reported in four people in the studies of Iranian and Italian patients (3%; 95% CI, 1–8%). None of the participants had a post-treatment Hb concentration <5 g/dL. The level of G6PD activity was measured in the Cambodian and Italian patients and was <30% normal in all participants.

We found 10 published studies in which patients with vivax (seven studies) or falciparum malaria (three studies) and confirmed G6PD deficiency were treated with primaquine; two of the studies included patients infected with both species. The total population comprised 178 adults and children, of whom 17 were lost to follow-up. The patients were Kadazan (Sabah), Chinese–Kadazan, Chinese, Bajau, Thai, Burmese, Afghan, Tanzanian and African American. In two studies in the United Republic of Tanzania, patients with falciparum malaria received a single transmission-blocking dose (0.75 mg/kg) of primaquine. Most of the other patients were given weekly treatments or daily doses of primaquine for 1–2 weeks. The other antimalarial drugs used were chloroquine, quinine or artesunate–SP. Seven episodes of clinical haemolysis were observed in the patients studied in Sabah, two of whom had renal failure, and five required a blood transfusion. The G6PD variants in these patients are unknown, although many variants have been reported in other Malaysian studies (including Mediterranean). Pretreatment Hb was reported infrequently but was >8 g/dL when stated. None of the studies reported post-treatment Hb <5 g/dL, but this cannot be excluded, as the Hb levels were not specified in the study in Sabah in which patients required blood transfusion. The maximum drop in Hb was 5 g/dL in Sabah (number not specified) and in one patient in Thailand.

3.2.8 Blackwater fever

Blackwater fever is a poorly understood constellation of signs and symptoms that may be observed in acute malaria and which overlaps with severe primaquine haemolysis in G6PD-deficient patients. The hallmark of this sometimes fatal condition is severe haemoglobinuria. Blackwater fever is strongly associated with quinine treatment epidemiologically, and reports of cases declined dramatically after quinine was replaced by chloroquine. In the context of severe malaria, blackwater fever is slightly more common after treatment with artesunate or artemether than after quinine. Blackwater fever may be due to oxidant haemolysis in G6PD-deficient individuals.

The blackwater fever syndrome, of unclear pathogenesis, was described more than a century ago. It is associated with malaria and characterized by severe intravascular haemolysis and anaemia, with dark urine and sometimes renal failure in patients who present with fever. Acute haemoglobinuria (Figure 17) indicates massive intravascular haemolysis and is caused by a variety of factors in patients with *P. falciparum* infections,

Figure 17. Blackwater or haemoglobinuria

Severe haemolysis and passage of black urine in a Burmese G6PD-deficient man with vivax malaria who had received primaquine at 22.5 mg base/day. Reproduced from reference *270*

including overwhelming malaria with high parasitaemia, G6PD deficiency and other causes of haemolysis. Differentiating these causes is not straightforward, and it may be difficult to make a correct diagnosis. Originally, blackwater fever was described not only as a complication of malaria but a general reaction in colonial expatriates (whites) who took intermittent quinine prophylactically against malaria. Mortality was very high but has been much lower in more recent series. The introduction of chloroquine in 1950 to replace quinine was associated with a dramatic fall in the incidence of blackwater fever; however, the development of resistance to chloroquine led to the reintroduction of quinine and the reappearance of blackwater fever in some areas. The mysterious association between quinine and haemolysis has not hitherto been explained. Quinine itself is not an oxidant. Occasionally, quinine is associated with antibody-mediated autoimmune haemolytic anaemia (thrombocytopenia is more common) and with the haemolytic uraemic syndrome. The recent identification of an oxidant metabolite of quinine may help explain G6PD-associated quinine-induced haemolysis (*271*). In a fatal case of pamaquine poisoning in Southern Rhodesia (now Zimbabwe) reported in 1935 after a post-mortem examination, the urinary findings were similar to those of blackwater fever, except that intact red cells were present. Although this death was attributed

to pamaquine, the deceased had had blackwater fever on two occasions previously, each time precipitated by a small dose of quinine. Consequently, he had been advised by his medical attendant to take mepacrine rather than quinine for any subsequent attack of malaria, which he did, but it was accompanied by pamaquine (*33*).

In a review published in 1996 of 50 Vietnamese patients with blackwater fever admitted prospectively to a research ward, all of whom had fever and haemoglobinuria, 40 (80%) were jaundiced, 25 (50%) had hepatomegaly, 17 (34%) had splenomegaly, and 9 (18%) had hepatosplenomegaly. Twenty-one patients (42%) had impaired renal function, but only four (8%) developed oliguric renal failure, three (6%) of whom required dialysis. Thirty-two patients (64%) developed severe anaemia (haematocrit <20%). One patient died, corresponding to a mortality rate of 2%. Blackwater fever was associated with quinine ingestion in 28 patients (56%), G6PD deficiency in 27 (54%) and concurrent malaria infection in 16 (32%), with considerable overlap of these factors, suggesting that they may interact and that it may not be justifiable to regard haemoglobinuria caused by G6PD deficiency as a separate syndrome (*272*). A report in 2001 described 21 cases of blackwater fever seen in France in 1990–1999 among European expatriates who had lived in sub-Saharan Africa. All the patients had macroscopic haemoglobinuria, jaundice and anaemia. Acute renal failure occurred in 15 (71%), 7 of whom required dialysis. The presumed triggers of blackwater fever were halofantrine (38%), quinine (24%), mefloquine (24%) and halofantrine or quinine (14%). G6PD activity was normal in the 14 patients tested. Low-level *P. falciparum* parasitaemia was found in 8 patients. All 21 patients survived (*273*). In a report in 2009 of a study in Viet Nam on the correlation between G6PD deficiency and haemoglobinuria, 25.6% of the haemoglobinuric patients hospitalized between 1993 and 1996 had confirmed malaria. The authors noted that, since the early 2000s, few patients had been admitted to hospital for haemoglobinuria associated with malaria, which might have been due to the national recommendation to replace quinine by artesunate in the treatment of malaria in Viet Nam after 1998 (*274*).

3.2.9 Methaemoglobinaemia

Methaemoglobinaemia was originally reported as an adverse event with most 8-aminoquinolines, including pamaquine and primaquine, and was commonly observed and measured in early studies. Later studies focused mainly on anaemia. Clinically, the presence of methaemoglobin manifests as cyanosis (seen as blueness of the skin and lips), which becomes evident when 1 g (7%) of Hb is converted to methaemoglobin. When peroxides accumulate, resulting in Hb denaturation and binding to the cell membrane, Heinz bodies can be seen as intraerythrocytic inclusion bodies (Figure 10). Although methaemoglobinaemia reflects an oxidative process, it is a different oxidative process from that involved in the haemolysis associated with G6PD deficiency. As methaemoglobin accumulates in older cells, G6PD-deficiency haemolysis may paradoxically lower methaemoglobin levels in the presence of oxidant drugs.

3.3 Reports of primaquine-induced haemolysis and other severe adverse events

All severe adverse events in case reports and in formal studies on the safety of primaquine were reviewed, with data analysis to estimate risk. A total of 14 deaths (four probably due to overdose of primaquine in G6PD-deficient children) were reported with primaquine and 17 deaths with pamaquine during the comparatively shorter time that the latter was used as an anti-malarial agent. Most severe adverse events were reported in confirmed G6PD-deficient individuals. As the severe events in the group that was not tested for G6PD all occurred in either African American or 'primaquine-sensitive' individuals (probable G6PD A– variant), they were included in the G6PD-deficient group in this analysis. No severe adverse events were reported in G6PD-normal individuals. Thus, the severe adverse events reported in the literature were probably in G6PD-deficient individuals. Most of the events were acute haemolytic anaemia, with or without a requirement for blood transfusion. Haemolysis was often accompanied by anaemia (not always severe), haemoglobinuria and in some cases renal failure.

Anaemia has been reported inconsistently in studies of 8-aminoquinolines. As mild-to-moderate anaemia does not usually result in clinical symptoms, any drug-induced adverse events might have been missed and not reported, unless haematocrit or Hb concentrations were measured before and after primaquine treatment; the maximum decrease is seen around day 7 after primaquine treatment. In some studies, one of the two blood parameters was measured, but no values or criteria were given, resulting in reports of "severe haemolysis" or "a considerable drop in haematocrit/haemoglobin", without necessarily meeting the WHO definition of <5 g/dL for severe anaemia. We therefore included all severe adverse events reported (when specific Hb values were provided, "severe" indicated Hb <5 g/dL) and cases reported as severe haemolysis, acute haemolytic anaemia or haematocrit drop, which might not have been classified as severe in all studies. In general, falls of <2 g/dL Hb or up to 20% of the initial haematocrit were considered not severe. They did not require discontinuation of primaquine, hospitalization or blood transfusion, and the blood parameters usually returned to pretreatment levels after completion of treatment. The timing of sampling also varied; measurements should be made around 7 days after the start of primaquine administration in order to measure the greatest reduction in Hb concentration.

We found isolated case reports of primaquine-induced anaemia and associated symptoms. These cases were not part of formal studies and are therefore not included in Annex 2 (which describes studies of the safety of primaquine), as the denominator was unknown. Such cases usually presented to a local clinic with symptoms typical of haemolytic anaemia, including dark urine, and with unknown G6PD status, which was usually tested on admission because of anaemia and lack of malaria parasitaemia; they may therefore have been "falsely normal". Below, we discuss both

isolated reports of primaquine-related haemolysis as well as the safety studies described in detail in Annex 2, including mass drug administration, in chronological order.

3.3.1 Cases of haemolysis attributed to primaquine

A total of 190 adverse events in 190 people were reported. Most were in individuals with confirmed or suspected G6PD deficiency, including African Americans, Ceylonese (Sri Lankan) children, Iranians and Sardinians, and individuals in Vanuatu, elsewhere in South-East Asia and the Americas (Brazil, Cuba, Venezuela). The primaquine doses received ranged from 15 to 30 mg daily for treatment of vivax malaria to a single 45-mg dose as a gametocytocide in falciparum malaria.

In a study reported in 1952 on the toxicity of primaquine in male African American volunteers, five of of 110 men receiving 30 mg primaquine daily developed severe haemolysis requiring discontinuation of treatment after 4–9 days. Four men had elevated bilirubin and dark urine; all recovered spontaneously within 2–3 weeks, and none required transfusion (*275*; see Annex 2). In 1961, acute haemolysis was characterized in six "primaquine-sensitive", G6PD-deficient African American male volunteers given 30 mg/day primaquine. The acute initial haemolysis, consisting of a rapid fall in haematocrit, was followed by a recovery phase, starting with an increase in reticulocyte count, followed by an equilibrium or "resistant" phase, during which Hb and haematocrit returned to pretreatment levels despite continued drug administration. A second haemolytic crisis could be induced during this phase with a higher primaquine dose (240 mg daily). Anaemia, reticulocytosis, haemoglobinuria, scleral icterus and other clinical signs of haemolysis were not apparent until 2–4 days after the beginning of primaquine treatment (*204*; see Annex 2). In a study reported in 1962 in the USA, all 12 primaquine-sensitive individuals given 30 mg primaquine daily experienced acute haemolysis during up to 20 days of administration. The level of haemolysis seen with a single 120-mg dose was similar to that with 30 mg daily in primaquine-sensitive individuals (*276*; see Annex 2). This was the first study in which it was observed that methaemoglobin levels were more than threefold lower in primaquine-sensitive individuals (African American, probably G6PD A– variant) than in G6PD-normal individuals, who did not experience acute haemolysis. The authors reported that older erythrocytes (destroyed by primaquine) preferentially accumulate methaemoglobin when exposed to agents that oxidize Hb (shown in this study by use of sodium nitrate).

In 1953, a case of haemolytic anaemia in a 19-year-old African American was reported in a study of the toxicity and curative effects of 10–30 mg primaquine daily for Korean vivax malaria. The patient had received 20 mg/day primaquine and developed moderately severe haemolytic anaemia (Hb fell from 14.6 g/dL on day 1 to 9.4 g/dL on day 8, and the RBC count fell from 4.4 to 3.2 million/μL, with moderate haemoglobinuria), although he was asymptomatic. Primaquine was withdrawn, and the patient was given oral sodium bicarbonate for the next 4 days. Two days after

treatment was stopped, his Hb was 8.2 g/dL; he was given 1 L of blood, and his Hb and RBC values returned to normal during the 3-month follow-up (*127*; see Annex 2). Haemolysis was reported in 10 G6PD-deficient individuals in Iran (now the Islamic Republic of Iran) in 1967, including six children under 12 years of age, treated with an adult primaquine dose of 45 mg weekly for 4 weeks or longer; one of the children required a blood transfusion. All the patients developed some degree of haemolysis, most showing pallor, jaundice and dark urine. Three of the children had a history of favism, and most individuals had a family history of favism (*277*; see Annex 2). A report from Zanzibar (now part of the United Republic of Tanzania) in 1968 described primaquine-induced haemolysis in a 12-year-old Arab girl who had received 30 mg primaquine and 300 mg amodiaquine. She vomited and felt giddy within 1 h; after 3 days she was pale, feverish and passed dark urine and was admitted with jaundice, dehydration and 50% Hb. She received 500 mL of blood on days 2 and 4 and was discharged after 2 weeks with 70% Hb. The patient was tested for G6PD 1 year later and found to have gross deficiency; she also had sickle-cell trait (*278*).

In a report from Ceylon (now Sri Lanka) in 1968, 21 children aged 2–12 years were admitted to hospital with acute intravascular haemolysis after receiving primaquine. Of these, 17 were tested for G6PD activity and found to be deficient. The authors noted that of 432 children given primaquine at the hospital (incidence of G6PD deficiency unknown), only one was included in the group of 21 who developed haemolysis; all the other children had been treated with primaquine elsewhere, and the doses they had received were often unknown. Most children ≤ 5 years were given half a tablet of 7.5 mg (3.75 mg) daily for 2–5 days, whereas children > 5 years received one tablet a day. Eight children had received doses higher than that recommended, mostly because the parents failed to follow instructions; therefore, some of the cases might have been due to primaquine overdosing. All 21 children had dark urine 1–5 days after treatment, presented with haemoglobinuria and anaemia (Hb measured in 11 children was 2.4–12.1 g/dL), were icteric and had hepatosplenomegaly. Treatment with primaquine was discontinued. Eight children had pain in the back, loin or abdomen, seven vomited, nine were in "acute cardiac failure", and five were drowsy, with oliguria and high blood urea. Urgent blood transfusions were required in 14 cases, 12 received steroid therapy, 9 were treated for congestive cardiac failure, and 5 were treated for renal failure. Four children died: three of hypoxia and severe cardiac failure due to anaemia and one from irreversible renal failure (*279*). In a study reported in 1969 on the haemolytic effect of a single dose of 45 mg primaquine given with 300 mg chloroquine weekly to 25 G6PD-deficient Sardinians, the mean drop in haematocrit ranged from 42.8% before to 33.2% after primaquine; there were six cases of severe haemolysis (>20% fall) (*212*; see Annex 2).

In 1981, several cases of primaquine-induced haemolysis were reported in a study in Malaysia, in which 23 G6PD-deficient malaria patients, including seven children, received either 75 mg primaquine over 3 days for falciparum malaria or 210 mg primaquine over 14 days for vivax or mixed

infections (children received proportionally less drug), all with chloroquine for 3 days. Seven patients (three aged 13, 14 and 16 years) had haemolysis 2–5 days after primaquine was started, and all had dark urine. Five required blood transfusions, and the other two had acute renal failure, one requiring peritoneal dialysis. The drop in Hb was 1–5 g/dL (*280*; see Annex 2).

A case of severe haemolysis and acute renal failure was reported in 1989 in a 28-year-old Thai soldier who had been taking pyrimethamine–dapsone weekly for 1 year without experiencing side-effects. When vivax parasitaemia was found in his blood, he received 3 days of chloroquine (total dose, 1500 mg) with primaquine (15 mg daily) for 4 days. He started vomiting and had dark urine and a pale appearance; renal failure was diagnosed, and he was admitted to hospital, where he received a blood transfusion on day 16 and renal dialysis. He recovered gradually. Four months later, tests showed he was G6PD deficient (*281*). In a report from Vanuatu in 1992, several patients presented to hospital with acute intravascular haemolysis after standard malaria treatment followed by a single dose of 45 mg primaquine as gametocytocidal treatment. All seven patients tested were G6PD deficient. Four patients had passed dark urine, most had marked reticulocytosis, and their Hb levels were 5.4, 4, 6.4 and 12.6 g/dL (*282*).

Three reports of acute haemolysis in patients in Cuba further illustrate the haemolytic risk posed by giving primaquine to G6PD-deficient individuals. The first report, in 1989, was of a comparative study of 1000 men returning to Cuba from malaria-endemic regions, 500 of whom were tested for G6PD deficiency by methylene blue and 500 were not. All the latter received 3 days of chloroquine (600 mg on day 1 and 450 mg on days 2 and 3, each with 30 mg primaquine daily) followed by 15 mg primaquine daily for 11 days. Careful chronological follow-up of the patients for haemolysis showed that 87% of cases occurred 5–7 days after the start of treatment with 105–135 mg primaquine. In the group that included G6PD-deficient patients receiving primaquine, all 16 G6PD-deficient men (3.2%) experienced haemolysis (*283*). The second report, in 1995, described two male Arab patients, presumably with the G6PD Mediterranean variant (assumed, not tested), who showed adverse symptoms 1–2 days after starting primaquine at 15 mg daily. The first patient presented 1 day later with jaundice, an Hb concentration of 7.5 g/dL, 15.2% reticulocytosis and diminished G6PD activity (no details given). He received 10 days of hydration and transfusions and recovered completely. The second patient presented with vomiting, anorexia, tachycardia, haematuria, oliguria, fever and pallor; he later developed uraemia and was admitted to a nephrology institute, where he stayed for 1 month (*284*). The third report, in 1997, described Cuban patients with vivax malaria who had been in Angola and received treatment with 15 mg primaquine daily for 14 days. They included eight G6PD-deficient patients (two severely deficient, with <10% normal enzyme activity, four moderate with 10–60% activity and two slightly deficient with 60–100%), probably with the A– variant. A decrease in Hb of 1–5% was attributed to haemolysis, and treatment was discontinued when a >2 g/dL decrease in

Hb was observed. Haemolysis occurred in seven G6PD-deficient patients between 1 and 7 days after the start of treatment, and four could not finish treatment because of the stringent withdrawal criteria, although no severe effects were observed (*285*). In a report from Venezuela in 2003, six vivax malaria patients who had acquired their infections when travelling to the same malaria-endemic region were admitted to hospital and given chloroquine–primaquine. A child aged 6 years with an initial Hb level of 8 g/dL developed a primaquine-triggered haemolytic reaction, with jaundice and fever (even after parasites had disappeared from the blood), and his Hb level dropped to 3.6 g/dL. After tests showed G6PD deficiency, primaquine was discontinued and treatment was re-started with chloroquine alone. The patient's Hb levels improved after transfusion, and he was well 1 month later (*286*).

A primaquine-related death was reported in 2002 in an 18-year-old man who was G6PD deficient with the Mediterranean variant, who had taken at least one dose of primaquine (45 mg). He developed severe haemolytic anaemia and acute renal failure and required blood transfusion (*287*).

In a study in Thailand reported in 2003, 18 of 44 G6PD-deficient patients received primaquine at 30 mg daily for 14 days after artesunate. Five patients left hospital before finishing treatment, nine completed treatment, and four were withdrawn from treatment on days 4–7 after primaquine because of significantly reduced haematocrits (with fractional reductions of 16%, 13%, 14% and 8%), with a mean change of 0.79% in non-G6PD-deficient patients (*146*; see Annex 2).

Haemolytic anaemia after primaquine prophylaxis for malaria was reported in 2005 in two soldiers (one African American, one of Asian descent) who had returned to the USA from Iraq. Both presented with headache, dark urine and haemolysis during treatment with primaquine; both proved to be G6PD-deficient, and both responded well to conservative treatment after cessation of therapy (*288*).

Overdose may cause serious haemolysis in G6PD deficiency. A report in 2010 described a case in a 35-year-old G6PD-deficient Burmese man who presented to a clinic on the Myanmar–Thailand border with intravascular haemolysis. Primaquine and chloroquine had been prescribed 4 days earlier for a *P. vivax* infection, but he had misunderstood the instructions and taken all the remaining primaquine tablets together on the fourth day for a total of 165 mg. He began to vomit and had severe abdominal pain with black urine. His blood urea (53.3 mg/dL) and serum creatinine (2.1 mg/dL) concentrations were elevated, his white blood cell count was 21 600/µL, his Hb concentration was 6.9 g/dL and his platelet count 13 600/µL (*270*).

A report in 2010 from a tertiary care unit in the western Brazilian Amazon described the development of haemolysis in 18 G6PD-deficient patients treated for vivax malaria with chloroquine and primaquine. The most

frequent findings accompanying haemolysis were fever and leukocytosis, in addition to anaemia requiring transfusion in 12/18 patients. Acute renal failure developed in three patients, which resolved well without haemodialysis. The main clinical symptoms were: jaundice (18/18), pallor (17/18), dark urine (14/18), fever (12/18), vomiting (10/18), dehydration (6/18), cyanosis (3/18) and low urinary output (1/18). Symptoms of haemolysis began 1–6 days after primaquine, and 12 patients had leukocytosis (leukocyte count >12 000/μL), which might have been due to the haemolytic crisis (*289*). In 2010, during mass drug administration, 564 Tanzanian children were given a single dose of 45 mg primaquine on day 3 of artesunate–SP. One case of severe anaemia was reported in a 5-year-old child, who had an Hb level of 4.8 g/dL (baseline, 8.3 g/dL), was given haematinic drugs and recovered without additional treatment: the Hb concentration was 7.8 g/dL after 2 weeks and 12.3 g/dL 3 months after the intervention (*219*).

A report from a tertiary care centre in the Brazilian Amazon in 2012 gave the results of the autopsies of 17 patients who died after a clinical diagnosis of *P. vivax* infection. Two deaths, both in G6PD-deficient individuals, were considered to be related to severe primaquine-related haemolysis (*290*).

3.3.2 Non-haemolytic severe adverse events due to administration of primaquine

A WHO evaluation in 1978 of the malaria control programme in Turkey, where G6PD deficiency is prevalent in several ethnic groups, described 60 admissions for severe complications and five deaths among G6PD-deficient patients who had received radical treatment for vivax malaria with primaquine (*291*). The nature of the severe adverse events was not specified.

One death due to hepatic necrosis was reported within the system of spontaneous adverse event reporting in the United Kingdom (*292*), which appears to be the same death in a 70-year-old man reported to the Uppsala Monitoring Centre in 1977 (*293*). No further details were provided. Another death was reported to the Uppsala Monitoring Centre from the USA in 1997, described as related to either primaquine or cefipime given to a man with thrombocytopenia and diarrhoea. He had also received didanosine, zidovudine and clindamycin (*293*), suggesting an underlying diagnosis of HIV infection and possibly *P. jiroveci* pneumonia, which is treated with primaquine and clindamycin.

Neuropsychiatric effects observed after administration of primaquine have been reported. A single case report from the USA in 1980 described depression and psychosis in a 55-year-old man with malaria who was treated with chloroquine and then primaquine at 15 mg daily, starting the day before discharge from hospital. The day after the second dose of primaquine, he was extremely depressed, anorectic, confused, forgetful and imagined events that had not occurred; none of these symptoms had been present before, and all disappeared within 24 h of discontinuation of primaquine

(*294*). In 1985, a doctoral thesis in France described mass administration of antimalaria drugs in Nicaragua in 1973–1983 to almost 2 million people (excluding infants). Chloroquine and primaquine were given over 3 days (adult dose, 15 mg primaquine per day, 600 mg chloroquine on day 1 and 450 mg of each on days 2 and 3; children were given proportionally less in age blocks). An undetermined number of cases of vertigo and rare cases of psychomotor agitation and transitory neurological problems were reported, which resulted in cessation of treatment (*295*).

Primaquine, like other aminoquinolines, has some effects on cardiac electrophysiology, but there is no evidence of significant cardiotoxicity at therapeutic doses (*296*). Laboratory studies suggest that primaquine blocks cardiac Na^+ channels by binding to open channels (*297*) and also affects outward K^+ channels (*268*), but neither effect is considered significant at therapeutic doses of primaquine.

3.3.3 Reports to the Uppsala Monitoring Centre of adverse events associated with administration of primaquine

The Uppsala Monitoring Centre, a WHO collaborating centre, provided all individual case reports submitted since 1969 in which primaquine was suspected of being a causative or possible interacting factor. A total of 1429 reports describing 4560 reactions or events were submitted to the Centre from all WHO regions. Many of the reporting countries had no pharmacovigilance system, and their systems relied on passive reporting of events from various sources; therefore, the amount of detail provided varies widely. This was the case for reports on primaquine, so that it was difficult to assess the severity of the reactions. Unlike case reports published in peer-reviewed journals, Monitoring Centre reports do not usually contain sufficient information from the original report to allow classification of events into categories for analysis, especially for the severity of the event.

In several Monitoring Centre reports on primaquine, other drugs were implicated as possible contributors to the reaction. For instance, many reports from Thailand mentioned dizziness in patients who had also received artesunate and mefloquine, which were listed as possible causes. Mefloquine is well known to cause dizziness, sleep disturbance and other neuropsychiatric reactions. In some reports, dapsone was also administered, which is known to pose a haemolytic risk to G6PD-deficient individuals. As noted above, information on the dose received and the G6PD status of the patient was lacking in most reports; these two categories of data were essential for our analyses (see below).

Given the limitations of the data in the Monitoring Centre reports and especially the uncertainty about the severity of events, we included in this review only the two deaths attributed to primaquine. One of the patients probably had HIV/AIDS and *P. jiroveci* pneumonia; therefore, the disease rather than its treatment might have caused death. All the other events reported that might have been categorized as life-threatening or severe if

more information had been available, events in which G6PD deficiency was listed as a factor and all events involving haemolytic anaemia, other types of anaemia and methaemoglobinaemia are listed in Annex 4.

3.4 Incidence of severe adverse events due to primaquine in various populations

The severe adverse events, including deaths, considered to have been caused by primaquine described in all the studies and case reports included in this review were used to calculate the approximate incidence in several categories. Populations treated by mass drug administration and otherwise and various subpopulations were analysed separately to determine the approximate risks of G6PD-deficient and G6PD-normal individuals associated with different regimens of primaquine.

3.4.1 Deaths reported after administration of pamaquine and primaquine

Table 7 lists all deaths attributed to pamaquine and primaquine. A total of 17 deaths were due to pamaquine and were all reported before 1950. At least 12 of these (70%) were associated with haemoglobinuria. During the past six decades, 14 deaths have been attributed to primaquine: ten among patients admitted to hospital and four among children who might have received overdoses of primaquine.

Table 7. Deaths attributable to iatrogenic haemolytic anaemia due to 8-aminoquinolines

Pamaquine	Primaquine
Four deaths reported before 1931 *(29–32)* One death reported in 1935 *(33)* Two deaths among four cases of haemoglobinuria reported in India in 1936 after 20 mg pamaquine daily for 3 days following mepacrine injections in patients also taking medication for syphilis *(34)* Six deaths among 14 cases of haemolysis and haemoglobinuria reported in 1943 after 30 mg pamaquine daily *(35)* Three deaths reported in 1946 among 13 Indian (mostly Punjabi) soldiers given "blanket treatment" with 10 mg pamaquine twice daily for 5 days, who developed haemoglobinuria *(298)* One death reported in 1949 from the USA of a vivax malaria patient who erroneously received three doses of 0.4 g pamaquine (total, 1.2 g) and died 7 days later *(40)*	Four deaths reported in 1968 among 21 G6PD-deficient children in Ceylon aged 2–12. Three were due to hypoxia and severe cardiac failure from anaemia and one to irreversible renal failure. One child had also received aspirin for 2 days. The children were admitted to hospital with acute intravascular haemolysis; however, treatment had been administered elsewhere, and the authors considered that some children might had been overdosed *(279)* Five deaths reported in Turkey in 1978 among 60 G6PD-deficient patients who had received primaquine for vivax malaria *(291)* One death due to hepatic necrosis reported spontaneously in the United Kingdom *(292)* Two deaths reported in 2012 in G6PD-deficient patients with vivax malaria, both found at autopsy to be due to primaquine-triggered haemolysis *(290)* One death resulting from acute renal failure was reported in 2002 in a G6PD-deficient (Mediterranean variant) 18-year-old man who had received at least one dose of 45 mg primaquine (Hb nadir, 2.8 d/dL), had been hospitalized for haemolytic anaemia and required blood transfusion *(287)* One death reported from the USA in 1997 to the Uppsala Monitoring Centre of a man with thrombocytopenia and diarrhoea and suspected HIV/AIDS with *P. jiroveci* pneumonia *(293)*
Total: 17 deaths	Total: 14 deaths

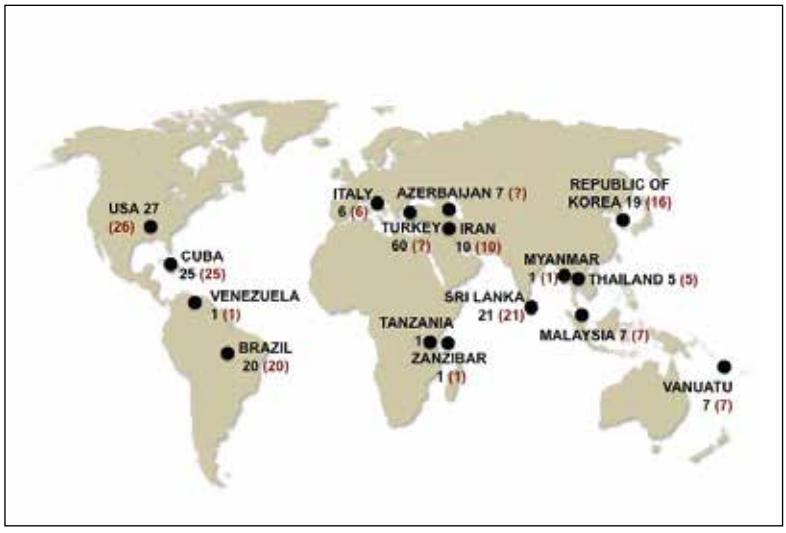
3.4.2 Severe adverse events reported after ingestion of primaquine

A total of 219 severe adverse events were attributed to primaquine, 78 in studies of its safety (27 in mass drug administration studies and 51 in other studies) and 141 in case reports. The distribution is worldwide (Figure 18). Details of the events, their location and the primaquine regimen are discussed below. Most of the events were acute haemolytic anaemia (see section 3.3.1); one case report described the only neurological event. Of the other cases, one was severe urticaria, two were methaemoglobinaemia, one was severe anaemia and 67 were hospitalizations for unknown reasons, which could have included some cases of acute haemolytic anaemia.

Studies of mass drug administration

The results of 12 studies of mass drug administration in which primaquine was given to a total of more than 9 million people in various countries are presented in Table 8 (for more details, see Annex 2). Of the 27 severe adverse events reported, 16 were confirmed haemolysis, giving incidences of three events and 1.9 confirmed cases of haemolysis per million people receiving primaquine. In five studies, primaquine was administered as a 15-mg daily dose for 14 days to a total of nearly 9 million people, and in seven studies it was administered as a single dose on the third day of artesunate or at a dose of 9, 30, 40 or 45 mg weekly or every 2 weeks to about 75 000 people. All the severe adverse events reported in these studies of mass drug administration were seen with 15 mg primaquine daily, except for one that occurred after a single 45-mg dose given with artesunate.

Figure 18. Geographical locations of reports of primaquine-related severe



The number of severe adverse events reported per country is indicated in black; the red number in brackets is the number of cases of acute haemolytic anaemia

Table 8. Severe adverse effects after mass administration of primaquine

Reference	Location and total population treated	Regimen	Prevalence of G6PD deficiency in population	Severe adverse events reported
Archambeault, 1954 (299)	Soldiers (*N* = 415 340) returning to the USA by ship from Korea. 332 925 completed the full course of 14 days	15 mg daily for 14 days with breakfast or lunch	Not determined	Two men reported to be allergic to primaquine; one man developed severe urticaria that disappeared when treatment was stopped and reappeared when resumed Two methaemoglobinaemia One haemolytic anaemia A few reports of mild-to-moderate dusky cyanosis that did not require drug withdrawal
Clyde, 1961 *(177)*	Tanganyika, *N* ≈ 15 000	30 mg weekly every 2 weeks or monthly with 150 mg amodiaquine	Not determined	None
Comer et al., 1971 *(104)*	Panama, *N* ≈ 2500	40 mg adult dose with pyrimeth-amine every 2 weeks	Not determined	None
Hodgkinson et al., 1961 *(1)*	East Africa and Congo, *N* = 235 children <12 years with starting mean Hb of 71%, indicating anaemia at presentation	Amodiaquine–primaquine tablets containing 15 mg primaquine administered weekly for 5 weeks at 0.25–0.5 mg	Not determined	None
Kaneko et al., 2000 *(105)*	Vanuatu (Aneityum Island), *N* = 718	45 mg chloroquine weekly with or without SP	Not determined	None
Vivona et al., 1961 *(300)*	US military and civilian personnel (n = 50 000) and Turkish troops (*n* = 250)	45 mg weekly combined in one tablet with 300 mg chloroquine at lunch with food	One Turkish man was G6PD-deficient; some G6PD deficiency A– predicted in African Americans	None
Shekalaghe et al., 2010 *(219)*	United Republic of Tanzania, *N* = 564	45 mg single dose as gametocyto-cide given on day 3 after 3 days of artesunate, SP given on day 1	By genotyp-ing only; 8.4% of *G202A* mutation heterozy-gotes and 3.9% of homo- or hemizygotes	Considerable but transient reduction in Hb; no cases required hospitalization. 5% of children became moderately anaemic (< 8 g/dL Hb) after SP–artesunate–primaquine: relative risk compared with G6PD B, 2.46 (95% CI, 0.77–7.84) for G6PD A and 8.87 (4.12–19.09) for G6PD A–. One 5-year-old developed severe anaemia, with Hb level of 4.8 g/dL (baseline, 8.3 g/dL), was given haematinic drugs and recovered with no additional treatment (Hb = 7.8 g/dL 2 weeks and 12.3 g/dL 3 months after intervention)

Other studies

Table 9 lists the severe adverse events reported in the studies and case reports included in this review, other than mass drug administration studies. Of 69 studies, 20 included G6PD-deficient individuals and in 49 the G6PD deficiency status was unknown or G6PD-deficient individuals were excluded (some in the older literature being "primaquine-sensitive" African Americans). No severe adverse events were reported in G6PD-normal individuals, with the possible exception of a psychotic reaction. Of the 192 severe adverse events reported, 25 occurred in 25 individuals whose G6PD status was not determined, and 167 were reported in G6PD-deficient individuals. The majority (87%) of events were reported in people with confirmed G6PD deficiency. Of the events in individuals whose G6PD status was not determined (13%), all except one occurred in either African American or "primaquine-sensitive" individuals (probably G6PD A– variant); they were therefore included in the G6PD-deficient group. Thus, all the severe adverse events reported in the literature were probably experienced by G6PD-deficient individuals.

Most of the events observed after primaquine were haemolysis, with or without a requirement for blood transfusion. In G6PD-deficient individuals, confirmed haemolysis represented 63.8% of all events, although more cases were probably included among the 60 Turkish patients with "other" severe complications, which were not described further (see Table 9). Haemolysis was often accompanied by anaemia, dark urine, haemoglobinuria, leukocytosis and in some severe cases by renal failure that required haemodialysis. The Hb concentration or fall in concentration was not always reported; therefore, the number of cases of severe anaemia might have been underestimated.

In order to estimate the global risk posed by primaquine administered to G6PD-deficient individuals, we included only studies of the effects in this population and excluded case reports. We then calculated the proportion of individuals who experienced severe adverse events in each G6PD category. No such events were reported in 5036 G6PD-normal individuals. Among 9696 individuals of unknown G6PD status, 12 events were reported in African Americans, who were probably G6PD-deficient, and 12 events in "primaquine-sensitive" individuals, for a total incidence of 24/9696 (0.25%). In the 241 confirmed G6PD-deficient individuals, the incidence of severe adverse events was 27/241 (11.2%).

In case reports, the severe adverse events described were one case of psychosis in a person of undefined G6PD status and 125 events in G6PD-deficient individuals.

3.4.3 Incidence of severe adverse events associated with different primaquine regimens

When we categorized all severe adverse events reported in the studies of the safety of primaquine and in case reports by the dose received (Table 10), we determined that 12% of all events occurred after a probable overdose, 75.5% after daily doses of either 15 or 30 mg (administered mainly for

Table 9. Severe adverse events due to primaquine reported in case reports and studies other than of mass drug administration

Severe adverse event	G6PD status not determined (primaquine dose taken)	G6PD-deficient individuals (primaquine dose taken)
Severe haemolysis	11 African American, 12 primaquine-sensitive (30 mg daily)	9 (6 children < 12 years) in Iran (45 mg weekly) 7 in Vanuatu (45 mg single dose); one case of anaemia 2 in Malaysia (15 mg daily); two cases of acute renal failure, one requiring peritoneal dialysis 6 Sardinians; self-contained haemolysis with decrease in haematocrit > 20% 16 Cubans (30 mg daily for 3 days, 15 mg daily afterwards) 7 Cubans (15 mg daily for 14 days) 1 Venezuelan child aged 6 years (vivax treatment); had severe anaemia, Hb fell from 8 to 3.6 g/dL 6 Brazilians (primaquine–chloroquine) 2 Brazilians (primaquine–chloroquine for vivax malaria); both died 4 Thais on primaquine (30 mg daily) had reduced haematocrit (8–16%) and were withdrawn on days 4–7 2 US soldiers (primaquine prophylaxis) 1 Burmese man (15 mg daily for 3 days then 165 mg single dose on day 4) 7 children in Ceylon aged 2–12 years (probably overdosed) 1 Arab in Cuba (15 mg daily for 14 days) presented with vomiting, anorexia, tachycardia, haematuria, oliguria, fever and pallor, later developed hyperazotaemia; was admitted for renal failure and discharged 1 month later Total, 71
Haemolysis with blood transfusion	1 African American (20 mg daily)	12-year-old Arab in Zanzibar (30 mg) Iranian child aged < 12 years (45 mg weekly adult dose) 14 children in Ceylon aged 2–12 years (probably overdosed); five had renal failure 5 Malaysians (15 mg daily) Thai soldier (15 mg daily for 4 days); had renal failure that required haemodialysis 12 Brazilians (primaquine–chloroquine); three had renal failure that required haemodialysis Arab in Cuba (15 mg daily for 14 days) with jaundice, Hb 7.5 g/dL, 15.2% reticulocytosis; recovered completely One 18-year-old Indian man who was G6PD-deficient with the Mediterranean variant received at least one dose of 45 mg, developed acute renal failure and died Total, 36
Other (unspecified)	One case of depression and psychosis in a 55-year-old man in the USA with malaria, who was treated with chloroquine and then primaquine 15 mg daily starting the day before discharge from hospital. Symptoms appeared after the second dose and disappeared within 24 h after primaquine was discontinued Total, 1	60 “severe complications” on admission to a Turkish hospital due to intake of primaquine for vivax malaria Total, 60
Total (%)	25 (13%)	167 (87%)

“Severe anaemia”, Hb concentration < 5 g/dL or reported as “severe anaemia”

Table 10. Severe adverse events after various doses of primaquine

Probable or confirmed overdose	For vivax malaria, 15 or 30 mg daily	Single or weekly doses of 30, 40 or 45 mg
One in Burma (15 mg daily for 3 days, then 165 mg single dose on day 4); haemolysis 21 children in Ceylon aged 2–12 years (probably overdosed); haemolysis requiring transfusion in 14 and no transfusion for 7 One 18-year-old Indian man who was G6PD-deficient with the Mediterranean variant received at least one dose of 45 mg, developed acute renal failure that required blood transfusion and died	23 African American and "primaquine-sensitive" individuals (30 mg); haemolysis One African American (20 mg); haemolysis requiring transfusion 16 Cubans (30 mg daily for 3 days, 15 mg daily afterwards); haemolysis Seven Cubans (15 mg daily for 14 days); haemolysis Four Thais (30 mg daily); haemolysis Two US soldiers (prophylaxis); haemolysis One Venezuelan child aged 6 years (vivax treatment); haemolysis with severe anaemia, Hb fell from 8 to 3.6 g/dL Five cases in Malaysia (15 mg daily); haemolysis requiring transfusion Two cases in Malaysia (15 mg daily); haemolysis with acute renal failure, one requiring peritoneal dialysis One Thai (15 mg daily for 4 days); haemolysis requiring transfusion and renal failure requiring dialysis 18 Brazilians (primaquine–chloroquine); 6 cases of haemolysis not requiring transfusion, 12 requiring transfusion; 3 cases of renal failure requiring haemodialysis Two Brazilians (chloroquine–primaquine for vivax malaria); death One case of depression and psychosis in the USA (15 mg for 2 days) Two Arabs in Cuba (15 mg daily for 14 days); one had jaundice, Hb 7.5 g/dL, 15.2% reticulocytosis, recovered completely after treatment; the other had vomiting, anorexia, tachycardia, haematuria, oliguria, fever and pallor, and later hyperazotaemia; recovered after 1 month 60 "severe complications" on admission to a Turkish hospital (vivax malaria)	Nine Iranians (6 children < 12 years) (45 mg weekly adult dose); haemolysis Seven cases in Vanuatu (45 mg single dose); haemolysis; one case of severe anaemia One 12 year-old in Zanzibar (30 mg); haemolysis requiring transfusion One Iranian child aged < 12 years (45 mg weekly adult dose); haemolysis requiring transfusion Six Sardinians; self-contained haemolysis with decrease in haematocrit > 20%
Total, 23 (12%) Children, 21 (91.3%)	Total, 145 (75.5%) Children, 2 (1.4%)	Total, 24 (12.5%) Children, 8 (33.3%)

Includes all severe adverse events shown in Table 9 and all events experienced by G6PD-deficient African American or "primaquine-sensitive" individuals, who were probably variant A– G6PD-deficient. We excluded 60 cases of "severe complications" admitted to a Turkish hospital in which the primaquine dose was unknown.

vivax malaria) and 12.5% after administration of 40 or 45 mg primaquine as a single dose or weekly. The majority of the events reported in cases in which primaquine might have been administered at a dose higher than that recommended were in children (91.3%), most of whom were Ceylonese and aged 2–12 years; only 1.4% of the events reported occurred in children given 15 or 30 mg primaquine daily. When primaquine was administered as a single or weekly dose, 33.3% of all severe adverse events were in children. As most of the studies involved only adults, however, except for the case reports of overdosing, these results are not representative of the risk of primaquine regimens in children.

Next, we excluded the case reports and used the results of safety studies to estimate the risk for severe adverse events and also for acute haemolytic anaemia by primaquine dose in two categories: in mass drug administration studies and in safety studies.

Mass drug administration

The incidence of severe adverse events after mass drug administration is shown in Table 11. The incidence was low with both daily regimens (2.9 per million) and single or weekly doses (13.3 per million, representing one case of severe anaemia). Of all the severe adverse events reported after daily administration of primaquine, 61.5% were haemolysis, giving an estimated incidence of significant haemolysis of 1.8 cases per million exposed individuals.

Table 11. Severe adverse events after mass drug administration of primaquine, by regimen

Primaquine regimen	No. of studies	Total population	Severe adverse events	Severe haemolysis or anaemia (% of all severe adverse events)
Daily dose (15 or 30 mg)	5	About 9 million	One severe urticaria, two methaemoglobinaemia, one haemolytic anaemia, seven hospitalizations, 15 cases of black urine with suspected haemolysis	16 (61.5%; 1.8 per million)
Single dose (9, 30 or 45 mg)	1 (45 mg) 6 (weekly, every 10 days, semi-weekly or monthly primaquine, administered with chloroquine, pyrimethamine or amodiaquine) Total, 6	564 (single dose 45 mg) 74 743 (weekly, every 10 days, semi-weekly or monthly primaquine, administered with chloroquine, pyrimethamine or amodiaquine) Total, 75 307	One case of severe anaemia in a Tanganyikan child; 5% of children in the same study showed moderate anaemia, < 8 g/dL, not requiring hospitalization Total, 1 (13.3 per million)	1 (100%)

Other studies

In the safety studies (with smaller cohorts than in studies of mass drug administration), the incidence of severe adverse events was 0.26% (95% CI, 0.17–0.35) when primaquine was given daily and 0.42% (95% CI, 0.22–0.63) when given as single or weekly doses (Table 12). In the latter category, 43.8% of all severe adverse events were in children under 12 years. All the events were acute severe haemolytic reactions. Case reports described 108 events with a daily regimen and 8 with a single 30- or 45-mg dose.

3.4.4 Limitations of the studies

The lack of detailed information in the studies and cases reports precluded precise estimates of the risk associated with primaquine. Few studies evaluated the safety of primaquine given as a falciparum gametocytocide at a single dose, and in none was it administered at a dose <30 mg. Thus, the efficacy and haemolytic risk of single doses <30 mg of primaquine as a gametocytocide, especially in G6PD-deficient individuals, are unknown, although they can be inferred from the studies with repeated doses and unpublished studies.

Table 12. Severe adverse events with different regimens of primaquine

Regimen	No. of studies	Population	Severe adverse events	Incidence
Daily (15 or 30 mg)	51	13 451	23 African American and primaquine-sensitive individuals (30 mg), haemolysis; one African American (20 mg), haemolysis requiring transfusion; four Thais (30 mg daily), haemolysis; five Malaysians (15 mg daily), haemolysis requiring transfusion; two Malaysians (15 mg) haemolysis and acute renal failure, one requiring peritoneal dialysis Total, 35	0.26%
Weekly or single dose (30, 40 or 45 mg)	13 single dose; 7 weekly or semi-weekly; 2 at 40 mg over 2 days and 30 mg over 3 days; 1 at a single dose of 120 mg Total, 23	1623, single dose; 1996, weekly or semi-weekly dose; 149, 40 mg over 2 days and 30 mg over 3 days; 3 at a single dose of 120 mg Total, 3771	Nine Iranians (six children < 12 years) (45 mg weekly), acute severe haemolysis; one Iranian child < 12 years (45 mg weekly), haemolysis requiring blood transfusion; six Sardinians, self-contained haemolysis with haematocrit fall of > 20% Total, 16 (seven children, 43.8%)	0.42%

Haemolysis and anaemia were not reported consistently in all studies, and neither Hb values nor cut-off values for severe anaemia were always specified. When Hb concentrations after administration of primaquine were reported, we used an Hb concentration of <5 g/dL to define severe anaemia in the context of malaria. Any haemolysis or anaemia described as "severe" or "acute" and which required hospitalization and blood transfusion was considered a severe haemolytic reaction. Therefore, the risk for haemolysis and anaemia cannot be estimated precisely. The risks for specific decreases in Hb concentration could also not be estimated.

Most of the detailed pathophysiological studies of haemolysis after primaquine in G6PD-deficient populations have been performed in people with the A– variant, many of whom did not have malaria. The effect of malaria on the risk for haemolysis is unclear. The frequency or severity of clinical haemolysis with primaquine treatment does not appear to differ between patients with malaria and people without malaria.

Few studies included G6PD-deficient individuals because of the known risk of this population for haemolysis. Most studies excluded confirmed G6PD-deficient individuals (or tested for G6PD status and excluded them) and used various methods to detect G6PD deficiency; only a small fraction gave genotyping results for G6PD variants. These limitations prevented us from estimating the risks associated with specific G6PD variants. The most information was available for the African A– variant. Moreover, the lack of genotyping and the few females in the reviewed studies mean that all the risk estimates are applicable only to G6PD-deficient males.

Because of concern about potential haemolysis, in none of the studies we reviewed was primaquine administered to pregnant or lactating women or infants, and there were no cases reported in these populations.

4. Conclusions

Primaquine is the only generally available drug with radical curative properties for *P. vivax* and *P. ovale* malaria. It is also a powerful gametocytocide in falciparum malaria. In gametocytaemic patients, a single dose added to effective ACT rapidly reduces the transmissibility of the treated infection. Primaquine causes dose-dependent haemolysis in G6PD-deficient individuals. The risks associated with a single dose are significantly lower than those associated with continued dosing. The risk for life-threatening haemolysis of a single gametocytocidal primaquine dose given to individuals with mild or moderate G6PD deficiency is very low.

5. References

1. Hodgkinson R, Courtney KO, Haggerty M. Effect of intermittent administration of a combination of amodiaquin and primaquine (Camoprim) on the hematocrit of primaquine-sensitive and non-sensitive children. *American Journal of Tropical Medicine and Hygiene*, 1961, 10:128–134.
2. Rawlins M. De testimonio: On the evidence for decisions about the use of therapeutic interventions. *Lancet*, 2008, 372:2152–2161.
3. James SP, Nicol MB, Shute PG. On the prevention of malaria with plasmoquine. *Lancet*, 1931, ii:341–342.
4. Kingsbury AN, Amies CR. A field experiment on the value of plasmoquine in the prophylaxis of malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1931, 25:159–172.
5. Jones R et al. A study of the prophylactic effectiveness of several 8-aminoquinolines in sporozoite-induced vivax malaria (Chesson strain). *Journal of Clinical Investigation*, 1948, 27:6–11.
6. Sweeney AW, Blackburn CR, Rieckmann KH. Short report: The activity of pamaquine, an 8-aminoquinoline drug, against sporozoite-induced infections of *Plasmodium vivax* (New Guinea strains). *American Journal of Tropical Medicine and Hygiene*, 2004, 71:187–189.
7. Sinton JA, Bird W. Studies in malaria with special reference to treatment; plasmoquine in treatment of malaria. *Indian Journal of Medical Research*, 1928, 16.
8. Sinton JA, Smith S, Potinger D. Studies in malaria, with special reference to treatment. XII. Further researches into treatment of chronic benign tertian malaria with plasmoquine and quinine. *Indian Journal of Medical Research*, 1930, 17.
9. Sinton JA. *Note on the treatment of chronic benign tertian malaria with plasmoqune and quinine*. Geneva: World Health Organization, 1931.
10. Vad BG, Mohile GB, The place of plasmochin on the treatment of malaria. *Indian Medical Gazette*, 1927, 62:430–434.
11. Most H et al. Combined quinine-plasmochin treatment of vivax malaria; effect of relapse rate. *American Journal of the Medical Sciences*, 1946, 212:550–560.
12. Ruhe DS et al. Studies in human malaria; the therapeutic action of pamaquine against St Elizabeth strain vivax malaria. *American Journal of Hygiene*, 1949, 49:367–373.
13. Barber, MA, Komp WHW. The viability of gametocytes in the blood of plasmochin-treated patients. In: *United Fruit Company 16th Annual Report*. Boston, Massachusetts, United Fruit Company, Medical Department, 1927:60–62.
14. Barber MA, Komp WH, Newman BM. The effect of small doses of plasmochin on the viability of gametocytes of malaria as measured by mosquito infection experiments. *Public Health Reports of the United Fruit Company*, 1929, 44: 1409–1420.

15. Green R. *The treatment of "crescent carriers" with plasmoquine compound*. Kuala Lumpur, Government Printing Office, 1929:1–20 (Bulletins Institute for Medical Research (Malaysia), Issue 3).
16. Green R. *The treatment of quartan malaria with plasmoquine*. Kuala Lumpur, Government Printing Office, 1929 (Bulletins Institute for Medical Research (Malaysia), Issue 3).
17. Kligler IJ, Mer G. Studies on malaria: V. Therapeutic value of mixtures of plasmochin and quinine. *Rivista di Malariologia*, 1930, 9:272–283.
18. Biggam AG, Arafa MA. Observations on a series of cases of atificially induced subtertian malaria with special reference to the effect of treatment by plasmoquine compound. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1930, 23:591–607.
19. Jerace F, Giovannola A. L'azione sterilizzante della plasmochina sui gameti dei parassiti malarigeni a sua importanza profilattica [The sterilizing action of plasmoquine on gametocytes of malaria parasites and its prophylactic importance]. *Rivista di Malariologia*, 1933, 12:457.
20. Carman JA. Atebrin, plasmoquine and quinine in the treatment of malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1935, 29:191–202.
21. Mackerras MJ, Ercole QN. Observations on the action of quinine, atebrin and plasmoquine on the gametocytes of *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1949, 42:455–463.
22. White NJ. Primaquine to prevent transmission of falciparum malaria. *Lancet Infectious Diseases*, 2013; 13:175–181.
23. Mackerras MJ, Ercole QN. Some observations on the action of quinine, atebrin, and plasmoquine on *Plasmodium vivax*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1949, 42:443–454.
24. Dick GW, Bowles RV. The value of plasmoquine as a gametocide in sub-tertian malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1947, 40:447–450.
25. Monk JF. Results of an investigation of the therapeutic action of paludrine and pamaquin on acute attacks of benign tertian malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1948, 41:657–662.
26. Berliner RW et al. Studies on the chemotherapy of the human malarias. Vii. The antimalarial activity of pamaquine. *Journal of Clinical Investigation*, 1948, 27:108–113.
27. Zubrod CG, Kennedy TJ, Shannon JA. Studies on the chemotherapy of the human malarias; the physiological disposition of pamaquine. *Journal of Clinical Investigation*, 1948, 27: 114–120.
28. Weniger H. *Toxicity and side effects of primaquine and other 8-aminoquinolines*. Geneva: World Health Organization, 1979.
29. Menk W. Combined quinine and plasmochin treatment for malaria in Haitian Negroes. In: *United Fruit Company 16th Annual Report*.

Boston, Massachusetts, United Fruit Company, Medical Department, 1927:78–81.

30. Cordes W. Experiences with plasmochin in malaria. Preliminary reports. In: *United Fruit Company 17th Annual Report*. Boston, Massachusetts, United Fruit Company, Medical Department, 1926:66–71.
31. Cordes W. Observations on the toxic effects of plasmochin. In: *United Fruit Company 16th Annual Report*. Boston, Massachusetts, United Fruit Company, Medical Department, 1927:62–67.
32. Manifold JA. Plasmoquine and quinine in the treatment of malaria. *Journal of the Royal Army Medical Corps*, 1931, 56:231–238; 410–423.
33. Blackie WK. A fatal case of plasmoquine poisoning. *South African Medical Journal*, 1935, 9:147–148.
34. Simeons ATW. Mass treatment with injectable atebrin. *Indian Medical Gazette*, 1936, 71:132–137.
35. Mann WN, Smith S. Haemoglobinuria following the administration of plasmoquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1943, 37:151–156.
36. Hardgrove M, Applebaum IL. Plasmochin toxicity; analysis of 258 cases. *Annals of Internal Medicine*, 1946, 25:103–112.
37. Craige B et al. Clinical standardization of Pamaquin (plasmochin) in mosquito-induced vivax malaria, Chesson strain: a preliminary report. *American Journal of Tropical Medicine and Hygiene*, 1947, s1-27:309–315.
38. Clayman CB et al. Toxicity of primaquine in Caucasians. *Journal of the American Medical Association*, 1952, 149:1563–1568.
39. Cooper WC et al. Studies in human malaria. XXXI. Comparison of primaquine, isopentaquine, SN-3883, and pamaquine as curative agents against Chesson strain vivax malaria. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:949–957.
40. Loken AC, Haymaker W. Pamaquine poisoning in man, with a clinicopathologic study of one case. *American Journal of Tropical Medicine and Hygiene*, 1949, 29:341–352.
41. Alving AS et al. The clinical trial of 18 analogues of pamaquin (plasmochin) in vivax malaria, Chesson strain. *Journal of Clinical Investigation*, 1948, 27:34–45.
42. Loeb RF. Activity of a new antimalarial agent, pentaquine (SN 13,276). *Journal of the American Medical Association*, 1946, 132:321–323.
43. Craige B et al. The toxicity of large doses of pentaquine (Sn-13,276), a new antimalarial drug. *Journal of Clinical Investigation*, 1948, 27:17–24.
44. Coatney GR et al. Studies in human malaria. XXVII. Observations on the use of pentaquine in the prevention and treatment of Chesson strain vivax malaria. *Journal of the National Malaria Society*, 1950, 9:222–233.
45. Alving AS et al. Pentaquine (Sn-13,276), a therapeutic agent effective in reducing the relapse rate in vivax malaria. *Journal of Clinical Investigation*, 1948, 27:25–33.

46. Strauss B, Gennis J. Radical cure of relapsing vivax malaria with pentaquine-quinine: A controlled study. *Annals of Internal Medicine*, 1950, 33:1413–1422.
47. Schmidt LH et al. Comparison of the curative antimalarial activities and toxicities of primaquine and its d and l isomers. *Antimicrobial Agents and Chemotherapy*, 1977, 12:51–60.
48. Vale N, Moreira R, Gomes P. Primaquine revisited six decades after its discovery. *European Journal of Medicinal Chemistry*, 2009, 44:937–953.
49. Tekwani BL, Walker LA. 8-Aminoquinolines: Future role as antiprotozoal drugs. *Current Opinion in Infectious Diseases*, 2006, 19:623–631.
50. *AIDSInfo drug database*. Rockville, Maryland: United States Department of Health and Human Services (http://aidsinfo.nih.gov/drugs/447/primaquine-phosphate/0/patient, accessed 7 February 2014).
51. Fletcher KA et al. Studies on the pharmacokinetics of primaquine. *Bulletin of the World Health Organization*, 1981, 59:407–412.
52. Ward SA et al. Pharmacokinetics of primaquine in man. II. Comparison of acute vs chronic dosage in Thai subjects. *British Journal of Pharmacology*, 1985, 19:751–755.
53. Bangchang KN et al. Pharmacokinetics of primaquine in G6PD deficient and G6PD normal patients with vivax malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1994, 88:220–222.
54. Kim YR et al. Pharmacokinetics of primaquine and carboxyprimaquine in Korean patients with vivax malaria. *Archives of Pharmacal Research*, 2004, 27:576–580.
55. Bhatia SC et al. Pharmacokinetics of primaquine in patients with *P. vivax* malaria. *European Journal of Clinical Pharmacology*, 1986, 31:205–210.
56. Cuong BT et al. Does gender, food or grapefruit juice alter the pharmacokinetics of primaquine in healthy subjects? *British Journal of Pharmacology*, 2006, 61:682–689.
57. Binh VQ et al. Sex affects the steady-state pharmacokinetics of primaquine but not doxycycline in healthy subjects. *American Journal of Tropical Medicine and Hygiene*, 2009, 81:747–753.
58. Edwards G et al. Interactions among primaquine, malaria infection and other antimalarials in Thai subjects. *British Journal of Pharmacology*, 1993, 35:193–198.
59. Mihaly GW et al. Pharmacokinetics of primaquine in man: Identification of the carboxylic acid derivative as a major plasma metabolite. *British Journal of Pharmacology*, 1984, 17:441–446.
60. Mihaly GW et al. Pharmacokinetics of primaquine in man. I. Studies of the absolute bioavailability and effects of dose size. *British Journal of Pharmacology*, 1985, 19:745–750.
61. Pybus BS et al. CYP450 phenotyping and accurate mass identification of metabolites of the 8-aminoquinoline, anti-malarial drug primaquine. *Malaria Journal*, 2012, 11:259.

62. Arnold J et al. The antimalarial action of primaquine against the blood and tissue stages of falciparum malaria (Panama, P-F-6 strain). *Journal of Laboratory and Clinical Medicine*, 1955, 46:391–397.
63. Geary TG, Divo AA, Jensen JB. Activity of quinoline-containing antimalarials against chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* in vitro. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1987, 81:499–503.
64. Baird JK et al. Short report: Therapeutic efficacy of chloroquine combined with primaquine against *Plasmodium falciparum* in north-eastern Papua, Indonesia. *American Journal of Tropical Medicine and Hygiene*, 2002, 66:659–660.
65. Baird JK, Fryauff DJ, Hoffman SL. Primaquine for prevention of malaria in travelers. *Clinical Infectious Diseases*, 2003, 37:1659–1667.
66. Baird JK, Rieckmann KH. Can primaquine therapy for vivax malaria be improved? *Trends in Parasitology*, 2003, 19:115–120.
67. Pukrittayakamee S et al. Activities of artesunate and primaquine against asexual- and sexual-stage parasites in falciparum malaria. *Antimicrobial Agents and Chemotherapy*, 2004, 48:1329–1334.
68. World Health Organization. *Guidelines for the treatment of malaria*, 2nd ed. Geneva, 2010.
69. Hallett RL et al. Combination therapy counteracts the enhanced transmission of drug-resistant malaria parasites to mosquitoes. *Antimicrobial Agents and Chemotherapy*, 2004, 48:3940–3943.
70. Piyaphanee W et al. Emergence and clearance of gametocytes in uncomplicated *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene*, 2006, 74:432–435.
71. Price RN et al. Effects of artemisinin derivatives on malaria transmissibility. *Lancet*, 1996, 347:1654–1658.
72. Chotivanich K et al. Transmission-blocking activities of quinine, primaquine, and artesunate. *Antimicrobial Agents and Chemotherapy*, 2006, 50:1927–1930.
73. Tangpukdee N et al. Gametocyte clearance in uncomplicated and severe *Plasmodium falciparum* malaria after artesunate–mefloquine treatment in Thailand. *Korean Journal of Parasitology*, 2008, 46:65–70.
74. Stepniewska K et al. *Plasmodium falciparum* gametocyte dynamics in areas of different malaria endemicity. *Malaria Journal*, 2008, 7:249.
75. Shekalaghe S et al. Primaquine clears submicroscopic *Plasmodium falciparum* gametocytes that persist after treatment with sulphadoxine–pyrimethamine and artesunate. *PLoS One*, 2007, 2:e1023.
76. Bousema T et al. Revisiting the circulation time of *Plasmodium falciparum* gametocytes: Molecular detection methods to estimate the duration of gametocyte carriage and the effect of gametocytocidal drugs. *Malaria Journal*, 2010, 9:136.
77. Global Malaria Programme. *Single dose primaquine as a gametocytocide in* Plasmodium falciparum *malaria. Updated WHO policy recommendation (October 2012)*. Geneva: World Health Organization (http://www.who.int/malaria/pq_updated_policy_recommendation_en_102012.pdf, accessed 7 February 2014).

78. Brondz I et al. Nature of the main contaminant in the anti malaria drug primaquine diphosphate: A qualitative isomer analysis. *Journal of Chromatography, B, Analytical Technologies in the Biomedical and Life Sciences*, 2004, 800:211–223.
79. Elbashir AA et al. Determination of quinocide as impurity in primaquine tablets by capillary zone electrophoresis. *Biomedical Chromatography*, 2009, 23:464–471.
80. Lysenko AY. Use of quinocide in treatment and prophylaxis of vivax malaria. *Bulletin of the World Health Organization*, 1960, 22:641–662.
81. Bruce-Chwatt LJ. Malaria research and eradication in the USSR. A review of Soviet achievements in the field of malariology. *Bulletin of the World Health Organization*, 1959, 21:737–772.
82. Coatney GR, Getz ME. Primaquine and quinocide as curative agents against sporozoite-induced Chesson strain vivax malaria. *Bulletin of the World Health Organization*, 1962, 27:290–293.
83. Mehrotra N et al. In vitro and in vivo pharmacokinetic studies of bulaquine (analogue of primaquine), a novel antirelapse antimalarial, in rat, rabbit and monkey—highlighting species similarities and differences. *Biopharmaceutics and Drug Disposition*, 2007, 28:209–227.
84. Krudsood S et al. Safety and tolerability of elubaquine (bulaquine, CDRI 80/53) for treatment of *Plasmodium vivax* malaria in Thailand. *Korean Journal of Parasitology*, 2006, 44:221–228.
85. Gogtay NJ et al. A randomized, parallel study of the safety and efficacy of 45 mg primaquine versus 75 mg bulaquine as gametocytocidal agents in adults with blood schizonticide-responsive uncomplicated falciparum malaria (ISCRTN50134587). *BMC Infectious Diseases*, 2006, 6:16.
86. Adak T, Valecha N, Sharma VP. *Plasmodium vivax* polymorphism in a clinical drug trial. *Clinical and Diagnostic Laboratory Immunology*, 2001, 8:891–894.
87. Brueckner RP et al. First-time-in-humans safety and pharmacokinetics of WR 238605, a new antimalarial. *American Journal of Tropical Medicine and Hygiene*, 1998, 58:645–649.
88. Shanks GD et al. A new primaquine analogue, tafenoquine (WR 238605), for prophylaxis against *Plasmodium falciparum* malaria. *Clinical Infectious Diseases*, 2001, 33:1968–1974.
89. Ponsa N et al. Transmission-blocking activity of tafenoquine (WR-238605) and artelinic acid against naturally circulating strains of *Plasmodium vivax* in Thailand. *American Journal of Tropical Medicine and Hygiene*, 2003, 69:542–547.
90. Coleman RE et al. Prevention of sporogony of *Plasmodium falciparum* and *P. berghei* in *Anopheles stephensi* mosquitoes by transmission-blocking antimalarials. *American Journal of Tropical Medicine and Hygiene*, 1994, 50:646–653.
91. Lell B et al. Malaria chemoprophylaxis with tafenoquine: A randomised study. *Lancet*, 2000, 355:2041–2045.

92. Walsh DS et al. Efficacy of monthly tafenoquine for prophylaxis of *Plasmodium vivax* and multidrug-resistant *P. falciparum* malaria. *Journal of Infectious Diseases*, 2004, 190:1456–1463.
93. Walsh DS et al. Randomized trial of 3-dose regimens of tafenoquine (WR238605) versus low-dose primaquine for preventing *Plasmodium vivax* malaria relapse. *Clinical Infectious Diseases*, 2004, 39:1095–1103.
94. Nasveld P et al. Comparison of tafenoquine (WR238605) and primaquine in the post-exposure (terminal) prophylaxis of vivax malaria in Australian Defence Force personnel. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:683–684.
95. Elmes NJ et al. The efficacy and tolerability of three different regimens of tafenoquine versus primaquine for post-exposure prophylaxis of *Plasmodium vivax* malaria in the Southwest Pacific. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2008, 102:1095–1101.
96. Jelinek T et al. Long-term efficacy of primaquine in the treatment of vivax malaria in nonimmune travelers. *American Journal of Tropical Medicine and Hygiene*, 1995, 52:322–324.
97. Rajgor DD et al. Efficacy of a 14-day primaquine regimen in preventing relapses in patients with *Plasmodium vivax* malaria in Mumbai, India. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2003, 97:438–440.
98. Bunnag D et al. High dose of primaquine in primaquine resistant vivax malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1994, 88:218–219.
99. Looareesuwan S et al. Primaquine-tolerant vivax malaria in Thailand. *Annals of Tropical Medicine and Parasitology*, 1997, 91:939–943.
100. Smoak BL et al. *Plasmodium vivax* infections in US Army troops: Failure of primaquine to prevent relapse in studies from Somalia. *American Journal of Tropical Medicine and Hygiene*, 1997, 56:231–234.
101. Duarte EC et al. Association of subtherapeutic dosages of a standard drug regimen with failures in preventing relapses of vivax malaria. *American Journal of Tropical Medicine and Hygiene*, 2001, 65:471–476.
102. Hill DR et al. Primaquine: report from CDC expert meeting on malaria chemoprophylaxis I. *American Journal of Tropical Medicine and Hygiene*, 2006, 75:402–415.
103. Weiss WR et al. Daily primaquine is effective for prophylaxis against falciparum malaria in Kenya: Comparison with mefloquine, doxycycline, and chloroquine plus proguanil. *Journal of Infectious Diseases*, 1995, 171:1569–1575.
104. Comer RD et al. Tratamiento colectivo con pirimetamina y primaquina para erradicar la malaria en Sambú, Panama [Mass treatment with pyrimethamine and primaquine for malaria eradication in Sambú, Panama]. *Boletín de la Oficina Sanitaria Panamericana*, 1971, 70:226–234.

105. Kaneko A et al. Malaria eradication on islands. *Lancet*, 2000, 356:1560–1564.
106. Fryauff DJ et al. Randomised placebo-controlled trial of primaquine for prophylaxis of falciparum and vivax malaria. *Lancet*, 1995, 346:1190–1193.
107. Baird JK et al. Primaquine for prophylaxis against malaria among nonimmune transmigrants in Irian Jaya, Indonesia. *American Journal of Tropical Medicine and Hygiene*, 1995, 52:479–484.
108. Soto J et al. Primaquine prophylaxis against malaria in nonimmune Colombian soldiers: Efficacy and toxicity. A randomized, double-blind, placebo-controlled trial. *Annals of Internal Medicine*, 1998, 129:241–244.
109. Soto J et al. Double-blind, randomized, placebo-controlled assessment of chloroquine/primaquine prophylaxis for malaria in nonimmune Colombian soldiers. *Clinical Infectious Diseases*, 1999, 29:199–201.
110. Baird JK et al. Randomized, parallel placebo-controlled trial of primaquine for malaria prophylaxis in Papua, Indonesia. *Clinical Infectious Diseases*, 2001, 33:1990–1997.
111. White NJ. Determinants of relapse periodicity in *Plasmodium vivax* malaria. *Malaria Journal*, 2011, 10:297.
112. Tiburskaja NA, Sergiev PG, Vrublevskaja OS. Dates of onset of relapses and the duration of infection in induced tertian malaria with short and long incubation periods. *Bulletin of the World Health Organization*, 1968, 38:447–457.
113. Coatney OR et al. Studies in human malaria. XVIII. The life pattern of sporozoite-induced St Elizabeth strain vivax malaria. *American Journal of Hygiene*, 1950, 51:200–215.
114. Coatney GR, Cooper WC, Young MD. Studies in human malaria. XXX. A summary of 204 sporozoite-induced infections with the Chesson strain of *Plasmodium vivax*. *Journal of the National Malaria Society*, 1950, 9:381–396.
115. Ungureanu E et al. Prepatent periods of a tropical strain of *Plasmodium vivax* after inoculations of tenfold dilutions of sporozoites. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1976, 70:482–483.
116. Coatney GR et al. Korean vivax malaria. V. Cure of the infection by primaquine administered during long-term latency. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:985–988.
117. Alving AS et al. Korean vivax malaria. II. Curative treatment with pamaquine and primaquine. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:970–976.
118. Di Lorenzo A et al. Korean vivax malaria. IV. Curative effect of 15 milligrams of primaquine daily for 7 days. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:983–984.
119. Alving AS et al. Potentiation of the curative action of primaquine in vivax malaria by quinine and chloroquine. *Journal of Laboratory and Clinical Medicine*, 1955, 46:301–306.

120. Collins WE, Jeffery GM. Primaquine resistance in *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene*, 1996, 55:243–249.
121. Arnold J, Alving AS, Clayman CB. Induced primaquine resistance in vivax malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1961, 55:345–350.
122. Chiang TY et al. Relapse of imported vivax malaria despite standard-dose primaquine therapy: An investigation with molecular genotyping analyses. *Clinical Microbiology and Infection*, 2012.
123. Carmona-Fonseca J, Alvarez G, Blair S. *Plasmodium vivax* malaria: Treatment of primary attacks with primaquine, in three different doses, and a fixed dose of chloroquine, Antioquia, Colombia, 2003–2004. *Biomedica*, 2006, 26:353–365.
124. Alvarez G et al. Efficacy of three chloroquine-primaquine regimens for treatment of *Plasmodium vivax* malaria in Colombia. *American Journal of Tropical Medicine and Hygiene*, 2006, 75:605–609.
125. Garrison PL et al. Cure of Korean vivax malaria with pamaquine and primaquine. *Journal of the American Medical Association*, 1952, 149:1562–1563.
126. Thaeler AD Jr, Arnold J, Alving AS. A clinical study of primaquine (S.N.13,272) in the treatment of malaria among the Miskito Indians of Nicaragua. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:989–999.
127. Jones R Jr et al. Korean vivax malaria. III. Curative effect and toxicity of primaquine in doses from 10 to 30 mg. daily. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:977–982.
128. Martelo OJ, Smoller M, Saladin TA. Malaria in American soldiers. *Archives of Internal Medicine*, 1969, 123:383–387.
129. Fisher GU et al. Malaria in soldiers returning from Vietnam. Epidemiologic, therapeutic, and clinical studies. *American Journal of Tropical Medicine and Hygiene*, 1970, 19:27–39.
130. Leslie T et al. A randomised trial of an eight-week, once weekly primaquine regimen to prevent relapse of *Plasmodium vivax* in Northwest Frontier Province, Pakistan. *PLoS One*, 2008, 3:e2861.
131. Basavaraj HR. Observations on the treatment of 678 malaria cases with primaquine in an area free from malaria transmission in Mysore State, India. *Indian Journal of Malariology*, 1960, 14:269–281.
132. Ungureanu E. *Note on the treatment of chronic benign tertian malaria with plasmoquine and quinine. The use of the association chloroquine–primaquine in the radical treatment of malaria*. Geneva, World Health Organization, 1962.
133. Contacos PG et al. Five day primaquine therapy—an evaluation of radical curative activity against vivax malaria infection. *American Journal of Tropical Medicine and Hygiene*, 1973, 22:693–695.
134. Miller LH et al. Sensitivity of four Central American strains of *Plasmodium vivax* to primaquine. *American Journal of Tropical Medicine and Hygiene*, 1974, 23:309–310.

135. Cedillos RA, Warren M, Jeffery GM. Field evaluation of primaquine in the control of *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene*, 1978, 27:466–472.
136. Singh J et al. Antirelapse treatment with primaquine and pyrimethamine. *Indian Journal of Malariology*, 1954, 8:127–136.
137. Roy RG et al. Results of 5-day course of radical treatment of *Plasmodium vivax* in six districts of Tamil Nadu. *Indian Journal of Medical Research*, 1979, 69:939–943.
138. Appavoo NC, Roy RG, Kapali V. Results of 3-day radical treatment of *Plasmodium vivax* in North Arcot and South Arcot Districts of Tamil Nadu. *Indian Journal of Malariology*, 1984, 21:21–24.
139. Sinha S, Dua VK, Sharma VP. Efficacy of 5 day radical treatment of primaquine in *Plasmodium vivax* cases at the BHEL industrial complex, Hardwar (UP). *Indian Journal of Malariology*, 1989, 26:83–86.
140. Singh N, Mishra AK, Sharma VP. Radical treatment of vivax malaria in Madhya Pradesh, India. *Indian Journal of Malariology*, 1990, 27:55–56.
141. Gogtay NJ et al. Efficacies of 5- and 14-day primaquine regimens in the prevention of relapses in *Plasmodium vivax* infections. *Annals of Tropical Medicine and Parasitology*, 1999, 93:809–812.
142. Dua VK, Sharma VP. *Plasmodium vivax* relapses after 5 days of primaquine treatment, in some industrial complexes of India. *Annals of Tropical Medicine and Parasitology*, 2001, 95:655–659.
143. Yadav RS, Ghosh SK. Radical curative efficacy of five-day regimen of primaquine for treatment of *Plasmodium vivax* malaria in India. *Journal of Parasitology*, 2002, 88:1042–1044.
144. Fernandopulle BM et al. Efficacy of a five-day course of primaquine in preventing relapses in *Plasmodium vivax* malaria—a pilot study. *Ceylon Medical Journal*, 2003, 48:32.
145. Solari-Soto L et al. Ensayo clinico del tratamiento de la malaria vivax con esquema acortado de primaquina comparado con el esquema tradicional [Clinical trial of treatment for vivax malaria with a short primaquine regimen compared with the usual regimen]. *Revista de la Sociedad Peruana de Medicina Interna*, 2002, 15:197–199.
146. Silachamroon U et al. Clinical trial of oral artesunate with or without high-dose primaquine for the treatment of vivax malaria in Thailand. *American Journal of Tropical Medicine and Hygiene*, 2003, 69:14–18.
147. Dao NV et al. Vivax malaria: Preliminary observations following a shorter course of treatment with artesunate plus primaquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2007, 101:534–539.
148. Krudsood S et al. High-dose primaquine regimens against relapse of *Plasmodium vivax* malaria. *American Journal of Tropical Medicine and Hygiene*, 2008, 78:736–740.
149. Carmona-Fonseca J, Maestre A. Prevention of *Plasmodium vivax* malaria recurrence: Efficacy of the standard total dose of primaquine administered over 3 days. *Acta Tropica*, 2009, 112:188–192.

150. Carmona-Fonseca J. Malaria vivax en niños: recurrencias con dosis estándar de primaquina administrada durante 3 frente a 7 días [Vivax malaria in children: recurrences with standard doses of primaquine administered for 3 versus 7 days]. *Iatreia*, 2010, 23:10–20.
151. Pukrittayakamee S et al. A comparison of two short-course primaquine regimens for the treatment and radical cure of *Plasmodium vivax* malaria in Thailand. *American Journal of Tropical Medicine and Hygiene*, 2010, 82:542–547.
152. Takeuchi R et al. Directly-observed therapy (DOT) for the radical 14-day primaquine treatment of *Plasmodium vivax* malaria on the Thai–Myanmar border. *Malaria Journal*, 2010, 9:308.
153. Maneeboonyang W et al. Directly observed therapy with primaquine to reduce the recurrence rate of *Plasmodium vivax* infection along the Thai–Myanmar border. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2011, 42:9–18.
154. Schmidt LH et al. Radical cure of infections with *Plasmodium cynomolgi*: A function of total 8-aminoquinoline dose. *American Journal of Tropical Medicine and Hygiene*, 1977, 26:1116–1128.
155. Clyde DF, McCarthy VC. Radical cure of Chesson strain vivax malaria in man by 7, not 14, days of treatment with primaquine. *American Journal of Tropical Medicine and Hygiene*, 1977, 26:562–563.
156. Griffin JT et al. Reducing *Plasmodium falciparum* malaria transmission in Africa: A model-based evaluation of intervention strategies. *PLoS Medicine*, 2010, 7.
157. Maude RJ et al. Modelling malaria elimination on the Internet. *Malaria Journal*, 2011, 10:191.
158. Jeffery GM, Eyles DE. Infectivity to mosquitoes of *Plasmodium falciparum* as related to gametocyte density and duration of infection. *American Journal of Tropical Medicine and Hygiene*, 1955, 4:781–789.
159. Jeffery GM, Young MD, Eyles DE. The treatment of *Plasmodium falciparum* infection with chloroquine, with a note on infectivity to mosquitoes of primaquine- and pyrimethamine-treated cases. *American Journal of Hygiene*, 1956, 64:1–11.
160. Young MD, Burgess RW. Pyrimethamine resistance in *Plasmodium vivax* malaria. *Bulletin of the World Health Organization*, 1959, 20: 27–36.
161. Rieckmann KH et al. Gametocytocidal and sporontocidal effects of primaquine and of sulfadiazine with pyrimethamine in a chloroquine-resistant strain of *Plasmodium falciparum*. *Bulletin of the World Health Organization*, 1968, 38:625–632.
162. Rieckmann KH et al. Gametocytocidal and sporontocidal effects of primaquine upon two strains of *Plasmodium falciparum*. *Military Medicine*, 1969, 134:802–819.
163. Clyde DF et al. Treatment of falciparum malaria caused by strain resistant to quinine. *Journal of the American Medical Association*, 1970, 213:2041–2045.
164. Clyde DF et al. Prophylactic and sporontocidal treatment of chloroquine-resistant *Plasmodium falciparum* from Vietnam. *American Journal of Tropical Medicine and Hygiene*, 1971, 20:1–5.

165. Gunders AE. The effect of a single dose of pyrimethamine and primaquine in combination upon gametocytes and sporogony of *Laverania falcipara* (*Plasmodium falciparum*) in Liberia. *Bulletin of the World Health Organization*, 1961, 24:650–653.
166. Burgess RW, Bray RS. The effect of a single dose of primaquine on the gametocytes, gametogony and sporogony of *Laverania falciparum*. *Bulletin of the World Health Organization*, 1961, 24:451–456.
167. White NJ et al. Rationale for recommending a lower dose of primaquine (0.25 mg base/kg) as a *Plasmodium falciparum* gametocytocide in populations where G6PD deficiency is common. *Malaria Journal*, 2012; 11:418e
168. Chomcharn Y et al. Effect of a single dose of primaquine on a Thai strain of *Plasmodium falciparum*. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1980, 11:408–412.
169. El-Sayed B et al. A randomized open-label trial of artesunate–sulfadoxine–pyrimethamine with or without primaquine for elimination of sub-microscopic *P. falciparum* parasitaemia and gametocyte carriage in eastern Sudan. *PLoS One*, 2007, 2:e1311.
170. Pukrittayakamee S et al. Effects of different antimalarial drugs on gametocyte carriage in *P. vivax* malaria. *American Journal of Tropical Medicine and Hygiene*, 2008, 79:378–384.
171. Smithuis F et al. Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: an open-label randomised trial. *Lancet Infectious Diseases*, 2010, 10:673–681.
172. Bunnag D et al. Effect of primaquine on gametocytes of *Plasmodium falciparum* in Thailand. *Lancet*, 1980, ii:91.
173. Ponnudurai T et al. Infectivity of cultured *Plasmodium falciparum* gametocytes to mosquitoes. *Parasitology*, 1989, 98:165–173.
174. Arango EM, Upegui YA, Carmona-Fonseca J. Efficacy of different primaquine-based antimalarial regimens against *Plasmodium falciparum* gametocytemia. *Acta Tropica*, 2012, 122:177–182.
175. Carmona-Fonseca J, Arango E, Blair S. Gametocitemia en malaria por *Plasmodium falciparum* tratada con amodiaquina o artesunato [Gametocytaemia in *Plasmodium falciparum* malaria treated with amodiaquine or artesunate]. *Biomedica*, 2008, 28:195–212.
176. Walker AJ. Potentialities of monthly doses of camoquin and a gametocidal drug in malaria control. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1955, 49:351–355.
177. Clyde DF. *Mass administration of an antimalarial drug combining 4-aminoquinoline and 8-aminoquinoline in Tanganyika*. Geneva, World Health Organization, 1961.
178. Gunders AE. *The effect of a single dose of pyrimethamine and primaquine in combination upon gametocytes and sporogony of* Laverania falcipara (=Plasmodium falciparum) *in Liberia*. Geneva: World Health Organization, 1961.

179. Kaneko A et al. Gametocytocidal effect of primaquine in a chemotherapeutic malaria control trial in North Sumatra, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1989, 20:351–359.

180. Kamtekar KD et al. A prospective study evaluating the efficacy of a single, 45-mg dose of primaquine, as a gametocytocidal agent, in patients with *Plasmodium falciparum* malaria in Mumbai, India. *Annals of Tropical Medicine and Parasitology*, 2004, 98:453–458.

181. Tangpukdee N et al. Efficacy of Artequick versus artesunate-mefloquine in the treatment of acute uncomplicated falciparum malaria in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2008, 39:1–8.

182. Song J et al. Rapid and effective malaria control in Cambodia through mass administration of artemisinin-piperaquine. *Malaria Journal*, 2010, 9:57.

183. Shah NK. *Reducing malaria transmission: the epidemiology and treatment of* Plasmodium falciparum *gametocytemia*. Doctoral thesis, Chapel Hill, North Carolina, University of North Carolina at Chapel Hill, 2012.

184. Weerasinghe KL et al. A safety and efficacy trial of artesunate, sulphadoxine-pyrimethamine and primaquine in *P. falciparum* malaria. *Ceylon Medical Journal*, 2002, 47:83–85.

185. Lederman ER et al. Combined chloroquine, sulfadoxine/pyrimethamine and primaquine against *Plasmodium falciparum* in Central Java, Indonesia. *Malaria Journal*, 2006, 5:108.

186. Vasquez AM et al. Estudio piloto de la eficacia y de los efectos sobre los gametocitos del esquema artesunato-mefloquina-primaquina para la malaria por *Plasmodium falciparum* [Pilot study of the efficacy and effects against gametocytes of a regimen of artesunate-mefloquine-primaquine in *Plasmodium falciparum* malaria]. *Biomedica*, 2009, 29:307–319.

187. Àlvarez G et al. Dynamics of *Plasmodium falciparum* parasitemia regarding combined treatment regimens for acute uncomplicated malaria, Antioquia, Colombia. *American Journal of Tropical Medicine and Hygiene*, 2010, 83:90–96.

188. Foy BD et al. Endectocides for malaria control. *Trends in Parasitology*, 2011, 27:423–428.

189. Clyde DF. Clinical problems associated with the use of primaquine as a tissue schizontocidal and gametocytocidal drug. *Bulletin of the World Health Organization*, 1981, 59:391–395.

190. Edgcomb JH et al. Primaquine, SN 13272, a new curative agent in vivax malaria; a preliminary report. *Journal of the National Malaria Society*, 1950, 9:285–292.

191. Betuela I et al. Tolerability and safety of primaquine in Papua New Guinean children 1 to 10 years of age. *Antimicrobial Agents and Chemotherapy*, 2012, 56:2146–2149.

192. Buchachart K et al. Effect of primaquine standard dose (15 mg/day for 14 days) in the treatment of vivax malaria patients in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2001, 32:720–726.
193. Ruwende C et al. Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria. *Nature*, 1995, 376:246–249.
194. Beutler E. The hemolytic effect of primaquine and related compounds: A review. *Blood*, 1959, 14:103–139.
195. Beutler E, Duparc S. Glucose-6-phosphate dehydrogenase deficiency and antimalarial drug development. *American Journal of Tropical Medicine and Hygiene*, 2007, 77:779–789.
196. Cappellini MD, Fiorelli G. Glucose-6-phosphate dehydrogenase deficiency. *Lancet*, 2008, 371:64–74.
197. Luzzatto L, Poggi V. Glucose-6-phosphate dehydrogenase deficiency. In: Orkin SH et al., eds. *Nathan and Oski's hematology of infancy and childhood*, 7th ed. Montreal, Saunders Canada, 2009.
198. Alvin R et al. Primaquine sensitivity. *Archives of Internal Medicine*, 1962, 109:137–162.
199. Dern RJ, Beutler E, Alving AS. The hemolytic effect of primaquine. II. The natural course of the hemolytic anemia and the mechanism of its self-limited character. *Journal of Laboratory and Clinical Medicine*, 1954, 44:171–176.
200. Kondrashin AV, Baranova AM, Sergiev VM. *Large scale use of primaquine in population with the G6PD deficiency (review of experiences)*. Moscow, Martzinovski Institute of Medical Parasitology and Tropical Medicine, Moscow Medical Academy, 2010.
201. Kondrashin AV, et al. Mass primaquine treatment to eliminate vivax malaria: Lessons from the past. *Malaria Journal*, 2014, 13:51.
202. Beutler E, Dern RJ, Alving AS. The hemolytic effect of primaquine. IV. The relationship of cell age to hemolysis. *Journal of Laboratory and Clinical Medicine*, 1954, 44:439–442.
203. Luzzatto L. The rise and fall of the antimalarial Lapdap: A lesson in pharmacogenetics. *Lancet*, 2010, 376:739–741.
204. Kellermeyer RW et al. The hemolytic effect of primaquine. XIII. Gradient susceptibility to hemolysis of primaquine-sensitive erythrocytes. *Journal of Laboratory and Clinical Medicine*, 1961, 58:225–233.
205. Beutler E, Dern RJ, Alving AS. The hemolytic effect of primaquine. III. A study of primaquine-sensitive erythrocytes. *Journal of Laboratory and Clinical Medicine*, 1954, 44:177–184.
206. Dern RJ, Beutler E, Alving AS. The hemolytic effect of primaquine. V. Primaquine sensitivity as a manifestation of a multiple drug sensitivity. *Journal of Laboratory and Clinical Medicine*, 1955, 45:30–39.
207. Kellermeyer RW et al. Hemolytic effect of therapeutic drugs. Clinical considerations of the primaquine-type hemolysis. *Journal of the American Medical Association*, 1962, 180:388–394.

208. Alving AS et al. Mitigation of the haemolytic effect of primaquine and enhancement of its action against exoerythrocytic forms of the Chesson strain of *Plasmodium vivax* by intermittent regimens of drug administration: A preliminary report. *Bulletin of the World Health Organization*, 1960, 22:621–631.
209. Brewer GJ, Zarafonetis CJ. The haemolytic effect of various regimens of primaquine with chloroquine in American Negroes with G6PD deficiency and the lack of an effect of various antimalarial suppressive agents on erythrocyte metabolism. *Bulletin of the World Health Organization*, 1967, 36:303–308.
210. Beutler E. Glucose-6-phosphate dehydrogenase deficiency: A historical perspective. *Blood*, 2008, 111:16–24.
211. George JN et al. Primaquine sensitivity in Caucasians: Hemolytic reactions induced by primaquine in G-6-PD deficient subjects. *Journal of Laboratory and Clinical Medicine*, 1967, 70:80–93.
212. Pannacciulli I et al. Hemolytic effects of standard single dosages of primaquine and chloroquine on G-6-PD-deficient Caucasians. *Journal of Laboratory and Clinical Medicine*, 1969, 74:653–661.
213. Aung-Than-Batu, Hla-Pe U, Thein-Than. Primaquine induced haemolysis in G-6-PD deficient Burmese (correspondence to the editor). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1970, 64:785–786.
214. Everett WD, Yoshida A, Pearlmen E. Hemoglobin E and glucose-6-phosphate deficiency in the Khmer Air Force (Cambodia). *American Journal of Tropical Medicine and Hygiene*, 1977; 26:597–601.
215. Bouma MJ et al. Prevalence and clinical presentation of glucose-6-phosphate dehydrogenase deficiency in Pakistani Pathan and Afghan refugee communities in Pakistan; implications for the use of primaquine in regional malaria control programmes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1995, 89:62–64.
216. Myat Phone K et al. The use of primaquine in malaria infected patients with red cell glucose-6-phosphate dehydrogenase (G6PD) deficiency in Myanmar. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1994, 25:710–713.
217. Calabro V et al. Glucose-6-phosphate dehydrogenase (G6PD) deficiency in southern Italy: A case of G6PD A(–) associated with favism. *Haematologica*, 1989, 74:71–73.
218. Vives Corrons JL, Pujades A. Heterogeneity of "Mediterranean type" glucose-6-phosphate dehydrogenase (G6PD) deficiency in Spain and description of two new variants associated with favism. *Human Genetics*, 1982, 60:216–221.
219. Shekalaghe SA et al. In Tanzania, hemolysis after a single dose of primaquine coadministered with an artemisinin is not restricted to glucose-6-phosphate dehydrogenase-deficient (G6PD A-) individuals. *Antimicrobial Agents and Chemotherapy*, 2010, 54:1762–1768.

220. Raupp P et al. Henna causes life threatening haemolysis in glucose-6-phosphate dehydrogenase deficiency. *Archives of Disease in Childhood*, 2001, 85:411–412.
221. Lau HKY, Li CH, Lee ACW. Acute massive haemolysis in children with glucose-6-phosphate dehydrogenase deficiency. *Hong Kong Medical Journal*, 2006, 12:149–151.
222. Dodoo A. Lapdap—a threat or an opportunity? *WHO Pharmaceuticals Newsletter*, 2004, 1:10.
223. Wootton DG et al. Open-label comparative clinical study of chlorproguanil–dapsone fixed dose combination (Lapdap) alone or with three different doses of artesunate for uncomplicated *Plasmodium falciparum* malaria. *PLoS One*, 2008, 3:e1779.
224. Bell DJ et al. Measurement of adherence, drug concentrations and the effectiveness of artemether–lumefantrine, chlorproguanil–dapsone or sulphadoxine–pyrimethamine in the treatment of uncomplicated malaria in Malawi. *Malaria Journal*, 2009, 8:204.
225. Tiono AB et al. Chlorproguanil–dapsone–artesunate versus chlorproguanil–dapsone: A randomized, double-blind, phase III trial in African children, adolescents, and adults with uncomplicated *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene*, 2009, 81:969–978.
226. Premji Z et al. Chlorproguanil–dapsone–artesunate versus artemether–lumefantrine: A randomized, double-blind phase III trial in African children and adolescents with uncomplicated *Plasmodium falciparum* malaria. *PLoS One*, 2009, 4:e6682.
227. Dunyo S et al. Randomized trial of safety and effectiveness of chlorproguanil–dapsone and lumefantrine–artemether for uncomplicated malaria in children in the Gambia. *PLoS One*, 2011, 6:e17371.
228. Fanello CI et al. High risk of severe anaemia after chlorproguanil–dapsone+artesunate antimalarial treatment in patients with G6PD (A–) deficiency. *PLoS One*, 2008, 3:e4031.
229. Pamba A et al. Clinical spectrum and severity of hemolytic anemia in glucose 6-phosphate dehydrogenase-deficient children receiving dapsone. *Blood*, 2012, 120:4123–4133.
230. Howes RE et al. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: A geostatistical model-based map. *PLoS Medicine*, 2012.
231. Chinevere TD et al. Prevalence of glucose-6-phosphate dehydrogenase deficiency in US Army personnel. *Military Medicine*, 2006, 171:905–907.
232. WHO Working Group. Glucose-6-phosphate dehydrogenase deficiency. *Bulletin of the World Health Organization*, 1989, 67:601–611.
233. Beutler E, Westwood B, Kuhl W. Definition of the mutations of G6PD Wayne, G6PD Viangchan, G6PD Jammu, and G6PD "LeJeune". *Acta Haematologica*, 1991, 86:179–182.
234. Beutler E, Vulliamy TJ. Hematologically important mutations: glucose-6-phosphate dehydrogenase. *Blood Cells, Molecules and Disease*, 2002, 28:93–103.

235. Minucci A et al. Glucose-6-phosphate dehydrogenase (G6PD) mutations database: Review of the "old" and update of the new mutations. *Blood Cells, Molecules and Disease*, 2012, 48:154–165.
236. Carter N et al. Frequency of glucose-6-phosphate dehydrogenase deficiency in malaria patients from six African countries enrolled in two randomized anti-malarial clinical trials. *Malaria Journal*, 2011, 10:241.
237. Santana MS et al. Glucose-6-phosphate dehydrogenase deficiency in an endemic area for malaria in Manaus: A cross-sectional survey in the Brazilian Amazon. *PLoS One*, 2009, 4:e5259.
238. Beutler E et al. Molecular heterogeneity of glucose-6-phosphate dehydrogenase A. *Blood*, 1989, 74:2550–2555.
239. Hirono A, Beutler E. Molecular cloning and nucleotide sequence of cDNA for human glucose-6-phosphate dehydrogenase variant A(–). *Proceedings of the National Academy of Sciences of the United States of America*, 1988, 85:3951–3954.
240. Oppenheim A et al. G6PD Mediterranean accounts for the high prevalence of G6PD deficiency in Kurdish Jews. *Human Genetics*, 1993, 91:293–294.
241. Tantular IS et al. Incidence and mutation analysis of glucose-6-phosphate dehydrogenase deficiency in eastern Indonesian populations. *Acta Medica Okayama*, 2010, 64:367–373.
242. Luzzatto L. Genetics of red cells and susceptibility to malaria. *Blood*, 1979, 54:961–976.
243. Ruwende C, Hill A. Glucose-6-phosphate dehydrogenase deficiency and malaria. *Journal of Molecular Medicine (Berlin)*, 1998, 76:581–588.
244. Luzzatto L, Bienzle U. The malaria/G-6-PD hypothesis. *Lancet*, 1979, i:1183–1184.
245. Louicharoen C et al. Positively selected G6PD-Mahidol mutation reduces *Plasmodium vivax* density in Southeast Asians. *Science*, 2009, 326:1546–1549.
246. Leslie T et al. The impact of phenotypic and genotypic G6PD deficiency on risk of *Plasmodium vivax* infection: A case–control study amongst Afghan refugees in Pakistan. *PLoS Medicine*, 2010, 7:e1000283.
247. Meissner PE et al. Diagnosis of red cell G6PD deficiency in rural Burkina Faso: Comparison of a rapid fluorescent enzyme test on filter paper with polymerase chain reaction based genotyping. *British Journal of Haematology*, 2005, 131:395–399.
248. Charoenlarp P et al. The course of primaquine-induced haemolysis in G-6-PD-deficient Thais. *Journal of the Medical Association of Thailand*, 1973, 56:392–397.
249. Tantular IS, Kawamoto F. An improved, simple screening method for detection of glucose-6-phosphate dehydrogenase deficiency. *Tropical Medicine and International Health*, 2003, 8:569–574.
250. Jalloh A et al. Rapid epidemiologic assessment of glucose-6-phosphate dehydrogenase deficiency in malaria-endemic areas in Southeast Asia using a novel diagnostic kit. *Tropical Medicine and International Health*, 2004, 9:615–623.

251. Kuwahata M et al. Population screening for glucose-6-phosphate dehydrogenase deficiencies in Isabel Province, Solomon Islands, using a modified enzyme assay on filter paper dried bloodspots. *Malaria Journal*, 2010, 9:223.
252. Tinley KE et al. Evaluation of a rapid qualitative enzyme chromatographic test for glucose-6-phosphate dehydrogenase deficiency. *American Journal of Tropical Medicine and Hygiene*, 2010, 82:210–214.
253. Kim S et al. Performance of the CareStart G6PD deficiency screening test, a point-of-care diagnostic for primaquine therapy screening. *PLoS One*, 2011, 6:e28357.
254. de Benoist B et al., eds. *Worldwide prevalence of anaemia 1993–2005. WHO global database on anaemia*. Geneva, World Health Organization, 2008.
255. Bienzle U, Guggenmoos-Holzmann I, Luzzatto L. *Plasmodium falciparum* malaria and human red cells. I. A genetic and clinical study in children. *International Journal of Epidemiology*, 1981, 10:9–15.
256. Abdalla S et al. The anaemia of *P. falciparum* malaria. *British Journal of Haematology*, 1980, 46:171–183.
257. Douglas NM et al. *Plasmodium vivax* recurrence following falciparum and mixed species malaria: Risk factors and effect of antimalarial kinetics. *Clinical Infectious Diseases*, 2011, 52:612–620.
258. Merat S et al. Case report: combination artemether–lumefantrine and haemolytic anaemia following a malarial attack. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2003, 97:433–434.
259. Aloni NM et al. Hémolyse intravasculaire après prise d'artéméther-luméfantrine [Intravascular haemolysis following artemether-lumefantrine intake]. *Bulletin de la Societé de Pathologie Exotique*, 2010, 103:296–298.
260. Tran TH et al. A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. *New England Journal of Medicine*, 1996, 335:76–83.
261. Dondorp A et al. Artesunate versus quinine for treatment of severe falciparum malaria: A randomised trial. *Lancet*, 2005, 366:717–725.
262. Dondorp AM et al. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): An open-label, randomised trial. *Lancet*, 2010, 376:1647–1657.
263. Rolling T et al. Artesunate versus quinine in the treatment of severe imported malaria: Comparative analysis of adverse events focussing on delayed haemolysis. *Malaria Journal*, 2013, 12:e241.
264. Phillips RE, Pasvol G. Anaemia of *Plasmodium falciparum* malaria. *Bailliere's Clinical Haematology*, 1992, 5:315–330.
265. Davies JN. Pathology of Central African natives; Mulago Hospital post mortem studies. *East African Medical Journal*, 1948, 25:454–467.
266. Obonyo CO et al. In-hospital morbidity and mortality due to severe malarial anemia in western Kenya. *American Journal of Tropical Medicine and Hygiene*, 2007, 77:23–28.

267. Grenfell P et al. Anaemia and malaria in Yanomami communities with differing access to healthcare. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2008, 102:645–652.
268. Carmona-Fonseca J, Uscategui RM, Correa AM. Malaria vivax en niños: aspectos clínicos y respuesta a la cloroquina [Vivax malaria in children: clinical aspects and response to chloroquine]. *Colombia Médica*, 2008, 39:3664–3377.
269. Uscategui RM, Correa AM, Carmona-Fonseca J. Cambios en las concentraciones de retinol, hemoglobina y ferritina en niños palúdicos colombianos [Changes in retinol, haemoglobin and ferritin concentrations in Colombian children with malaria.] *Biomedica*, 2009, 29:270–281.
270. Burgoine KL, Bancone G, Nosten F. The reality of using primaquine. *Malaria Journal*, 2010, 9:376.
271. Marcsisin SR et al. CYP450 phenotyping and metabolite identification of quinine by accurate mass UPLC–MS analysis: A possible metabolic link to blackwater fever. *Malaria Journal*, 2013; 12:e214
272. Tran TH et al. Blackwater fever in southern Vietnam: A prospective descriptive study of 50 cases. *Clinical Infectious Diseases*, 1996, 23:1274–1281.
273. Bruneel F et al. Resurgence of blackwater fever in long-term European expatriates in Africa: Report of 21 cases and review. *Clinical Infectious Diseases*, 2001, 32:1133–1140.
274. Hue NT et al. Glucose-6-phosphate dehydrogenase (G6PD) mutations and haemoglobinuria syndrome in the Vietnamese population. *Malaria Journal*, 2009, 8:152.
275. Hockwald RS et al. Toxicity of primaquine in Negroes. *Journal of the American Medical Association*, 1952, 149:1568–1570.
276. Brewer GJ et al. The hemolytic effect of primaquine. XV. Role of methemoglobin. *Journal of Laboratory and Clinical Medicine*, 1962, 59:905–917.
277. Ziai M et al. Malaria prophylaxis and treatment in G-6-PD deficiency. An observation on the toxicity of primaquine and chloroquine. *Clinical Pediatrics (Philadelphia)*, 1967, 6:242–243.
278. Chopra SA. Haemolytic crisis in a Zanzibari Arab girl with G6PD deficiency and sickle cell trait. *East African Medical Journal*, 1968, 45:726–727.
279. Abeyaratne KP, Halpe NL. Sensitivity to primaquine in Ceylonese children due to deficiency of erythrocytic glucose-6-phosphate dehydrogenase. *Ceylon Medical Journal*, 1968, 13:134–138.
280. Khoo K-K. The treatment of malaria in glucose-6-phosphate dehydrogenase patients in Sabah. *Annals of Tropical Medicine and Parasitology*, 1981, 75:591–195.
281. Karwacki JJ et al. Primaquine induced hemolysis in a Thai soldier. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1989, 20:555–556.

282. Reeve PA et al. Acute intravascular haemolysis in Vanuatu following a single dose of primaquine in individuals with glucose-6-phosphate dehydrogenase deficiency. *Journal of Tropical Medicine and Hygiene*, 1992, 95:349–351.
283. Martinez-Perez JL, Hadad-Melendez P. Sindrome hemolitico por primaquina y deficiencia de glucosa 6 fosfato deshidrogenasa [Primaquine-induced haemolytic syndrome and glucose-6-phosphate dehydrogenase deficiency]. *Revista Cubana de Medicina Tropical*, 1989, 41:299–306.
284. Menendez-Capote R, Caña-Lugo C, Fernandez-Nuñez A. Primaquina y viajeros del mundo arabe. Reporte y recomendacion [Primaquine and travellers from the Arab world. Report and recommendation]. *Revista Cubana de Medicina Tropical*, 1995, 47:221–223.
285. Menendez-Capote R, Diaz-Perez L, Luzardo-Suarez C. Hemolisis y tratamiento con primaquina. Informe preliminar [Haemolysis and treatment with primaquine. Preliminary report]. *Revista Cubana de Medicina Tropical*, 1997, 49:136–138.
286. Navarro P et al. Paludismo como infeccion del viajero adquirido en el estado Sucre [Malaria infection of travellers acquired in Sucre State]. *Revista de la Facultad de Medicina*, 2003, 26:34–38.
287. Sukumar S, Colah R, Mohanty D. G6PD gene mutations in India producing drug-induced haemolytic anaemia. *British Journal of Haematology*, 2002, 116:671–672.
288. Carr ME Jr, Fandre MN, Oduwa FO. Glucose-6-phosphate dehydrogenase deficiency in two returning Operation Iraqi Freedom soldiers who developed hemolytic anemia while receiving primaquine prophylaxis for malaria. *Military Medicine*, 2005, 170:273–276.
289. Ramos WM Jr et al. Clinical aspects of hemolysis in patients with *P. vivax* malaria treated with primaquine, in the Brazilian Amazon. *Brazilian Journal of Infectious Diseases*, 2010, 14:410–412.
290. Lacerda MV et al. Postmortem characterization of patients with clinical diagnosis of *Plasmodium vivax* malaria: To what extent does this parasite kill? *Clinical Infectious Diseases*, 2012, 55:e67–e74.
291. Onori E, Muir D. *Report on a visit to Euro-Mal TUR/MPD/001 (Turkey)*. Geneva, World Health Organization, 1978.
292. Department of Health. *Yellow card scheme. Primaquine ADR reports*. London, 2011.
293. Uppsala Monitoring Centre. *Primaquine ADRs (suspected/interacting)*. Uppsala, 2012.
294. Schlossberg D. Reaction to primaquine. *Annals of Internal Medicine*, 1980, 92:435.
295. Verdier R. *Nicaragua: Historique de la lutte contre le paludisme de 1973 a 1983 [Nicaragua: History of malaria control from 1973 to 1983]*. Doctoral thesis, Paris, Université Paris VII, 1985.
296. White NJ. Cardiotoxicity of antimalarial drugs. *Lancet Infectious Diseases*, 2007, 7:549–558.

297. Orta-Salazar G et al. Inhibition of cardiac Na+ current by primaquine. *British Journal of Pharmacology*, 2002, 135:751–763.
298. Dimson SB, McMartin RB. Pamaquin haemoglobinuria. *Quarterly Journal of Medicine*, 1946, 15:25–46.
299. Archambeault CP. Mass antimalarial therapy in veterans returning from Korea. *Journal of the American Medical Association*, 1954, 154:1411–1415.
300. Vivona S et al. The concurrent weekly administration of chloroquine and primaquine for the prevention of Korean vivax malaria. *Bulletin of the World Health Organization*, 1961, 25:267–269.

Annex 1. New WHO recommendation on primaquine as a *P. falciparum* gametocytocide

While this review was in press, WHO revised its previous recommendation on the use of primaquine as a *P. falciparum* gametocytocide on the basis of an extensive review of the evidence on the efficacy and safety of primaquine transmission-blocking activity, including when it was given to G6PD-deficient individuals. The new WHO recommendation of a lower dose of 0.25 mg base/kg primaquine (instead of 0.75 mg/kg), without a requirement for G6PD testing (*1*), reads as follows:

> In: (1) areas threatened by artemisinin resistance where single dose primaquine as a gametocytocide for *P. falciparum* malaria is not being implemented, and (2) elimination areas which have not yet adopted primaquine as a gametocytocide for *P. falciparum* malaria: A single 0.25 mg base/kg primaquine dose should be given to all patients with parasitologically confirmed *P. falciparum* malaria on the first day of treatment in addition to an ACT, except for pregnant women and infants <1 year of age.

The evidence reviewed here to support the efficacy and safety of primaquine as a *P. falciparum* transmission-blocking antimalarial agent and the rationale for use of the newly recommended lower dose has been recently discussed and summarized elsewhere (*2*, *3*).

References

1. World Health Organization. *Updated WHO policy recommendation (October 2012): single dose Primaquine as a gametocytocide in* Plasmodium falciparum *malaria*. Geneva, Global Malaria Programme (http://www.who.int/malaria/diagnosis_treatment/treatment/who_pq_policy_recommendation/ en/; accessed 20 November 2012).
2. White NJ. Primaquine to prevent transmission of falciparum malaria. *Lancet Infectious Diseases*, 2013; 13:175–181.
3. White NJ et al. Rationale for recommending a lower dose of primaquine (0.25 mg base/kg) as a *Plasmodium falciparum* gametocytocide in populations where G6PD deficiency is common. *Malaria Journal*, 2012; 11:418e.

Annex 2. Studies of the safety of primaquine

Details of the studies of the safety of primaquine are described in chronological order, from the oldest to the most recent. First, we list studies that did not specifically address the effect of antimalarial therapy in G6PD-deficient individuals and then studies that did so.

Published studies of the safety of primaquine but with no evaluation of G6PD deficiency

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Reference and location: Edgcomb et al., 1950 (*1*), USA

Study question: Curative effect of primaquine against mosquito-transmitted *P. vivax* (Chesson strain) and toxicity in intravenously infected prison inmate volunteers

Primaquine dose or regimen: Alone at 22.5 or 45 mg daily for 14 days; with quinine during primary attacks at 15, 22.5, 30 or 60 mg (in six subdivided doses) daily for 14 days; in toxicity studies at 120 or 240 mg, alone or with quinine (in six subdivided daily doses)

Other antimalarial agents given: 1.64 g quinine daily with primaquine for 14 days

Adverse events surveillance: Patients hospitalized during the trial, and parasitaemia evaluated daily during immediate follow-up and regularly up to 1 year; toxicity studied in all infected volunteers

G6PD deficiency status: Not determined

Severe adverse events reported: None in about 50 men receiving primaquine at various doses. Severe toxicity at 240 mg (severe abdominal cramps and 9–10% methaemoglobin) but no irreversible damage; when primaquine was given at 120 mg with quinine, methaemoglobinaemia (mean, 10%) was half that without quinine (20%)

Reference and location: Alving, Arnold and Robinson, 1952 (*2*), US soldiers in Japan and in ships sailing from Japan to the USA

Study question: Use of primaquine in 1493 officers and men before and on a military ship sailing from Japan, to determine whether primaquine toxicity increased the incidence of motion sickness and vice versa

Primaquine dose or regimen: (1) *n* = 742 (16.9% African American) on board ship given 15 mg daily for 12 days immediately before lunch and *n* = 751 given placebo; (2) *n* = 2060 (7.6% African American) men who spent 2–10 days in Japan before sailing received primaquine at the same regimen and *n* = 725 received placebo. 82% of men received 13–20 pills consecutively, and an additional 8.8% received all but one; thus, 90.9% received 12 or more pills with an interruption of not more than 1 day.

Other antimalarial agents given: None

Adverse events surveillance: Seasickness was measured from the number of men at mess: those who missed mess were considered to have gastrointestinal distress; men at sick call each day were examined for possible toxicity.

G6PD deficiency status: Not determined

Severe adverse events reported: (1) No signs of toxicity, no evidence of haemolysis in the African American men; (2) no evidence of toxicity; seasickness in primaquine group close to that of controls

Reference and location: Clayman et al., 1952 (*3*), USA

Study question: Toxicity of primaquine in 699 white male volunteer prison inmates; 335 were either uninfected or treated in the interval between clinical malaria attacks, the other 364 were treated during a clinical attack. Similar regimens of pamaquine were used for comparison (see Annex 3).

Primaquine dose or regimen: Single or divided daily doses of 10–240 mg for 5–14 days or a single dose of 30 mg weekly and semi-weekly for 52 weeks with chloroquine

Other antimalarial agents given: Quinine, chloroquine or other drugs (methylene blue, nicotinamide, thionine, Bindschedler green, toluidine blue, theobromine, aminophylline, ephedrine sulfate, chloramphenicol, Ordaraprim®)

Adverse events surveillance: Hb and methaemoglobin were measured before drug administration, every other day during clinical malaria and then daily; during interim therapy, Hb, methaemoglobin and white blood cells were usually measured on days 1, 7, 10 and 14 of drug administration; urinalyses were done when toxicity was anticipated. The authors describe a "side" study in which an additional 10 volunteers were bitten by infected mosquitoes and the next day took single weekly doses of 30 mg primaquine with 300 mg chloroquine for 14 weeks.

G6PD deficiency status: Not determined

Severe adverse events reported: None among 699 men who received primaquine. Mild decreases in Hb but no severe haemolytic anaemia even at a daily dose as high as 240 mg. At doses of 15, 22.5 and 30 mg, only mild-to-moderate abdominal cramps; at 30 mg, ≤15% patients lost 2 g/dL Hb and 3.8% showed leukocytosis (10 000–17 000 per mL3), no leukopenia; at 60 mg (n = 7), mild-to-severe abdominal cramps, nausea, anorexia, vomiting, mild epigastric distress, cyanosis caused by methaemoglobinaemia (with primaquine alone, not with primaquine + chloroquine), leukocytosis (13 500/μL in one volunteer, no Hb loss or leukopenia; at 120 mg, abdominal cramps and with primaquine only methaemoglobinaemia with cyanosis and a variation in Hb ± 1 g/dL of control. Only two of six men given primaquine + quinine appeared cyanotic, two of five men lost 1.1–2 g/dL Hb, two cases of leukopenia. Weekly and semi-weekly doses of 30 mg primaquine for up to 52 weeks were not dangerously toxic when given with 300 mg chloroquine.

Reference and location: Garrison et al., 1952 (*4*) preliminary results at 11 months; Alving et al., 1953 (*5*) 18 months' observations; USA

Study question: Comparison of curative effectiveness of primaquine and pamaquine when given with chloroquine to US veterans of the Korean conflict with vivax malaria (see also Annex 3)

Primaquine dose or regimen: 15 mg daily for 14 consecutive days with chloroquine

Other antimalarial agents given: Chloroquine, total dose of 1.5 g in three doses of 300 mg during the first 24 h, followed by a single dose of 300 mg daily for 2 days; given either alone or with primaquine

Adverse events surveillance: All patients hospitalized during treatment and observed daily for toxicity. White blood count, Hb and urinalysis measured on admission, after 1 week for men given primaquine and at the end of therapy; all men followed every 6 weeks with interviews and a thick film for measuring parasites. Patients who relapsed were given the same treatment.

G6PD deficiency status: Not determined

Severe adverse events reported: No significant toxicity was observed in 348 patients who received primaquine + chloroquine, with no evidence of haemolytic anaemia or cyanosis; 15% patients had mild-to-moderate anaemia (Hb, 6.4–12 g/dL) at beginning of treatment. Hb increased during therapy as the acute attack was controlled; methaemoglobin was not measured.

Reference and location: Hockwald et al., 1952 (*6*), USA

Study question: Toxicity of primaquine in African American volunteers

Primaquine dose or regimen: 110 men received a single dose of 30 mg for 14 days, and 50 men took 15 mg daily

Other antimalarial agents given: 50 men received no other drug, 25 received 2 g quinine for the 14 days, 25 received 0.6 grains (0.04 g) chloroquine on day 1 and 0.45 g on day 2, and 10 men received 0.6 g chloroquine on day 1, 0.45 grains (0.03 g) on day 2 and 0.3 g on each of the following 12 days.

Adverse events surveillance: Daily questionnaire; Hb measured daily and 3 and 7 days after end of treatment

G6PD deficiency status: Not determined

Severe adverse events reported: Of the 110 men who received 30 mg primaquine daily, 105 completed 14 days without acute haemolysis, 17 showed mild anaemia (mean Hb decrease of 1.8 g/dL ± 0.72). The other five men showed severe haemolysis requiring discontinuation of treatment at 4–9 days, with elevated bilirubin and dark urine in four. All recovered spontaneously within 2–3 weeks without requiring transfusion. Of the 50 men who completed treatment with 15 mg daily, 12 showed mild anaemia with a mean Hb decrease of 2 g/dL ± 0.75.

Reference and location: Cooper et al., 1953 (*7*), USA

Study question: Comparison of 8-aminoquinolines (primaquine, isopentaquine, SN-3883 and pamaquine) in prison inmate volunteers infected with *P. vivax* Chesson strain by mosquito bites (see also Annex 3)

Primaquine dose or regimen: 20 or 10 mg with quinine

Other antimalarial agents given: Quinine at 1 g daily started on day 3–5 of patent parasitaemia; all drugs given in four equal doses at 06:00, 12:00, 18:00 and 24:00 for 14 days

Adverse events surveillance: Blood smears every other day until 72 days after bites, twice weekly through day 180, once weekly until day 270. 102 men (half of each group) were observed clinically for 505 days, the remaining 102 for 350 days after exposure. Hb, methaemoglobin and haematocrit were measured immediately before treatment and again on days 7 and 14

G6PD deficiency status: Not determined

Severe adverse events reported: None among 34 men who took 10 mg and 34 who took 20 mg daily. No haemolysis seen, primaquine well tolerated especially for abdominal discomfort. Methaemoglobin was lowest with primaquine. Cyanosis occurred in 2/34 men at 20 mg. At the end of the study, the authors gave groups of 10 men a shorter regimen of a single dose of 20 or 30 mg primaquine (with 3 days of chloroquine, 1.5 g total dose) for 7 days; no severe adverse events were seen, but 80–90% relapse rate 40–50 days after chloroquine

Reference and location: Di Lorenzo et al., 1953 (*8*), USA

Study question: Curative effect of a week of primaquine for Korean vivax malaria

Primaquine dose or regimen: 15 mg daily for 7 days

Other antimalarial agents given: 1.5 grains (0.11 g) chloroquine over 3 days: three doses of 300 mg during the first 24 h and a single dose of 300 mg on each of the next 2 days; chloroquine given with (n = 31) or without primaquine (n = 46)

Adverse events surveillance: Follow-up of at least 90 days for relapse

G6PD deficiency status: Not determined

Severe adverse events reported: None in 31 patients who received primaquine with chloroquine. No specific measures of blood parameters

Reference and location: Thaeler, Arnold and Alving, 1953 (*9*), Nicaragua

Study question: Effectiveness of primaquine in preventing vivax malaria relapse and assessment of toxicity in Miskito Indians

Primaquine dose or regimen: 10 mg (n = 121), 15 mg (n = 151) or 20 mg (n = 49) daily for 14 days; controls received only chloroquine

Other antimalarial agents given: 900 mg chloroquine over 2 days as adult dose; smaller doses given to individuals weighing less

Adverse events surveillance: All patients observed clinically during treatment and re-examined 60, 90, 120, 150 and 360 days later

G6PD deficiency status: Not determined

Severe adverse events reported: None in 321 people who received primaquine. No haemolysis, and primaquine well tolerated in all age groups. Hb measurements revealed some anaemia (4.1–8.0 g/dL) in 16% of individuals and high incidences of intestinal parasites and malnutrition

Reference and location: Archambeault, 1954 (*10*), US soldiers (and a few civilians) returning by ship from Korea

Study question: Effects of mass drug administration

Primaquine dose or regimen: 15 mg for 14 days on board ship at breakfast or lunch

Other antimalarial agents given: None

Adverse events surveillance: Primaquine was given under direct observation: a card issued to each man was punched for each tablet received, or reasons for not taking it were recorded. A total of 415 340 military passengers received primaquine in 1952 and 1953; 332 925 completed the full course of 14 days, 21 499 completed 13 days, 24 932 12 days, 21 493 11 days, 9348 10 days and 5143 < 10 days

G6PD deficiency status: Not determined

Severe adverse events reported: Two men reported to be allergic to the drug, with no further information. Severe urticaria developed in one man, which disappeared on discontinuation of the drug and reappeared when therapy was resumed. Methaemoglobinaemia developed in two men and haemolytic anaemia in one. A few reports of mild-to-moderate dusky cyanosis that did not require discontinuation of primaquine

Reference and location: Singh et al., 1954 (*11*), India

Study question: Comparison of primaquine with pyrimethamine for preventing vivax relapse

Primaquine dose or regimen: 15 mg daily in two 7.5 mg doses for 5 days (n = 50), 46 completed treatment

Other antimalarial agents given: Two quinine–pyrimethamine tablets every 12 h (27 grains [1.75 g] quinine hydrochloride and 30 mg pyrimethamine), followed by 25 mg pyrimethamine weekly for 8 weeks (n = 126); 100 patients followed-up

Adverse events surveillance: All patients observed twice daily for toxic manifestations and followed up for relapses for 6–7.5 months

G6PD deficiency status: Not determined

Severe adverse events reported: None among 50 patients who received primaquine

Reference and location: Basavaraj, 1960 (*12*), India

Study question: Health records of migrant workers given primaquine in a malaria-free region

Primaquine dose or regimen: 15 mg daily for 5 days

Other antimalarial agents given: Camaquin (4-aminoquinoline), 0.6 g/adult single dose on day 1

Adverse events surveillance: Monthly surveillance of health records

G6PD deficiency status: Not determined

Severe adverse events reported: None in 2314 people given primaquine (approximately equal gender proportion and all age groups)

Reference and location: Clyde, 1961 (*13*), Tanganyika (now part of the United Republic of Tanzania)

Study question: Effect of mass administration of amodiaquine–primaquine to mainly Bantu, highly immune adults in three malaria-holoendemic locations

Primaquine dose or regimen: 15 mg for children aged 0–5 years, 30 mg for those aged ≥ 6 years, weekly for 39 weeks, fortnightly eight times or monthly for 9 months; treatment administered by trained medical staff

Other antimalarial agents given: Children aged 0–5 years received 75 mg amodiaquine, those aged ≥ 6 years received 150 mg

Adverse events surveillance: Periodic individual assessment of parasitaemia, gametocyte prevalence, species distribution, anopheline vector assessment

G6PD deficiency status: Not determined

Severe adverse events reported: None among 5000–7000 people at each of three locations, each with > 90% coverage. No haemolytic effects, no gastric disturbance even in small children and no severe or other adverse events

Reference and location: Gunders, 1961 (*14*), Liberia

Study question: Effect on gametocytes and sporogonia of naturally acquired *P. falciparum* infections after a single dose of pyrimethamine and primaquine (mostly children)

Primaquine dose or regimen: 40 mg for adults >100 lb (45.4 kg), 20 mg for 51–100 lb (23.1–45.4 kg) and 10 mg for 20–50 lb (9.1–22.7 kg)

Other antimalarial agents given: 50 mg pyrimethamine for adults >45.4 kg, 25 mg for 23.1–45.4 kg and 12.5 mg for 9.1–22.7 kg

Adverse events surveillance: Gametocyte counts before medication and daily afterwards; mosquitoes fed before drug administration and daily up to 3 days were dissected at 7–10 days for sporogonies and 11–14 for sporozoites

G6PD deficiency status: Not determined

Severe adverse events reported: None among 19 children (average age, 3.5 years) who received a minimum dose, two adults who received a maximum dose and one who received an intermediate dose

Reference and location: Ungureanu, 1962 (*15*), Romania

Study question: Comparison of two primaquine regimens for radical cure of patients infected with *P. vivax*: 15 days at 15 mg daily (n = 15) and 5 days at 225 mg total dose (n = 10); five volunteer uninfected controls; an additional 10 were infected with blood containing *P. vivax, P. malariae* or *P. falciparum*

Primaquine dose or regimen: 15 mg daily for 15 days or 45 mg daily for 5 days

Other antimalarial agents given: 600 mg chloroquine over 3 days (300 mg on day 1 and 150 mg each on days 2 and 3), or 3 days of 150 mg chloroquine daily or 300 mg nivaquine daily

Adverse events surveillance: Patients kept under observation during and after treatment

G6PD deficiency status: Not determined

Severe adverse events reported: None reported in 40 individuals received primaquine, even at the high dose

Reference and location: Comer et al., 1971 (*16*), Panama

Study question: Mass administration of primaquine with pyrimethamine every 2 weeks to ~2500 people (75% indigenous, 20% black, 5% white) in a rural river valley for malaria eradication

Primaquine dose or regimen: Every 2 weeks: 40 mg for people weighing >100 lb (45.4 kg), 20 mg for 50–100 lb (22.7–45.4 kg), 10 mg for 20–49 lb (9.1–22.2 kg) and 5 mg for infants aged 6 months weighing <20 lb (<9.1 kg)

Other antimalarial agents given: Pyrimethamine for cycles 1–25: 50 mg for >45.4 kg, 25 mg for 22.7–45.4 kg, 12.5 mg for 9.1–22.2 kg and 6.25 mg for <9.1 kg; for cycles 26–49: 75 mg for >45.4 kg, 37.5 mg for 22.7–45.4 kg, 18.75 mg for 9.1–22.2 kg and 9.38 mg for <9.1 kg

Adverse events surveillance: Door-to-door population census by the drug distributors every 2 weeks; thick blood slides every 8 weeks

G6PD deficiency status: Not determined

Severe adverse events reported: None in about 2500 recipients. Only headache and nausea were reported as adverse events; nothing that obviated a new cycle of treatment was registered.

Reference and location: Clyde and McCarthy, 1977 (*17*), USA

Study question: Efficacy as radical cure for mosquito-borne vivax Chesson strain infection in 11 healthy nonimmmune male volunteers aged 23–41 years

Primaquine dose or regimen: 60 mg daily for 7 days after chloroquine, when acute infection had subsided

Other antimalarial agents given: Chloroquine at 1.5 grains (0.11 g) over 3 days to patients with symptoms of parasitaemia and after two consecutive blood smears with parasites

Adverse events surveillance: Not described

G6PD deficiency status: Measured and positives excluded, but test not reported

Severe adverse events reported: None in 11 volunteers. Two men had moderate abdominal cramps and nausea towards the end of treatment.

Reference and location: Cedillos, Warren and Jeffery, 1978 (*18*), El Salvador

Study question: Field tests to compare amodiaquine alone with either amodiaquine and 5 days' primaquine or amodiaquine with a single dose of primaquine for *P. vivax* infections

Primaquine dose or regimen: 15 mg daily for 5 days (n = 90; children <5 years received 7.5 mg daily) or single dose of 45 mg (n = 67; children received 30 mg (7–12 years) 22.5 mg (3–7) or 15 mg (1–3))

Other antimalarial agents given: Amodiaquine given either alone (600 mg single dose) or with primaquine

Adverse events surveillance: Follow-up for ≥ 3 months and ≥ 9 months in some cases; visit every 2 weeks for thick blood films from people reporting fever and every 4–6 weeks from asymptomatic patients

G6PD deficiency status: Not determined

Severe adverse events reported: None among 157 people who received primaquine, including many children ≤14 years. Although adverse events were not monitored, none were reported at visits during follow-up.

Reference and location: Appavoo, Roy and Kapali, 1984 (*19*), India

Study question: Efficacy of a 3-day primaquine regimen for radical treatment of vivax malaria with chloroquine

Primaquine dose or regimen: 30 mg on day 0 and day 1, 15 mg on day 2 (adult dose, 75 mg); children > 1 year received proportionally less: 9–14-year-olds received 75%, those aged 5–8 years received 50% and those aged 1–4 years received 25% of adult dose

Other antimalarial agents given: Presumptive 600 mg chloroquine given 7–10 days before radical treatment and 600 mg on day 0 with first dose of primaquine

Adverse events surveillance: Blood smears taken on day 7 and monthly for 12 months and adverse side-effects noted

G6PD deficiency status: Not determined

Severe adverse events reported: None among 1203 people who received primaquine. Immediate results of treatment revealed no adverse events. The authors stated that, since 1977, 300 000 people had been treated with this regimen, with no deaths or severe adverse events; however, mild haemolysis was not evaluated.

Reference and location: Kaneko et al., 1989 (*20*), Indonesia

Study question: Gametocytocidal effect of primaquine when given with SP for falciparum malaria

Primaquine dose or regimen: 45 mg single dose for adults, proportionally less for those weighing <50 kg

Other antimalarial agents given: Single dose of 1500 mg sulfadoxine + 75 mg pyrimethamine adult dose and proportionally less to people weighing <50 kg

Adverse events surveillance: Slides to detect parasites prepared before drug administration (day 0), and patients followed clinically and parasitologically on days 2, 7 and once a week until gametocytaemia was cleared. Gametocyte counts also done for 87 patients given primaquine + SP

G6PD deficiency status: Not determined

Severe adverse events reported: None among about 489 patients given primaquine + SP, 318 with SP alone

Reference and location: Bunnag et al., 1994 (*21*), Thailand

Study question: Relapse rate of *P. vivax* malaria after primaquine treatment in 167 adults aged 15–60 years (47 women) with vivax malaria

Primaquine dose or regimen: After chloroquine, recovery from acute malaria symptoms and clearance of parasitaemia, patients were allocated by double-blind randomization to primaquine at 15 mg (n = 81) or 22.5 mg (n = 86) daily for 14 days.

Other antimalarial agents given: 300 mg chloroquine given on day 1 by open randomization to 87 patients and 450 mg to 80 patients

Adverse events surveillance: Monitored daily by physical examination and laboratory tests, including daily haematocrit; blood cell counts and biochemistry weekly. Follow up at 1, 3, 6 and 12 months

G6PD deficiency status: Measured

Severe adverse events reported: None among 167 patients who received primaquine

Reference and location: Baird et al., 1995 (*22*), Indonesia

Study question: Use of primaquine for malaria prophylaxis in nonimmune migrants from Java and Bali

Primaquine dose or regimen: 30 mg every other day (N = 45) for 16–19 weeks

Other antimalarial agents given: Chloroquine at 300 mg weekly to 54 people in the same village as those receiving primaquine

Adverse events surveillance: People interviewed once a week about presence of a list of physical complaints, and blood films taken

G6PD deficiency status: All participants screened for G6PD deficiency and found to be negative

Severe adverse events reported: None among 45 people who received primaquine

Reference and location: Fryauff et al., 1995 (*23*), Indonesia

Study question: Randomized double-blind placebo-controlled trial of primaquine, chloroquine and placebo for prophylaxis of falciparum and vivax malaria in males >15 years

Primaquine dose or regimen: 0.5 mg/kg daily for 1 year (30 mg daily adult dose). All three treatments started the day after the end of radical cure with 100 mg doxycycline twice daily for 10 days, quinine sulfate thrice daily for 4 days and 0.5 mg/kg primaquine daily for 14 days

Other antimalarial agents given: 300 mg chloroquine

Adverse events surveillance: Weekly malaria smears or, if symptoms appeared, weekly questionnaire for 1 year. Blood counts and biochemistry on days 0 and 3–5 of radical cure and at weeks 10, 20, 40 and 52 of prophylaxis

G6PD deficiency status: Measured

Severe adverse events reported: None in 42 patients who received primaquine. Mean methaemoglobin increase in primaquine group 5.8, versus 1.2 with placebo and 0.8 with chloroquine, but no clinical signs of methaemoglobinaemia; 7 days after last primaquine dose, methaemoglobin decreased to 2.4%

Reference and location: Weiss et al., 1995 (*24*), Kenya

Study question: Use of primaquine for falciparum malaria prophylaxis in 9–14-year-old schoolchildren by intermittent or daily dosing

Primaquine dose or regimen: First prophylactic dose given the day after curative therapy; 12 weeks of multivitamins or 15 mg primaquine on 3 days/week with food, or 11 weeks randomized to daily multivitamins, 15 mg primaquine or three regimens not including primaquine

Other antimalarial agents given: Curative therapy for pre-existing malaria: 7 days of quinine bisulfate at 300 mg three times daily and doxycycline at 50 mg twice daily

Adverse events surveillance: Symptoms assessed daily for 3 weeks by questionnaire, blood samples taken weekly for parasitaemia. During daily treatment, blood samples were taken at 6 and 11 weeks 1 h before medication for cell count, biochemistry and levels of primaquine and its major metabolite carboxyprimaquine in serum

G6PD deficiency status: Measured

Severe adverse events reported: None in about 40 patients who received primaquine intermittently and 32 who received primaquine. None of the adverse events reported was worse with primaquine.

Reference and location: Soto et al., 1998 (*25*), Colombia

Study question: Randomized, blinded study of 176 18–42-year-old male volunteer soldiers given primaquine prophylactically for vivax or falciparum malaria in a malaria-endemic area; one third received placebo

Primaquine dose or regimen: 30 mg daily for 16 weeks with breakfast

Other antimalarial agents given: None

Adverse events surveillance: Parasitaemia was determined by microscopy; malaria assumed at ≥ 1500 parasites/μL or at least two symptoms: fever, headache, myalgia, nausea, vomiting, diarrhoea or icterus. Toxicity was assessed daily from answers to questions.

G6PD deficiency status: Measured

Severe adverse events reported: None among 122 men who received primaquine. Three participants given primaquine (2.5%) had epigastric pain, abdominal pain or vomiting severe enough to cause withdrawal; six others (5.0%) had mild or moderate gastrointestinal symptoms. Only one placebo recipient (2.0%) had mild gastrointestinal symptoms.

Reference and location: Gogtay et al., 1999 (*26*), India

Study question: Efficacy of two primaquine regimens in preventing relapse of vivax malaria in 16–63-year-old patients with parasitaemia for 14 days

Primaquine dose or regimen: 5 days (n = 80) or 14 days (n = 81) at 15 mg/kg primaquine; treatment given under supervision

Other antimalarial agents given: Chloroquine for 3 days at 10 mg/kg on days 1 and 2, 5 mg/kg on day 3

Adverse events surveillance: Parasitaemia evaluated on admission; patients with 480–19 200 asexual parasites/μL blood included. Blood smears done on days 4, 8, 15, 22, 29 and then monthly. Patients followed-up up to 6 months

G6PD deficiency status: Measured but test not specified; cost, 15 rupees per test

Severe adverse events reported: None among 161 patients who received primaquine. Only mild adverse events, such as nausea and skin rash

Reference and location: Rowland and Durrani, 1999 (*27*), Pakistan

Study question: Randomized controlled trial of 5 and 14 days' primaquine against relapses of vivax malaria in an Afghan refugee settlement

Primaquine dose or regimen: 0.5 mg/kg for 5 (n = 128) or 14 days (n = 32) after chloroquine

Other antimalarial agents given: 25 mg/kg chloroquine in divided doses over 3 days

Adverse events surveillance: Each case followed-up daily by health workers, who supervised treatment until treatment was completed; blood smears taken at 1–3-day intervals for 28 days

G6PD deficiency status: Measured

Severe adverse events reported: None among 128 patients who received primaquine for 5 days and 32 for 14 days. Haemolysis was not specifically monitored. Frequency of recurrence of vivax malaria similar after 5 days' primaquine and placebo but lower after 14 days' primaquine (32% second episodes versus 49% without primaquine and 2% third episodes versus 25%), with longer intervals

Reference and location: Soto et al., 1999 (*28*), Colombia

Study question: Double-blind, randomized, placebo-controlled assessment of chloroquine + primaquine for malaria prophylaxis in nonimmune Colombian soldiers

Primaquine dose or regimen: 30 mg daily for 17 weeks with breakfast

Other antimalarial agents given: Chloroquine as one 300-mg tablet weekly

Adverse events surveillance: Parasitaemia determined by microscopy, malaria assumed at ≥ 1500 parasites/μL or at least two symptoms: fever, headache, myalgia, nausea, vomiting, diarrhoea or icterus. Toxicity assessed daily by questions

G6PD deficiency status: Measured

Severe adverse events reported: None among 100 men who received chloroquine + primaquine. Two (2%) men given chloroquine + primaquine (none given placebo) had to terminate prophylaxis prematurely because of gastrointestinal side-effects

Reference and location: Kaneko et al., 2000 (*29*), Vanuatu

Study question: Mass administration of primaquine for malaria elimination on Aneityum Island ($N = 718$); impregnated bed nets also distributed and retreated with permethrin once a year

Primaquine dose or regimen: 45 mg weekly for 8 weeks with chloroquine and SP or chloroquine only. Doses for children were calculated as one third the adult dose for children aged 1–4 years, one half for those aged 5–8 years and two thirds for those aged 9–15 years; pregnant women received chloroquine only; infants <3 months were not given primaquine.

Other antimalarial agents given: 45 and 600 mg chloroquine and three tablets of SP (1500 mg sulfadoxine plus 75 mg pyrimethamine) in weeks 1, 5 and 9 of primaquine, 300 mg chloroquine in weeks 2–4 and 6–8 of primaquine

Adverse events surveillance: Each dose administered under close supervision by staff. Villagers asked about possible side-effects of the previous week's drug intake before the next round; follow-up for malaria incidence and parasite rates for 9 years

G6PD deficiency status: Not determined

Severe adverse events reported: None among 718 people who received weekly primaquine. Some villagers reported vomiting after taking the tablets, and others complained that too many tablets had to be taken (13 for an adult in the first round). After a meeting with villagers, chloroquine removed from the fifth and the ninth rounds, and side-effects seldom reported

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Reference and location: Baird et al., 2001 (*30*), Papua, Indonesia

Study question: Randomized, parallel placebo-controlled trial of primaquine for malaria prophylaxis

Primaquine dose or regimen: 30 mg daily with morning meal for 20 weeks. Placebo given to 25% of participants

Other antimalarial agents given: None. Before primaquine, all individuals received radical curative therapy with single daily doses of 1 g atovaquone with 400 mg proguanil on days 0, 1 and 2; primaquine given on days 3–16

Adverse events surveillance: Blood films examined after any complaint consistent with malaria (headache, fever, chills, nausea, vomiting or malaise), and routine blood films collected once a week, regardless of symptoms. Blood taken for haematology and chemistry at weeks 4 and 20

G6PD deficiency status: Measured

Severe adverse events reported: None among 82 patients who received primaquine. The only adverse events for which the relative risk was significant (primaquine compared with placebo, $p < 0.05$) were headache, cough and sore throat, which were less frequent with primaquine. Haematological and chemical parameters indistinguishable before prophylaxis and at 4 or 20 weeks or within normal ranges. Mild methaemoglobinaemia on the last day of prophylaxis resolved within 18 days.

Reference and location: Nasveld et al., 2002 (*31*), Australia

Study question: Open randomized comparison of tafenoquine and primaquine in terminal prophylaxis of vivax malaria in Australian Defence Force personnel returning from Papua New Guinea

Primaquine dose or regimen: 214 volunteers received primaquine at 22.5 mg daily for 14 days

Other antimalarial agents given: 292 volunteers received tafenoquine at 400 mg daily for 3 days, and 86 received tafenoquine at 200 mg base twice daily for 3 days

Adverse events surveillance: Drug tolerability assessed by direct questioning and completion of a study diary by volunteers

G6PD deficiency status: Measured

Severe adverse events reported: None among 214 volunteers who received primaquine. Gastrointestinal disturbances such as nausea and abdominal pain in all groups; tafenoquine resulted in more adverse events than primaquine, which caused more intense but transient events

Reference and location: Solari-Soto et al., 2002 (*32*), Peru

Study question: Randomized open comparison of two regimens of primaquine in patients mainly aged 15–45 years

Primaquine dose or regimen: Either 15 mg daily for 14 days (n = 30) or 30 mg daily for 7 days (n = 30)

Other antimalarial agents given: Chloroquine at 10 mg/kg per day for 2 days followed by 1 day at 5 mg/kg

Adverse events surveillance: Follow-up at 7, 14, 28 and 60 days with a questionnaire on symptoms and adverse events, and thick blood slides every 2 weeks for 8 weeks

G6PD deficiency status: Not determined

Severe adverse events reported: None among 60 patients who received primaquine

Reference and location: Weerasinghe et al., 2002 (*33*), Sri Lanka

Study question: Safety and efficacy of artesunate, SP and primaquine in falciparum malaria

Primaquine dose or regimen: 45 mg single dose on day 0

Other antimalarial agents given: Artesunate at 4 mg/kg, sulfadoxine at 25 mg/kg, pyrimethamine at 1.25 mg/kg on day 0 and only artesunate on days 1 and 2 (4 mg/kg each day)

Adverse events surveillance: Blood examined for parasites, and patients assessed on days 1, 2, 7, 14, 21 and 28, including severity of selected symptoms; biochemical analyses on days 0, 7 and 28

G6PD deficiency status: Not determined

Severe adverse events reported: None among 30 patients who received primaquine

Reference and location: Rajgor et al., 2003 (*34*), India

Study question: Efficacy of a 14-day primaquine regimen in preventing relapse of vivax malaria

Primaquine dose or regimen: 15 mg/day primaquine for 14 days starting on day 4, administered by the investigator after lunch (n = 131); no therapy, n = 142

Other antimalarial agents given: All patients received initial treatment with chloroquine at 25 mg/kg per days on days 1–3

Adverse events surveillance: Patients hospitalized for entire treatment or asked to come daily for directly observed therapy; after treatment, patients asked to return for follow-up every month for 6 months and if they developed fever

G6PD deficiency status: Measured

Severe adverse events reported: None among 131 patients who received primaquine

Reference and location: Giao et al., 2004 (*35*), Viet Nam

Study question: Randomized clinical trial of efficacy and tolerance of CV8 (32 mg dihydroartemisinin + 320 mg piperaquine phosphate + 90 mg trimethoprim + 5 mg primaquine) for falciparum malaria

Primaquine dose or regimen: Two tablets of CV8 at 0, 8, 24 and 48 h

Other antimalarial agents given: Four tablets of Malarone (250 mg atovaquone + 100 mg proguanil hydrochloride) at 0, 24 and 48 h

Adverse events surveillance: Daily for 7 days, then 14, 21 and 28 days

G6PD deficiency status: Not determined, but authors noted that the prevalence of G6PD deficiency in southern Viet Nam was relatively low (*36*)

Severe adverse events reported: None among 84 people receiving CV8 containing primaquine

Reference and location: Walsh et al., 2004 (*37*), Thailand

Study question: Randomized, open-label, prospective comparison of tafenoquine and primaquine to assess safety, tolerability and ability to prevent vivax malaria relapse

Primaquine dose or regimen: 15 mg daily for 14 days

Other antimalarial agents given: Tafenoquine given at 300 mg daily for 7 days, 600 mg daily for 3 days or 600 mg single dose; 1500 mg chloroquine given over 3 days before tafenoquine or chloroquine; one arm placebo after chloroquine

Adverse events surveillance: Parasitaemia for a minimum of 8 weeks, with additional follow-up to 24 weeks, daily assessment of adverse events, methaemoglobin and blood smear during drug administration, at least every 2 weeks to week 8 and every 2–4 weeks up to 24 weeks

G6PD deficiency status: Measured

Severe adverse events reported: None among 12 people who received primaquine. More mild adverse events experienced with tafenoquine than primaquine

Reference and location: Carmona-Fonseca, Alvarez and Blair, 2006 (*38, 39*), Colombia

Study question: Evaluation of three doses of primaquine in chloroquine–primaquine treatment for uncomplicated vivax malaria in adults

Primaquine dose or regimen: 15 mg daily for either 3 days (45 mg total), 7 days (105 mg) or 14 days (210 mg) starting with chloroquine

Other antimalarial agents given: Chloroquine at 600 mg on day 1, 450 mg each on days 2 and 3 (with or without primaquine)

Adverse events surveillance: Clinical evaluation before treatment and on days 1, 2, 3 and 28

G6PD deficiency status: Not determined

Severe adverse events reported: None among 210 patients who received primaquine

Reference and location: Gogtay et al., 2006 (*40*), India

Study question: Randomized, parallel study of safety and efficacy of primaquine and bulaquine as gametocytocidal agents in adults with blood schizonticide-responsive uncomplicated falciparum malaria

Primaquine dose or regimen: 45 mg primaquine single dose on day 4 of treatment (because the incidence of nausea and vomiting is higher in the first few days of schizonticidal therapy, and primaquine was given regardless of parasite clearance)

Other antimalarial agents given: Patients with a gametocyte count >55/μL within 72 h of diagnosis, regardless of asexual parasite count, admitted to hospital and treated under observation with quinine orally at 10 mg/kg thrice daily for 7 days and doxycycline at 100 mg once daily for 7 days

Adverse events surveillance: On day 8, patients assessed for gametocytaemia, discharged and asked to return on days 15, 22 and 29 for further safety and parasitological checks. Malaria blood smears done twice a day for the first 72 h and once daily thereafter until discharge and at follow-up

G6PD deficiency status: Measured

Severe adverse events reported: None among 31 patients who received primaquine

Reference and location: Lederman et al., 2006 (*41*), Indonesia

Study question: Use of chloroquine + SP and primaquine as gametocytocidal treatment for *P. falciparum* malaria

Primaquine dose or regimen: 45 mg single dose given on day 0 (n = 28) or day 2 (n = 28)

Other antimalarial agents given: Chloroquine at 25 mg/kg over 3 days and 25 mg/kg sulfadoxine + 1.25 mg/kg pyrimethamine single dose on day 0

Adverse events surveillance: 28-day follow-up; personnel visited patients on days 1, 2, 3, 4, 7, 11, 14, 18, 21 and 28 to assess symptoms, clinical recovery and adverse events and to obtain finger-prick blood samples. Blood blot specimens also prepared on days 0, 2, 7, 14, 21, 28 or day of recurrent parasitaemia from finger-prick blood samples

G6PD deficiency status: Measured

Severe adverse events reported: None among 56 patients who received primaquine

Reference and location: Dao et al., 2007 (*42*), Viet Nam

Study question: Preliminary study on safety and efficacy of a shorter artesunate–primaquine regimen for vivax malaria in adult patients

Primaquine dose or regimen: Three primaquine tablets (7.5 mg per tablet, total dose 22.5 mg) twice a day for 7 days starting 48 h after artesunate; drug taken immediately after breakfast and dinner with water and administration directly observed by one of the study physicians

Other antimalarial agents given: Artesunate at 200 mg at 0, 12, 24 and 36 h after admission to study

Adverse events surveillance: Safety first assessed in 15 male soldier inpatients with 28-day follow-up for recurrence of parasitaemia; 1 year later, 13 civilian patients (7 male, 6 female) were admitted to health stations for at least 5 days and returned twice daily to receive their medication under supervision, with follow-up for 28 days

G6PD deficiency status: Measured

Severe adverse events reported: None among 28 patients (22 men, 6 women). Artesunate–primaquine well tolerated; mild abdominal discomfort and diarrhoea experienced by two patients; Hb, haematocrit, RBC and white blood cell values not significantly different before and after treatment

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Reference and location: El-Sayed et al., 2007 (*43*), eastern Sudan

Study question: Two-arm, open-label randomized controlled trial of efficacy of artesunate–SP–primaquine in clearing falciparum parasitaemia and gametocytes

Primaquine dose or regimen: 0.75 mg/kg primaquine on day 3

Other antimalarial agents given: 25 mg/kg sulfadoxine–1.25 mg/kg pyrimethamine as a single dose; 200 mg artesunate to adults and children weighing ≥50 kg, 4 mg/kg for children ≤50 kg once daily for 3 days

Adverse events surveillance: On days 1, 2, 3, 7 and 14

G6PD deficiency status: Not determined

Severe adverse events reported: None among 44 patients who received primaquine. Three given artesunate–SP–primaquine vomited, two complained of insomnia and another two of itching. The authors noted that the low frequency of gametocytes at the beginning (asymptomatic subpatent falciparum parasitaemia) obviated assessment of primaquine.

Reference and location: Elmes et al., 2008 (*44*), Australia

Study question: Comparison of post-exposure prophylactic efficacy of tafenoquine alone as a single or twice daily dose and primaquine at 22.5 mg daily plus doxycycline in defence personnel aged 18–55 years returning to Australia after serving in Bougainville and Timor-Leste

Primaquine dose or regimen: 22.5 mg daily for 14 days (as 7.5 mg thrice daily)

Other antimalarial agents given: Tafenoquine at 400 mg daily, 200 mg twice a day or 200 mg daily, all for 3 days; 100 mg doxycycline daily for 14 days with primaquine

Adverse events surveillance: Interview and diary from day 4 after start of therapy; all individuals monitored for 12 months for relapse of vivax malaria after leaving the endemic area

G6PD deficiency status: Measured

Severe adverse events reported: None among 464 personnel who received primaquine. With both primaquine and tafenoquine, the commonest adverse events were related to the gastrointestinal system, the most frequent in all groups being nausea, abdominal distress and diarrhoea; most events were of mild or moderate severity.

Reference and location: Krudsood et al., 2008 (*45*), Thailand

Study question: Efficacy, safety and tolerability of primaquine combined with artesunate to prevent relapse in patients with acute, symptomatic vivax malaria

Primaquine dose or regimen: After artesunate, patients randomly assigned into six groups: groups 1–5 received 30 mg primaquine daily for 5, 7, 9, 11 and 14 days, respectively; group 6 received 30 mg primaquine twice a day (60 mg daily) for 7 days

Other antimalarial agents given: All patients received 600 mg artesunate over 5 days before assignment to primaquine groups

Adverse events surveillance: Signs and symptoms of malaria and adverse events were monitored daily for the first 7 days and weekly thereafter. All patients closely monitored for clinical signs of intravascular haemolysis and haemoglobinuria; total follow-up, 28 days

G6PD deficiency status: Measured

Severe adverse events reported: None among 322 patients who received primaquine

Reference and location: Tangpukdee et al., 2008 (*46*), Thailand

Study question: Comparison of the efficacy, safety and tolerability of Artequick® (80 mg artemisinin, 400 mg piperaquine and 4 mg primaquine) with artesunate–mefloquine for acute uncomplicated falciparum malaria in patients ≥ 15 years

Primaquine dose or regimen: Artequick® once a day for 3 days

Other antimalarial agents given: Artesunate at 4 mg/kg and mefloquine at 8 mg/kg once a day for 3 days

Adverse events surveillance: Daily assessment during 28 days' follow up with non-suggestive questioning; routine haematology and blood biochemistry on day 0 and weekly for 4 weeks

G6PD deficiency status: Measured by fluorescence spot method

Severe adverse events reported: None among 65 patients who received Artequick® containing primaquine, during treatment or during the 28-day follow-up. Other adverse events (headache, dizziness, anorexia, weakness, nausea, abdominal pain, diarrhoea) during the first 3 days of drug administration could not be differentiated from those of malaria and disappeared within the first 5 days of treatment.

Reference and location: Carmona-Fonseca, Alvarez and Maestre, 2009 (*47*), Colombia

Study question: Occurrence of methaemoglobinaemia and other adverse events due to high doses of primaquine to prevent relapse of vivax malaria in adults aged about 30 years (66% men; no pregnant women)

Primaquine dose or regimen: 3 days' consecutive supervised course of two, three and five times the standard dose: 0.58 mg/kg (n = 27), 0.83 mg/kg (n = 28) and 1.17 mg/kg (n = 65)

Other antimalarial agents given: Chloroquine at 10 mg/kg on day 1 and 7.5 mg/kg on days 2 and 3

Adverse events surveillance: Examination by a general physician before and after treatment; methaemoglobin measured on days 1 and 15, and follow-up on days 4 and 18 after completion of 3-day primaquine treatment

G6PD deficiency status: Measured by normalized Beutler method and spectrophotometry; levels < 2.29 IU/g Hb considered G6PD-deficient

Severe adverse events reported: None among 120 patients who received primaquine. Methaemoglobin levels were 6.01 ± 6.88% at twice the standard dose, 5.29 ± 6.17% at three times and 4.84 ± 4.13% at five times. Methaemoglobin >4% was observed in 46–50% of patients in all groups on day 1 and in 4–9% of patients in all groups on day 15, showing a significant reduction. Random blood tests for 20 patients after 24 h of primaquine showed normal values. Although clinical findings such as jaundice, choluria, splenomegaly and palm pallor were more frequent in patients at five times the standard dose, the authors considered that they more likely to be associated with malaria than with primaquine. The adverse events seen with the higher daily doses were mild and short-lived, and treatment was not terminated for any patient.

Reference and location: Carmona-Fonseca and Maestre (*48*), 2009, Colombia

Study question: Efficacy of a 3-day primaquine regimen with chloroquine to prevent relapse of malaria

Primaquine dose or regimen: Four regimens: standard total dose of 0.25 mg/kg daily for 14 days (n = 68), 1.17 mg/kg daily for 3 days (standard adult total dose) (n = 65), 0.83 mg/kg daily for 3 days (71% total dose) (n = 28) or 1.75 mg/kg daily (50% total dose) for 3 days (n = 27)

Other antimalarial agents given: Chloroquine at 10 mg/kg on day 1 and 7.5 mg/kg daily on days 2 and 3; chloroquine and primaquine given simultaneously

Adverse events surveillance: Close observation during the first 28 days to detect chloroquine treatment failure, and monitoring during 120-day follow-up by active surveillance at clinic or home; capillary blood taken before treatment and then daily until a negative thick smear, then daily for 3 days and subsequently on days 28, 60 and 120

G6PD deficiency status: Measured

Severe adverse events reported: None among 188 patients who received primaquine

Reference and location: Vasquez et al., 2009 (*49*), Colombia

Study question: Efficacy and gametocytocidal effect of artesunate–mefloquine regimen with (n = 25) and without (n = 25) primaquine for falciparum malaria

Primaquine dose or regimen: 45-mg single dose after day 3 of artesunate–mefloquine (22.5 mg for children aged 7–13 years and 0.3–0.6 mg/kg for children aged 1–6 years)

Other antimalarial agents given: 200 mg artesunate and 500 mg mefloquine on days 0, 1 and 2 (100 mg artesunate and 250 mg mefloquine daily for children aged 7–13 years; for children aged 1–6 years, 50 mg artesunate on days 0, 1 and 2 and 250 mg mefloquine only on day 1)

Adverse events surveillance: Medical, parasitological and adverse events monitored on days 1, 2, 3, 7, 14, 21, 28, 35 and 42

G6PD deficiency status: Not determined

Severe adverse events reported: None among 25 patients who received primaquine. Common adverse events were vertigo (42%) and motor imbalance, such as unstable walk (36%), on days 2 and 3; gastrointestinal adverse events such as nausea, diarrhoea and vomiting were present both with and without primaquine

Reference and location: Carmona-Fonseca, 2010 (*50*), Colombia

Study question: Randomized controlled comparison of two primaquine regimens to prevent vivax malaria recurrence in children < 18 years

Primaquine dose or regimen: 0.50 mg/kg daily for 7 days (n = 41) or 1.17 mg/kg daily for 3 days (n = 38), given with food as directly observed therapy

Other antimalarial agents given: 10 mg/kg chloroquine on day 1 and 7.5 mg/kg on days 2 and 3 with primaquine

Adverse events surveillance: Follow-up during treatment, on day 28 and then monthly until day 120

G6PD deficiency status: Measured

Severe adverse events reported: None among 79 patients who received primaquine

Reference and location: Pukrittayakamee et al., 2010 (*51*), Thailand

Study question: Randomized comparison of two short-course primaquine regimens for the treatment and radical cure of vivax malaria in men

Primaquine dose or regimen: 0.50 mg/kg daily (adult dose, 30 mg/day) for 7 days or 1.0 mg/kg daily (adult dose, 60 mg base/day) for 7 days

Other antimalarial agents given: None

Adverse events surveillance: Gastrointestinal and any other symptoms assessed daily during and after treatment (days 0–7, 14, 28). Haematocrit measured daily during and after treatment. Blood count and biochemistry performed before treatment and on days 7, 14 and 28

G6PD deficiency status: Measured

Severe adverse events reported: None among 85 patients who received primaquine. All recovered fully. One developed acute tonsillitis on day 5 but responded to antibiotics. One patient at 60 mg had persistent mild abdominal pain until day 7, responded well to antacids, and pain resolved on day 8, indicating that it was related to primaquine. Transient localized rashes in three patients on days 0–3 lasted 24 h. Gastrointestinal symptoms on days 0–3 (acute malaria) were commoner at the lower dose (33/43 versus 22/44; $p = 0.033$).

Reference and location: Smithuis et al., 2010 (*52*), Myanmar

Study question: Open-label randomized trial to compare the effectiveness of four ACT components in adults and children with acute uncomplicated falciparum malaria or mixed infection

Primaquine dose or regimen: 0.75 mg/kg single dose on day 0 as gametocytocide taken after dinner

Other antimalarial agents given: All four WHO-recommended fixed-dose ACT components (artesunate–mefloquine, artesunate–amodiaquine, dihydroartemisinin–piperaquine, artemether–lumefantrine) and loose artesunate–mefloquine

Adverse events surveillance: The first dose was supervised; all subsequent doses were self- administered. Patients were asked to return weekly for 9 weeks or if they became ill. Hb measured on day 63

G6PD deficiency status: Not determined

Severe adverse events reported: None among 397 patients who received primaquine. Frequency of adverse events no different with and without primaquine, except more abdominal pain with primaquine. No haemolysis but a small adverse event on recovery from anaemia with primaquine

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Reference and location: Ebringer et al., 2011 (*53*), Timor-Leste

Study question: Open-label study of safety and tolerability of primaquine in 18–55-year-old Australian Defence Force personnel for presumptive anti-relapse therapy

Primaquine dose or regimen: 60 mg/day (30 mg twice a day) for 7 days

Other antimalarial agents given: Either daily doxycycline (100 mg) or atovaquone/proguanil (250/100 mg) for malaria prophylaxis

Adverse events surveillance: Blood samples taken at days 0 and 7; adverse events ascertained from daily diary and an interview on day 6

G6PD deficiency status: Measured

Severe adverse events reported: None among 203 personnel who received primaquine. Only mild and moderate adverse events observed, some attributable to physical and mental stress

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Reference and location: Maneeboonyang et al., 2011 (*54*), Thailand

Study question: Comparison of directly observed and self-administered therapy with primaquine to reduce vivax malaria recurrence

Primaquine dose or regimen: 15 mg daily for 14 days after chloroquine

Other antimalarial agents given: Chloroquine at 1500 mg total dose over 3 days

Adverse events surveillance: All patients followed up on days 14, 21, 28, 60 and 90, with blood smears taken at those times and before treatment for vivax and falciparum parasitaemia

G6PD deficiency status: Measured

Severe adverse events reported: None among 92 patients who received primaquine. Mean haematocrit was 37.6% in directly observed therapy group and 38.6% in self-administered therapy group.

Reference and location: Betuela et al., 2012 (*55*), Papua New Guinea

Study question: Two paediatric cohorts (5–10 and 1–5 years of age) randomized to assess safety and tolerability of primaquine

Primaquine dose or regimen: 5–10-year-olds received 0.5 mg/kg daily for 14 days with chloroquine; 1–5-year-olds received 0.5 mg/kg daily for 14 days with artesunate

Other antimalarial agents given: 5–10-year-olds randomized to receive chloroquine (standard 25 mg/kg total dose divided over 3 days) with either primaquine or placebo; 1–5-year-old cohorts randomized to receive artesunate (4 mg/kg per day) for 7 days with or without primaquine

Adverse events surveillance: Symptoms, adverse events and general tolerability of the drugs were recorded daily from standardized questionnaires

G6PD deficiency status: Measured

Severe adverse events reported: None among 252 children aged 5–10 years and 141 children aged 1–5 years who received primaquine. This study constitutes the first published evidence of acceptable safety and tolerability of primaquine given for 14 days at a high dose (0.5 mg/kg) to G6PD-normal children aged 1–10 years. In children aged 5–10 years, adverse events were infrequent, with a notable reduction in Hb level thought to be associated with primaquine after 7 days in only one child, The younger cohort had a slightly higher rate of adverse events due to more pre-existing illness. Gastrointestinal effects were rare even in children aged 1–5 years who did not receive the drug with food.

Reference and location: Kolaczinski et al., 2012 (*56*), Pakistan

Study question: Single-blinded, randomized trial to compare chloroquine and SP with either artesunate or primaquine for failure rates and effect on gametocyte carriage; six arms: chloroquine, chloroquine–artesunate, chloroquine–primaquine, SP, SP–artesunate, SP–primaquine

Primaquine dose or regimen: 0.5 mg/kg on day 3 of chloroquine and on day 0 of SP

Other antimalarial agents given: Chloroquine at 25 mg/kg for 3 days, SP at 25 mg/kg sulfadoxine + 1.25 mg/kg pyrimethamine on day 0 and artesunate at 4 mg/kg daily for 3 days

Adverse events surveillance: Patients followed-up on each day of treatment (days 0–2) and then on days 3, 7, 14, 21 and 28. Blood smears, spots taken and clinical symptoms recorded; haematocrit measured and blood spots taken on day of failure or day 28

G6PD deficiency status: Not determined

Severe adverse events reported: None among 100 patients who received primaquine. The 28-day failure rate was 81% with chloroquine and 73% with chloroquine–primaquine. Trophozoite clearance time was lowest with artesunate; addition of primaquine to either chloroquine or SP did not affect clearance times. Primaquine and artesunate with chloroquine or SP were more effective than chloroquine or SP alone in reducing gametocytaemia; primaquine more effective against older gametocyte infections

Published studies to evaluate the safety of primaquine, including G6PD-deficient individuals

Reference and location: Jones et al., 1953 (*57*); USA

Study question: Toxicity and curative effects of 10–30 mg primaquine daily for Korean vivax malaria (963 patients with a late acute attack diagnosed by parasitaemia and fever >101 °F [>38.3 °C])

Primaquine dose or regimen: Daily dose of 10, 15, 20 or 30 mg starting on day 1 of chloroquine, for 7 or 14 days

Other antimalarial agents given: Chloroquine at 1.5 g over 3 days (three doses of 300 mg during the first day and a single 300-mg dose on each of the next 2 days)

Adverse events surveillance: Follow-up for at least 4 months for relapse

G6PD deficiency status: Not measured, but several African American were included in the study, who might have been G6PD-deficient: 14 were treated with 20 mg, 8 with 15 mg and 9 with 10 mg

Effect in G6PD-deficient individuals: One 19-year-old African American treated with 20 mg developed moderately severe haemolytic anaemia: Hb fell from 14.6 g at start of treatment to 9.4 g on day 8, and his RBC count fell from 4.4 to 3.2 million/μL, with moderate haemoglobinuria. He was asymptomatic; however, the drug was withdrawn. Two days after the patient was given oral sodium bicarbonate for 4 days, Hb was 8.2 g/dL; he was given 1 litre of blood, and Hb and RBC count returned to normal for 3 months

Severe adverse events reported: One case of haemolytic anaemia in one of 510 men who received primaquine. No significant toxicity with 10 or 15 mg daily; 20 mg caused acute severe intravascular haemolysis in one of 14 African Americans but was well tolerated by all other patients. Mild abdominal pain and cyanosis due to methaemoglobinaemia occurred in ~25% of white patients given 30 mg: 20% were cyanotic with methaemoglobin 8–14% on the last day, but cyanosis cleared promptly after treatment was completed.

Reference and location: Arnold et al., 1954 (*58*), USA

Study question: Toxicity of continuous and intermittent primaquine + chloroquine therapy to prevent relapse of Chesson vivax malaria in African Americans

Primaquine dose or regimen: 30 mg weekly as a single dose for 12 months (n = 60) or 9 months (n = 37)

Other antimalarial agents given: Chloroquine at 300 mg weekly with primaquine

Adverse events surveillance: Monthly analysis of blood and urine; symptoms of toxicity monitored (no details provided)

G6PD deficiency status: Not measured, but all the men were African Americans

Effect in G6PD-deficient individuals: None

Severe adverse events reported: None among 97 African Americans who received primaquine. No symptoms of toxicity. Study performed to confirm safety of weekly versus daily therapy for G6PD-deficient individuals

Reference and location: Hodgkinson, Courtney and Haggerty, 1961 (*59*), USA, East Africa and Congo

Study question: Effect of intermittent administration of a combination of amodiaquine and primaquine (Camoprim) on the haematocrit of primaquine-sensitive and non-sensitive children: 11 African American primaquine-sensitive children, 64 white and 28 African American non-sensitive children; and mass drug administration to 235 African children aged 6 months to 12 years

Primaquine dose or regimen: Amodiaquine–primaquine tablets containing 15 mg primaquine administered weekly for at least 5 weeks (two studies in 2 consecutive years) at 0.2–0.5 mg/kg to primaquine-sensitive children; for 5 week at 0.3–0.5 mg/kg for non-sensitive children; and for 5 weeks to African children

Other antimalarial agents given: Tablets contained 5 mg amodiaquine

Adverse events surveillance: Haematocrit and reticulocyte counts before and 3, 5 and 8 days after drug administration

G6PD deficiency status: Measured in 11 African American children (probably

G6PD A– variant: seven hemizygous boys and two homozygous and two heterozygous girls). Not measured in the 235 African children

Effect in G6PD-deficient individuals: No severe haemolysis in any group. In primaquine-sensitive children, the lowest mean haematocrit was 3.5% below pretreatment level (on day 17) at the lowest dose and 4.2% on day 12 with the higher doses. After mass administration, the Hb levels unexpectedly rose and remained elevated during drug administration; however, the mean initial Hb was 71%, indicating anaemia at presentation.

Severe adverse events reported: None among 338 children who received primaquine

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Reference and location: Kellermeyer et al., 1961 (*60*), USA

Study question: Characterization of haemolysis caused by primaquine at 30 mg daily in healthy African American male volunteers

Primaquine dose or regimen: 30 mg daily for 14 days

Other antimalarial agents given: None

Adverse events surveillance: Erythrocyte life span and G6PD activity measured. After recovery from haemolysis, three of six sensitive men were given primaquine at a higher dose of 120 mg daily (after 30 mg daily for either 5 weeks or 1 year)

G6PD deficiency status: Measured in six primaquine-sensitive men

Effect in G6PD-deficient individuals: Anaemia, increased reticulocytosis, haemoglobinuria, scleral icterus and other clinical signs of haemolysis were seen 2–4 days after beginning of primaquine treatment.

Severe adverse events reported: Acute haemolysis observed in six sensitive men. The acute initial phase of haemolysis (rapid fall in haematocrit) was followed by a recovery phase, starting with an increase in reticulocyte count, followed by an equilibrium or "resistant" phase, during which Hb and haematocrit returned to pretreatment levels. A second haemolytic crisis could be induced during this phase with a higher dose of primaquine (240 mg daily).

Reference and location: Vivona et al., 1961 (*61*), Korea (now the Republic of Korea)

Study question: Feasibility and toxicity of a primaquine–chloroquine regimen for US military and civilian personnel (*n* = 50 000, including many African Americans) and Turkish troops (*n* = 250)

Primaquine dose or regimen: 45 mg weekly with chloroquine with food (the two drugs in one tablet)

Other antimalarial agents given: 300 mg chloroquine weekly with primaquine

Adverse events surveillance: Any toxic reactions were followed-up by meaurements of Hb and haematocrit.

G6PD deficiency status: Only 104 Turkish troops were tested, with one found to be G6PD-deficient but no detectable activity by the Glock and McLean method. Presumably, many of the African American men had G6PD A–.

Effect in G6PD-deficient individuals: After 14 weekly doses, no clinical toxic reactions were observed in any of the 250 Turkish soldiers, including the G6PD-deficient one; at 4.5 months, he had a normal haemogram

Severe adverse events reported: None among soldiers who received primaquine. No haemolysis or any other toxic reaction was observed during the first 22 weeks of this trial.

Reference and location: Brewer et al., 1962 (*62*), USA

Study question: Presence of methaemoglobin as a result of primaquine therapy in African American prison inmate volunteers

Primaquine dose or regimen: 30 mg daily (*n* = 24; 12 normal and 12 primaquine-sensitive) and single dose 120 mg (*n* = 3 primaquine-sensitive individuals)

Other antimalarial agents given: None

Adverse events surveillance: Three men who took a single 120-mg dose observed every 3 h for 6 days

G6PD deficiency status: Not measured; however, the 12 primaquine-sensitive men who received 30 mg daily and the three who received a single 120-mg dose were probably G6PD-deficient

Effect in G6PD-deficient individuals: All 12 primaquine-sensitive men who received 30 mg daily experienced acute haemolysis and methaemoglobin levels more than three times lower than those of 12 G6PD-normal men. The severity of haemolysis at 120 mg was no greater than at 30 mg in sensitive individuals. The Hb level dropped from 13–14% in G6PD-normal men to 10% on day 7 (starting at 11% on day 4) and then slowly returned to 12–13% on days 18–20.

Severe adverse events reported: Acute haemolysis in all sensitive individuals (probably G6PD-deficient). This study showed, contrary to what was believed previously, that methaemoglobinaemia is lower in primaquine-sensitive than in G6PD-normal individuals. The authors showed that that older erythrocytes that are destroyed by primaquine are those that preferentially accumulate methaemoglobin when exposed to agents that oxidize Hb, such as sodium nitrate.

Reference and location: Cahn and Levy, 1962 (*63*), USA

Study question: Tolerance of primaquine-sensitive versus insensitive men to large weekly doses of primaquine and amodiaquine

Primaquine dose or regimen: 45 mg weekly at single dose for 8 weeks

Other antimalarial agents given: Amodiaquine at 300 mg single dose weekly for 8 weeks

Adverse events surveillance: RBCs, haematocrit and Hb measured and urinalyses performed before the beginning of the study and at weekly intervals

G6PD deficiency status: Measured by methaemoglobin reduction test

Effect in G6PD-deficient individuals: No significant differences in haematocrit or Hb between G6PD-normal and G6PD-deficient men

Severe adverse events reported: None among 27 men who completed treatment. One case of mild nausea and one of mild burning on urination

Reference and location: Brewer and Zarafonetis, 1967 (*64*), USA

Study question: Effects of various primaquine + chloroquine regimens on haemolysis in G6PD-deficient individuals of African American descent in prisons

Primaquine dose or regimen: Groups of eight men received 45 mg primaquine once weekly, twice weekly or once weekly for 4 weeks then twice weekly.

Other antimalarial agents given: 300 mg chloroquine at the same time as primaquine

Adverse events surveillance: Blood samples taken three times a week for Hb and haematocrit measurements

G6PD deficiency status: 24 volunteers were African American and G6PD-deficient (presumably G6PD A– variant)

Effect in G6PD-deficient individuals: Most men dosed weekly had minimal, asymptomatic haemolytic episodes, with complete recovery within 8 weeks; one complained of upset stomach, nervousness and loss of sleep early during haemolysis. Men dosed twice weekly had slightly more acute haemolytic episodes, but the symptoms were minimal; recovery was complete within 8 weeks, except for one man who recovered at 10 weeks. Men who received primaquine once weekly for 4 weeks then twice weekly had less acute decreases in Hb and haematocrit, except for one individual.

Severe adverse events reported: None among 24 men who received primaquine. The haematological toxicity after twice-weekly primaquine + chloroquine was somewhat greater than after once-weekly treatment, but the temporary anaemia was well tolerated by G6PD-deficient men. One man treated twice weekly showed symptoms of upper respiratory infection with no exacerbation of haemolysis. One who received primaquine once weekly for 4 weeks then twice weekly had a marked haemolytic reaction with fever and chills, considered to be due to an infection, which was treated with salicylates.

Reference and location: George et al., 1967 (*65*), USA

Study question: Primaquine-induced haemolysis in three white (English, Sicilian and Ashkenazi Jewish) and one African American G6PD-deficient individuals

Primaquine dose or regimen: 45 mg single dose

Other antimalarial agents given: None

Adverse events surveillance: Baseline parameters observed for 1 week, then primaquine-related haemolytic episodes and recovery evaluated daily for 11 days and at 1–4-day intervals for 27 days

G6PD deficiency status: G6PD activity measured in RBC haemolysates before and after primaquine. Classification of one A– and three Mediterranean B– variants by enzyme activity and electrophoretic mobility

Effect in G6PD-deficient individuals: All individuals had a normal baseline RBC mass and haematocrit. The African American and the Englishman had 10–20% G6PD activity, and the other two had no activity. The activity of the first two increased after primaquine, coincident with reticulocytosis; no increase was seen in those with no activity.

Severe adverse events reported: Moderate haemolysis in three of four G6PD-deficient men who received primaquine. After primaquine ingestion, the haematocrit fell in all men, with a nadir at 5–7 days, returning to normal within 2–3 weeks. The duration of haemolysis was similar in all men (4–5 days), but fewer RBC were destroyed in the African American (8%) than in the whites (~20%).

Reference and location: Ziai et al., 1967 (*66*), Iran (now the islamic Republic of Iran)

Study question: Effects of short and prolonged chloroquine–primaquine therapy in G6PD-deficient individuals

Primaquine dose or regimen: 45 mg adult dose weekly for 4 weeks or longer unless severe adverse events occurred

Other antimalarial agents given: 300 mg chloroquine adult dose weekly

Adverse events surveillance: Initial Hb and haematocrit measured and then after first dose

G6PD deficiency status: Measured by enzymatic activity of RBCs, G6PD deficiency <115 U/dL; three children had history of favism, and most individuals had family history of favism

Effect in G6PD-deficient individuals: All developed some clinical or laboratory evidence of haemolysis (most showed pallor, jaundice and dark urine), which was not uniform and not necessarily related quantitatively to G6PD levels

Severe adverse events reported: Haemolysis observed in all 10 individuals (six children <12 years); one child required a blood transfusion

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Reference and location: Pannacciulli et al., 1969 (*67*), Italy

Study question: Haemolytic effects of primaquine + chloroquine in 25 G6PD-deficient Sardinians

Primaquine dose or regimen: 45 mg single dose; in a few cases, a second dose was given in the second week.

Other antimalarial agents given: 300 mg chloroquine weekly

Adverse events surveillance: RBC survival and haematocrit measured before and after treatment

G6PD deficiency status: 25 individuals (one woman, 24 men) found to have severe deficiency; measured in RBC (Kornberg and Horecker method)

Effect in G6PD-deficient individuals: 19 of the 25 individuals receiving primaquine showed shortened survival of erythrocytes (mean half-life, 26 days, and 9.6 days after primaquine) and drop in mean haematocrit from 42.8% before to 33.2% after primaquine

Severe adverse events reported: Moderate-to-severe hameolysis in 19 of 25 G6PD-deficient people who received primaquine. No haemolysis in three G6PD-normal individuals. The crises were self-limited, and the haematocrit began to rise within a few days.

Reference and location: Fisher et al., 1970 (*68*), USA

Study question: Epidemiology of vivax malaria among US Army troops in Viet Nam and among returned soldiers in the USA

Primaquine dose or regimen: Unsupervised 45-mg dose weekly for 8 weeks (n = 94) or supervised 15-mg dose daily for 14 days (n = 133)

Other antimalarial agents given: Chloroquine at 300 mg weekly for 8 weeks

Adverse events surveillance: 6-month follow-up for relapse. Routine laboratory examinations, three stool examinations for ova and parasites, three blood films per week

G6PD deficiency status: All African American patients screened for G6PD deficiency by the methaemoglobin reduction method; three found to be G6PD-deficient

Effect in G6PD-deficient individuals: One of the two G6PD-deficient patients who received 15 mg primaquine for 14 days developed mild Coombs-negative haemolytic anaemia, which was self-limiting, Hb never <9 g/dL and with no azotaemia or haemoglobinuria. The third G6PD-deficient man received the 8-week regimen with no overt haemolysis.

Severe adverse events reported: Adverse events reported only in the three G6PD-deficient men.

Reference and location: Everett et al., 1977 (*69*), Cambodia

Study question: Use of primaquine for radical cure in Khmer Air Force troops

Primaquine dose or regimen: 15 mg daily in the morning for 14 days to 15 G6PD-deficient men and 31 G6PD-normal controls

Other antimalarial agents given: None

Adverse events surveillance: All patients seen daily by a physician and followed for haemoglobinuria and changes in haematocrit during treatment (blood drawn on days 7 and 15)

G6PD deficiency status: 15 men were G6PD-deficient (test not described) with Mahidol variant and 4–11% of normal enzymatic activity (except one variant with 22%); six had Hb E.

Effect in G6PD-deficient individuals: Significant fall in haematocrit, from 43% (day 1) to 34% (days 7 and 15); G6PD-normal: 45% before primaquine, 44% after. Haemolysis was significant but not dangerous.

Severe adverse events reported: None among 46 men who received primaquine. No significant side-effects seen in controls or the G6PD-deficient group; no nausea, weakness or change in urine colour. The lowest haematocrits (at end of first week) were 26% and 28%, which rose to 30% at week 2 and 35% by day 21. This study shows that the G6PD deficiency Mahidol variant and Hb E are linked.

Reference and location: Khoo, 1981 (*70*), Malaysia

Study question: Primaquine-induced haemolysis in G6PD-deficient malaria patients ($N = 23$, including seven children)

Primaquine dose or regimen: 75 mg over 3 days for falciparum malaria, 210 mg over 14 days for vivax or mixed infections (children received proportionally less drug)

Other antimalarial agents given: Chloroquine at 1500 mg over 3 days with primaquine

Adverse events surveillance: Thick films for parasitaemia taken on days 1, 4, 7, 14, 21 and 28

G6PD deficiency status: Measured by Brewer methaemoglobin reduction test

Effect in G6PD-deficient individuals: The seven patients with haemolysis had "complete" G6PD deficiency.

Severe adverse events reported: Seven of 23 patients who received primaquine showed haemolysis 2–5 days after start, all with dark urine (three were 13, 14 and 16 years old, the rest adults). Five required a blood transfusion, and the other two had acute renal failure, one requiring peritoneal dialysis; the fall in Hb was 1–5 g/dL.

Reference and location: Bangchang et al., 1994 (*71*), Thailand

Study question: Controlled trial to study the pharmacokinetics of primaquine in *P. vivax* patients aged 19–35

Primaquine dose or regimen: Daily doses of 15 mg for 14 days after chloroquine

Other antimalarial agents given: Total oral dose of 1500 mg chloroquine over 3 days

Adverse events surveillance: Daily assessment until discharge

G6PD deficiency status: Measured; 13 G6PD-deficient patients included (control, 13 G6PD-normal patients)

Effect in G6PD-deficient individuals: Haemolysis observed only in G6PD-deficient patients, with >20% or <20% maximum drop in haematocrit from baseline between days 4 and 7, and nausea, vomiting, abdominal pain and diarrhoea

Severe adverse events reported: None among 13 patients who received primaquine. Values and percentage haemolysis observed not reported; all patients responded well to treatment, with complete parasite and fever clearance within 16 days.

Reference and location: Myat Phone et al., 1994 (*72*), Myanmar

Study question: Efficacy of 45 mg primaquine weekly with 15 mg daily for 14 days for vivax malaria patients with severe G6PD deficiency

Primaquine dose or regimen: 45 mg weekly for 8 weeks for patients infected with *P. vivax* (n = 31) and 45 mg single dose for patients with *P. falciparum* (n = 32)

Other antimalarial agents given: Quinine at 600 mg three times a day for 7 days, followed by primaquine

Adverse events surveillance: Blood smears taken and haematological parameters measured for 5 days, then on days 7 and 14

G6PD deficiency status: Methaemoglobin reduction test used to screen before treatment on admission. G6PD variants characterized electrophoretically

Effect in G6PD-deficient individuals: No haemolysis observed in 22 G6PD-deficient patients (two with the B– variant resulting in mild (40–60%) enzyme activity, 20 with the severe Myanmar variant resulting in enzyme activity <5% normal

Severe adverse events reported: None in 63 patients who received primaquine. No marked change in methaemoglobin in 32 G6PD-deficient patients

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Reference and location: Buchachart et al., 2001 (*73*), Thailand

Study question: Effect of primaquine in 12–60-year-old patients with vivax malaria

Primaquine dose or regimen: 15 mg daily for 14 days after chloroquine

Other antimalarial agents given: 1500 mg chloroquine for 3 days: 600 mg initially, 300 mg at 6, 24 and 48 h, for a total dose of approximately 20 mg/kg

Adverse events surveillance: Malaria symptoms monitored daily for first 7 days, then weekly, including signs of haemolysis, blood count and chemistry; blood smears daily until discharge

G6PD deficiency status: Determined by fluorescence spot test with ultraviolet light (Beutler)

Effect in G6PD-deficient individuals: No severe adverse events, but haematocrit significantly lower on days 7 and 14, returning to baseline on day 21 and higher on day 28; minimum was 20% (14% in G6PD normal). No blood transfusions required, but required for three G6PD-normal patients

Severe adverse events reported: None in 364 patients who received primaquine, 22 of whom were G6PD-deficient. 100% cure rate in both groups but only 28-day follow-up; parasite clearance time was comparable (59.4 ± 17.5 for G6PD-normal and 59.8 ± 15.0 h for G6PD-deficient; $p = 0.91$), but fever clearance time significantly longer in the G6PD-deficient group (45.2 ± 35.2 h) than in G6PD-normal (28.0 ± 22.2 h) ($p < 0.01$)

Reference and location: Silachamroon et al., 2003 (*74*), Thailand

Study question: Clinical trial of oral artesunate and primaquine for prevention of relapse of vivax malaria, optimum duration of artesunate treatment (5 or 7 days) and safety, tolerability and effectiveness of primaquine at a high dose (0.6 mg/kg a day for 14 days)

Primaquine dose or regimen: 0.6 mg/kg given after a meal or soft drink; maximum dose, 30 mg once a day for 14 days after artesunate

Other antimalarial agents given: Artesunate for 5 or 7 days: 200 mg on day 1 and 100 mg on the following 4 or 6 days, with or without primaquine for 14 days. Patients treated with artesunate alone were given primaquine at 15 mg/day for 14 days at follow-up on day 28 of the study to complete radical cure.

Adverse events surveillance: Signs and symptoms of malaria monitored daily for the first 7 days of admission and weekly thereafter; G6PD-deficient patients monitored closely during primaquine treatment: haematocrit assessed daily and examination for clinical evidence of intravascular haemolysis or haemoglobinuria. All patients hospitalized for 28 days or until reappearance of parasites

G6PD deficiency status: Measured. Patients with G6PD deficiency not excluded; those with a history of dark urine or significant haemoglobinuria related to primaquine treatment during a previous episode of malaria were excluded.

Effect in G6PD-deficient individuals: Of 44 G6PD-deficient patients, 18 received primaquine. Five of the 18 left hospital before finishing treatment, nine completed treatment, and four had significantly reduced haematocrits and were withdrawn on days 4–7 after primaquine; the decreases in haematocrit were 16%, 13%, 14% and 8% (mean change in G6PD-normal patients, 0.79%)

Severe adverse events reported: None in 394 patients who received primaquine. Some patients had nausea, vomiting or abdominal discomfort, which was self-limiting or required only symptomatic treatment. The only other adverse events were decreases of 8–16% in haematocrit in four G6PD-deficient patients.

Reference and location: Krudsood et al., 2006 (*75*), Thailand

Study question: Randomized, open-label, prospective comparison of safety and tolerability of bulaquine and primaquine for prevention of relapse of vivax malaria in 71 patients aged 16–51 years

Primaquine dose or regimen: 30 mg once daily for 7 days

Other antimalarial agents given: Chloroquine at 1500 mg over 3 days, followed by bulaquine or primaquine

Adverse events surveillance: Monitoring for signs and symptoms of malaria daily for the first 7 days of admission and weekly thereafter; all patients were closely monitored for evidence of intravascular haemolysis and haemoglobinuria.

G6PD deficiency status: Screening for G6PD deficiency on admission; in G6PD-deficient patients, haematocrit measured on days 0–10, 14, 21 and 28

Effect in G6PD-deficient individuals: The four G6PD-deficient patients treated with primaquine had clinically significant falls in haematocrit on days 7–9 (from 35% to 20%; lowest on day 8). Methaemoglobin was not measured.

Severe adverse events reported: None in 71 patients who received primaquine. During the 28-day follow-up, both drugs were well tolerated.

Reference and location: Shekalaghe et al., 2007 (*76*), United Republic of Tanzania

Study question: Two-armed, randomized controlled trial in children aged 3–15 years with fever and *P. falciparum* infection

Primaquine dose or regimen: 0.75 mg/kg on day 3

Other antimalarial agents given: 25 mg/kg sulfadoxine + 1.25 mg/kg pyrimethamine as a single dose; artesunate at 4 mg/kg once daily for 3 days

Adverse events surveillance: On days 1, 2, 3, 7, 14, 28 and 42. Anaemia symptoms (fatigue, weakness, dizziness, headache, heart palpitations) or allergic drug reactions (rash) were assessed.

G6PD deficiency status: Measured. G6PD A–: 5.6% in SP + artesunate arm, 7.5% in SP + artesunate + primaquine arm

Effect in G6PD-deficient individuals: Although Hb reduction shortly after SP + artesunate + primaquine was pronounced in G6PD A– children, none had clinical symptoms of anaemia or Hb <5 g/dL. Hb on days 28 and 42 was equal to or higher than at enrolment.

Severe adverse events reported: None in 53 children who received primaquine

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Reference and location: Leslie et al., 2008 (*77*), Pakistan

Study question: Open-label, randomized, placebo-controlled study with once weekly primaquine for 8 weeks to prevent relapse of vivax malaria

Primaquine dose or regimen: 0.5 mg/kg daily for 14 days or 0.75 mg/kg weekly for 8 weeks

Other antimalarial agents given: Initial 3-day chloroquine (25 mg/ kg in divided doses over 3 days) for acute disease, with either supervised weekly placebo once a week for 8 weeks or primaquine

Adverse events surveillance: Patients monitored for 8 weeks and for 9 additional months by active surveillance (home visits every 2 weeks) and passively at health centre. Patients presenting with febrile illness assessed by blood smear

G6PD deficiency status: Measured by Sigma colorimetric test: only one 13-year-old boy was G6PD-deficient and was assigned to 8 weeks of primaquine

Effect in G6PD-deficient individuals: The G6PD-deficient patient showed a slight, not clinically significant drop in Hb: Hb was 10.0 g/dL (versus 12.6 g/dL in G6PD-normal controls matched by age and sex; 95% CI, 11.8–13.4)) on day 7 and 10.6 (versus 12.6; 95% CI, 12.0–13.3) on day 14.

Severe adverse events reported: None in 54 patients who received primaquine. The Hb profile in the three arms was similar; no patient became seriously anaemic (Hb <7.0 g/dL), and no observed anaemia was clinically significant.

Reference and location: Shekalaghe et al., 2010 (*78*), United Republic of Tanzania

Study question: Part of population receiving mass drug administration randomized to either placebo or a gametocytocidal combination

Primaquine dose or regimen: 0.75 mg/kg on day 3 with last dose of artesunate

Other antimalarial agents given: The gametocytocidal drug combination also contained 25 mg sulfadoxine + 1.25 mg/kg pyrimethamine on day 1 and artesunate at 4 mg/kg daily for 3 days.

Adverse events surveillance: Hb concentration measured before treatment and at day 7

G6PD deficiency status: Measured at beginning by the presence of *G202A* mutation: 8.4% G6PD A heterozygotes and 3.9% G6PD A– homo- and hemizygotes

Effect in G6PD-deficient individuals: All children had Hb >8 g/dL at day 0. Moderate anaemia (Hb, <8 g/dL) in 4.5% (18/399) G6PD B children after the intervention, 11.1% (3/27) G6PD A and 40.0% (6/15) G6PD A–; the relative risk for moderate anaemia in comparison with G6PD B was 2.46 (95% CI, 0.77–7.84) for G6PD A and 8.87 (95% CI, 4.12–19.09) for G6PD A– children; however, all variants experienced considerable reductions in Hb.

Severe adverse events reported: One case of severe anaemia among 564 individuals who received primaquine. Considerable reductions in Hb concentrations, although these were transient and no patients required hospitalization or had clinical symptoms of anaemia. 5% of children became moderately anaemic after SP + artesunate + primaquine. This study suggests that the primaquine-related haemolytic risk extends beyond G6PD-deficient individuals. One 5-year-old child developed severe anaemia, with an Hb level of 4.8 g/dL (baseline, 8.3 g/dL), was given haematinic drugs and recovered with no need for additional treatment (Hb concentration, 7.8 g/dL 2 weeks after initiation of the intervention and 12.3 g/dL 3 months later).

Reference and location: Song et al., 2010 (*79*), Cambodia

Study question: Mass administration of ACT followed by primaquine to reduce transmission. Two studies: one in 17 villages (n = 3653) and one among mostly Khmer people (n = 2387)

Primaquine dose or regimen: 9 mg for adults at 10-day intervals for 6 months; first dose taken with artemisinin–piperaquine. Children aged 11–15 years took 3/4 tablet, those aged 7–10 years 1/2 tablet, those aged 3–6 years 1/3 tablet and those aged 1–2 years 1/4 tablet

Other antimalarial agents given: Two tablets of 62.5 mg artemisinin and 375 mg piperaquine for adults ≥ 16 years at 0 and 24 h, 1.5 tablets for children aged 11–15 years, one tablet for children aged 6–10 years, complete two-dose artemisinin–piperaquine granules (artemisinin 24 mg, piperaquine 144 mg per sachet) for children aged 5–6 years, 1.5 sachets for those aged 3–4 years and one sachet for children aged 1–2 years

Adverse events surveillance: After the start of mass treatment, periodic surveys to monitor parasite rates in villages with initial parasite rates ≥ 20% every 6 months; rates in 50 adults and 50 children determined randomly in every village. Severe adverse events reported passively, with no active monitoring

G6PD deficiency status: Entire population included, with no testing for G6PD deficiency. 252 villagers (one from each family) were screened with the G-6-PDH Kit Visual Colour. The rate was 17.1% (43/252); 18.6% male and 13.3% female

Effect in G6PD-deficient individuals: Not specifically followed, but, given the high incidence of G6PD deficiency, no severe adverse events in G6PD-deficient individuals

Severe adverse events reported: None in 6040 people who received primaquine. No indication of clinically significant adverse drug reactions

Reference and location: Takeuchi et al., 2010 (*80*), Thailand

Study question: Effectiveness of directly observed therapy with chloroquine + primaquine for radical cure of vivax malaria and prevention of relapse within 90-day follow-up of individuals >3 years of age

Primaquine dose or regimen: 216 patients randomized to directly observed therapy (n = 109) or self-administered therapy (n = 107): 15 mg daily for 14 days after chloroquine; for patients aged 8–13 years, the dose was 10 mg daily for 14 days; for those aged 3–7 years, 5 mg daily for 14 days

Other antimalarial agents given: Chloroquine at 1500 mg over 3 days followed by primaquine; for patients aged 8–13 years, the dose was 900 mg over 3 days, for those aged 3–7 years 750 mg over 3 days

Adverse events surveillance: Patients with microscopically confirmed vivax infection were either visited at home (directly observed therapy) or given drugs with instructions (self-administered therapy). During 90-day follow-up, both groups were visited by staff on days 7, 14, 28, 60 and 90 to determine parasitaemia

G6PD deficiency status: Commonest variants in the Karen population (G6PD Mahidol and Viangchan) determined by PCR and restriction fragment length polymorphism

Effect in G6PD-deficient individuals: G6PD-deficient mutations found in 22% of patients (2% homozygous, 9% hemizygous, 11% heterozygous), all Mahidol variant

Severe adverse events reported: None in 54 children aged 3–7 years and 39 children aged 8–13 years who received primaquine. Although adverse events were not specifically monitored, the authors reported that all participants recovered without severe adverse events.

Reference and location: Reviewed by Kondrashin, Baranova and Sergiev, 2010 (*81*), Azerbaijan

Study question: Post-eradication malaria therapy with primaquine, excluding infants and pregnant and lactating women, in 1971 ($n > 67\,000$ or 90% of targeted population) and 1972 (with a target of $n = 110\,000$; coverage, 87–93%)

Primaquine dose or regimen: "Intermittent" treatment (considered to be "safe"), consisting of 4 days of 15 mg primaquine daily, no drug on days 5–7 (when most primaquine-related haemolysis occurs) and 15 mg primaquine daily again on days 8–17 (total duration, 17 days); primaquine given after food and with water

Other antimalarial agents given: None

Adverse events surveillance: Directly observed therapy and daily surveillance of side-effects

G6PD deficiency status: Not tested; prevalence of G6PD deficiency in the region assessed in 1971–1972 shown to vary widely (0–38.4%)

Effect in G6PD-deficient individuals: Intermittent treatment given to 30 000 people in areas of high incidence of G6PD deficiency, with no severe adverse events. A small fraction of the treated population had light or moderate side-effects, like dizziness, headache, back pain and change of urine colour on days 3–6, which did not progress further. A few G6PD-deficient individuals had more serious complications, like severe headache, back pain, fatigue, red or black urine and jaundice; primaquine was stopped and all necessary treatment administered, with rapid recovery. Seven individuals were hospitalized, but no blood transfusion was required.

Severe adverse events reported: None in about 170 000 people who received primaquine. Everyone had reduced Hb (1–2 g/dL for G6PD-normal, 3–5 g/dL for G6PD-deficient, with increased reticulocytes), which correlated with age: more pronounced in children <11 years. G6PD-deficient malaria patients tolerated primaquine better than uninfected G6PD-deficient patients

Reference and location: Reviewed by Kondrashin, Baranova and Sergiev, 2010 (*81*), northern Afghanistan

Study question: Post-eradication malaria therapy with primaquine in the early 1970s, field trials in 1972 (n = 1937) and later the same year in 14 villages (n = 14 028 targeted, coverage >90%) and in 1973–1974, covering a total population of 78 000

Primaquine dose or regimen: "intermittent" treatment in high G6PD-deficiency areas: 4 days at 15 mg primaquine daily, no drug on days 5–7 and 15 mg daily again on days 8–17

Other antimalarial agents given: None

Adverse events surveillance: Directly observed therapy and daily surveillance of side-effects

G6PD deficiency status: Not tested; G6PD prevalence in different ethnic groups in the region assessed in 1973 and ranged from 0 to 21.6%, Pathans showing the highest incidence

Effect in G6PD-deficient individuals: No severe adverse effects reported, although an average of ≥ 4% G6PD deficiency expected in the regions where primaquine was administered

Severe adverse events reported: None in >90 000 people who received primaquine. Low incidence of side effects; the main symptoms were fatigue, headache, back pain, gastrointestinal disorders, change in urine colour. Two women with black urine reported 4–5 days after starting treatment did not require hospitalization.

Reference and location: Reviewed by Kondrashin, Baranova and Sergiev, 2010 (*81*), Tajikistan

Study question: Antimalaria therapy with primaquine in 1998–2008

Primaquine dose or regimen: 15 mg daily for 14 days

Other antimalarial agents given: None

Adverse events surveillance: Directly observed therapy and daily surveillance of side-effects

G6PD deficiency status: Not tested; the prevalence of G6PD deficiency in three regions of Tajikistan was 2.1%, ranging from 0.8% in Dangara to 1.6% in Dushanbe and 4% in Kabadiyon

Effect in G6PD-deficient individuals: No severe adverse events reported

Severe adverse events reported: None among 1 386 756 people who received primaquine. Only one adverse event (nature not determined) and 11 refusals of treatment were reported, all in 1999.

Reference and location: Reviewed by Kondrashin, Baranova and Sergiev, 2010 (*81*); Kondrachine, 2001, 2008 (*82*, *83*); Phu, 2008 (*84*); Democratic People's Republic of Korea

Study question: Antimalaria proliferation strategy in populations, excluding pregnant women and children <5 years old; 62 567 patients treated in May 2000 (*83*)

Primaquine dose or regimen: 15 mg daily (adult dose) for 14 days after breakfast with water. The safety and effectiveness of a shorter, higher dose of primaquine was also tested: twice 30 mg/day (N = 10 022) for 7 days in two doses (after breakfast and after dinner).

Other antimalarial agents given: None

Adverse events surveillance: Recipients of each directly observed dose were interviewed about side-effects the previous day.

G6PD deficiency status: Not tested; region considered to have a low prevalence (0.5–2.9%)

Effect in G6PD-deficient individuals: See below.

Severe adverse events reported: None among 62 567 who received primaquine in 2000 and almost 7 million in 2002–2007. The frequency of side-effects in 2002 was 4% (including headache, epigastric pain, nausea and vomiting, dizziness, anorexia and urine colour change), with no severe haemolysis. 15 people with black urine and suspected haemolysis recovered fully within 3–4 days after withdrawal of primaquine, with no hospitalization required. The fewest side-effects were seen in children <16 years, at <1.5% in 2003 and 2004. More adverse effects were seen with the shorter, higher dose (9.4%) than with 15 mg/day for 14 days (5.3%) (*84*).

References

1. Edgcomb JH et al. Primaquine, SN 13272, a new curative agent in vivax malaria; a preliminary report. *Journal of the National Malaria Society*, 1950, 9:285–292.
2. Alving AS, Arnold J, Robinson DH. Mass therapy of subclinical vivax malaria with primaquine. *Journal of the American Medical Association*, 1952, 149:1558–1562.
3. Clayman CB et al. Toxicity of primaquine in Caucasians. *Journal of the American Medical Association*, 1952, 149:1563–1568.
4. Garrison PL et al. Cure of Korean vivax malaria with pamaquine and primaquine. *Journal of the American Medical Association*, 1952, 149:1562–1563.
5. Alving AS et al. Korean vivax malaria. II. Curative treatment with pamaquine and primaquine. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:970–976.
6. Hockwald RS et al. Toxicity of primaquine in Negroes. *Journal of the American Medical Association*, 1952, 149:1568–1570.
7. Cooper WC et al. Studies in human malaria. XXXI. Comparison of primaquine, isopentaquine, SN-3883, and pamaquine as curative agents against Chesson strain vivax malaria. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:949–957.
8. Di Lorenzo A et al. Korean vivax malaria. IV. Curative effect of 15 milligrams of primaquine daily for 7 days. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:983–984.
9. Thaeler AD Jr, Arnold J, Alving AS. A clinical study of primaquine (S.N.13,272) in the treatment of malaria among the Miskito Indians of Nicaragua. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:989–999.
10. Archambeault CP. Mass antimalarial therapy in veterans returning from Korea. *Journal of the American Medical Association*, 1954, 154:1411–1415.
11. Singh J et al. Antirelapse treatment with primaquine and pyrimethamine. *Indian Journal of Malariology*, 1954, 8:127–136.
12. Basavaraj HR. Observations on the treatment of 678 malaria cases with primaquine in an area free from malaria transmission in Mysore State, India. *Indian Journal of Malariology*, 1960, 14:269–281.
13. Clyde DF. *Mass administration of an antimalarial drug combining 4-aminoquinoline and 8-aminoquinoline in Tanganyika*. Geneva, World Health Organization, 1961.
14. Gunders AE. The effect of a single dose of pyrimethamine and primaquine in combination upon gametocytes and sporogony of *Laverania falcipara* (*Plasmodium falciparum*) in Liberia. *Bulletin of the World Health Organization*, 1961, 24:650–653.

15. Ungureanu E. *Note on the treatment of chronic benign tertian malaria with plasmoqune and quinine. The use of the association chloroquine–primaquine in the radical treatment of malaria*. Geneva, World Health Organization, 1962.
16. Comer RD et al. Tratamiento colectivo con pirimetamina y primaquina para erradicar la malaria en Sambu, Panama [Mass treatment with pyrimethamine and primaquine for malaria eradication in Sambú, Panama]. *Boletín de la Oficina Sanitaria Panamericana*, 1971, 70:226–234.
17. Clyde DF, McCarthy VC. Radical cure of Chesson strain vivax malaria in man by 7, not 14, days of treatment with primaquine. *American Journal of Tropical Medicine and Hygiene*, 1977, 26:562–563.
18. Cedillos RA, Warren M, Jeffery GM. Field evaluation of primaquine in the control of *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene*, 1978, 27:466–472.
19. Appavoo NC, Roy RG, Kapali V. Results of 3-day radical treatment of *Plasmodium vivax* in North Arcot and South Arcot Districts of Tamil Nadu. *Indian Journal of Malariology*, 1984, 21:21–24.
20. Kaneko A et al. Gametocytocidal effect of primaquine in a chemotherapeutic malaria control trial in North Sumatra, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1989, 20:351–359.
21. Bunnag D et al. High dose of primaquine in primaquine resistant vivax malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1994, 88:218–219.
22. Baird JK et al. Primaquine for prophylaxis against malaria among nonimmune transmigrants in Irian Jaya, Indonesia. *American Journal of Tropical Medicine and Hygiene*, 1995, 52:479–484.
23. Fryauff DJ et al. Randomised placebo-controlled trial of primaquine for prophylaxis of falciparum and vivax malaria. *Lancet*, 1995, 346:1190–1193.
24. Weiss WR et al. Daily primaquine is effective for prophylaxis against falciparum malaria in Kenya: comparison with mefloquine, doxycycline, and chloroquine plus proguanil. *Journal of Infectious Diseases*, 1995, 171:1569–1575.
25. Soto J et al. Primaquine prophylaxis against malaria in nonimmune Colombian soldiers: efficacy and toxicity. A randomized, double-blind, placebo-controlled trial. *Annals of Internal Medicine*, 1998, 129:241–244.
26. Gogtay NJ et al. Efficacies of 5- and 14-day primaquine regimens in the prevention of relapses in *Plasmodium vivax* infections. *Annals of Tropical Medicine and Parasitology*, 1999, 93:809–812.
27. Rowland M, Durrani N. Randomized controlled trials of 5- and 14-days primaquine therapy against relapses of vivax malaria in an Afghan refugee settlement in Pakistan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1999, 93:641–643.

28. Soto J et al. Double-blind, randomized, placebo-controlled assessment of chloroquine/primaquine prophylaxis for malaria in nonimmune Colombian soldiers. *Clinical Infectious Diseases*, 1999, 29:199–201.
29. Kaneko A et al. Malaria eradication on islands. *Lancet*, 2000, 356:1560–1564.
30. Baird JK et al. Randomized, parallel placebo-controlled trial of primaquine for malaria prophylaxis in Papua, Indonesia. *Clinical Infectious Diseases*, 2001, 33:1990–1997.
31. Nasveld P et al. Comparison of tafenoquine (WR238605) and primaquine in the post-exposure (terminal) prophylaxis of vivax malaria in Australian Defence Force personnel. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2002, 96:683–684.
32. Solari-Soto L et al. Ensayo clinico del tratamiento de la malaria vivax con esquema acortado de primaquina comparado con el esquema tradicional [Clinical trial of treatment for vivax malaria with a short primaquine regimen compared with the usual regimen]. *Revista de la Sociedad Peruana de Medicina Interna*, 2002, 15:197–199.
33. Weerasinghe KL et al. A safety and efficacy trial of artesunate, sulphadoxine-pyrimethamine and primaquine in *P. falciparum* malaria. *Ceylon Medical Journal*, 2002, 47:83–85.
34. Rajgor DD et al. Efficacy of a 14-day primaquine regimen in preventing relapses in patients with *Plasmodium vivax* malaria in Mumbai, India. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2003, 97:438–440.
35. Giao PT et al. CV8, a new combination of dihydroartemisinin, piperaquine, trimethoprim and primaquine, compared with atovaquone-proguanil against falciparum malaria in Vietnam. *Tropical Medicine and International Health*, 2004, 9:209–216.
36. Youel DB et al. Low incidence of erythrocyte G-6-P D deficiency in Vietnamese and Montagnards of South Vietnam. *Vox Sanguinis*, 1971, 20:555–558.
37. Walsh DS et al. Randomized trial of 3-dose regimens of tafenoquine (WR238605) versus low-dose primaquine for preventing *Plasmodium vivax* malaria relapse. *Clinical Infectious Diseases*, 2004, 39:1095–1103.
38. Carmona-Fonseca J, Alvarez G, Blair S. *Plasmodium vivax* malaria: treatment of primary attacks with primaquine, in three different doses, and a fixed dose of chloroquine, Antioquia, Colombia, 2003–2004. *Biomedica*, 2006, 26:353–365.
39. Alvarez G et al. Efficacy of three chloroquine-primaquine regimens for treatment of *Plasmodium vivax* malaria in Colombia. *American Journal of Tropical Medicine and Hygiene*, 2006, 75:605–609.
40. Gogtay NJ et al. A randomized, parallel study of the safety and efficacy of 45 mg primaquine versus 75 mg bulaquine as gametocytocidal agents in adults with blood schizonticide-responsive uncomplicated falciparum malaria (ISCRTN50134587). *BMC Infectious Diseases*, 2006, 6:16.

41. Lederman ER et al. Combined chloroquine, sulfadoxine/pyrimethamine and primaquine against *Plasmodium falciparum* in Central Java, Indonesia. *Malaria Journal*, 2006, 5:108.
42. Dao NV et al. Vivax malaria: preliminary observations following a shorter course of treatment with artesunate plus primaquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2007, 101:534–539.
43. El-Sayed B et al. A randomized open-label trial of artesunate–sulfadoxine–pyrimethamine with or without primaquine for elimination of sub-microscopic *P. falciparum* parasitaemia and gametocyte carriage in eastern Sudan. *PLoS One*, 2007, 2:e1311.
44. Elmes NJ et al. The efficacy and tolerability of three different regimens of tafenoquine versus primaquine for post-exposure prophylaxis of *Plasmodium vivax* malaria in the Southwest Pacific. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2008, 102:1095–1101.
45. Krudsood S et al. High-dose primaquine regimens against relapse of *Plasmodium vivax* malaria. *American Journal of Tropical Medicine and Hygiene*, 2008, 78:736–740.
46. Tangpukdee N et al. Efficacy of Artequick versus artesunate-mefloquine in the treatment of acute uncomplicated falciparum malaria in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2008, 39:1–8.
47. Carmona-Fonseca J, Alvarez G, Maestre A. Methemoglobinemia and adverse events in *Plasmodium vivax* malaria patients associated with high doses of primaquine treatment. *American Journal of Tropical Medicine and Hygiene*, 2009, 80:188–193.
48. Carmona-Fonseca J, Maestre A. Prevention of *Plasmodium vivax* malaria recurrence: efficacy of the standard total dose of primaquine administered over 3 days. *Acta Tropica*, 2009, 112:188–192.
49. Vasquez AM et al. Estudio piloto de la eficacia y de los efectos sobre los gametocitos del esquema artesunato-mefloquina-primaquina para la malaria por *Plasmodium falciparum* [Therapeutic efficacy of a regimen of artesunate-mefloquine-primaquine treatment for *Plasmodium falciparum* malaria and treatment effects on gametocytic development]. *Biomedica*, 2009, 29:307–319.
50. Carmona-Fonseca J. Malaria vivax en niños: recurrencias con dosis estándar de primaquina administrada durante 3 frente a 7 días [Vivax malaria in children: recurrences with standard doses of primaquine administered for 3 versus 7 days]. *Iatreia*, 2010, 23:10–20.
51. Pukrittayakamee S et al. A comparison of two short-course primaquine regimens for the treatment and radical cure of *Plasmodium vivax* malaria in Thailand. *American Journal of Tropical Medicine and Hygiene*, 2010, 82:542–547.
52. Smithuis F et al. Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: an open-label randomised trial. *Lancet Infectious Diseases*, 2010, 10:673–681.

53. Ebringer A et al. Evaluation of the safety and tolerability of a short higher-dose primaquine regimen for presumptive anti-relapse therapy in healthy subjects. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2011, 105:568–573.
54. Maneeboonyang W et al. Directly observed therapy with primaquine to reduce the recurrence rate of *Plasmodium vivax* infection along the Thai–Myanmar border. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2011, 42:9–18.
55. Betuela I et al. Tolerability and safety of primaquine in Papua New Guinean children 1 to 10 years of age. *Antimicrobial Agents and Chemotherapy*, 2012.
56. Kolaczinski K et al. Defining *Plasmodium falciparum* treatment in South West Asia: a randomized trial comparing artesunate or primaquine combined with chloroquine or SP. *PLoS One*, 2012, 7:e28957.
57. Jones R Jr et al. Korean vivax malaria. III. Curative effect and toxicity of primaquine in doses from 10 to 30 mg. daily. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:977–982.
58. Arnold J et al. The effect of continuous and intermittent primaquine therapy on the relapse rate of Chesson strain vivax malaria. *Journal of Laboratory and Clinical Medicine*, 1954, 44:429–438.
59. Hodgkinson R, Courtney KO, Haggerty M. Effect of intermittent administration of a combination of amodiaquin and primaquine (Camoprim) on the hematocrit of primaquine-sensitive and non-sensitive children. *American Journal of Tropical Medicine and Hygiene*, 1961, 10:128–134.
60. Kellermeyer RW et al. The hemolytic effect of primaquine. XIII. Gradient susceptibility to hemolysis of primaquine-sensitive erythrocytes. *Journal of Laboratory and Clinical Medicine*, 1961, 58:225–233.
61. Vivona S et al. The concurrent weekly administration of chloroquine and primaquine for the prevention of Korean vivax malaria. *Bulletin of the World Health Organization*, 1961, 25:267–269.
62. Brewer GJ et al. The hemolytic effect of primaquine. XV. Role of methemoglobin. *Journal of Laboratory and Clinical Medicine*, 1962, 59:905–917.
63. Cahn MM, Levy EJ. The tolerance to large weekly doses of primaquine and amodiaquine in primaquine-sensitive and non-sensitive subjects. *American Journal of Tropical Medicine and Hygiene*, 1962, 11:605–606.
64. Brewer GJ, Zarafonetis CJ. The haemolytic effect of various regimens of primaquine with chloroquine in American Negroes with G6PD deficiency and the lack of an effect of various antimalarial suppressive agents on erythrocyte metabolism. *Bulletin of the World Health Organization*, 1967, 36:303–308.
65. George JN et al. Primaquine sensitivity in Caucasians: hemolytic reactions induced by primaquine in G-6-PD deficient subjects. *Journal of Laboratory and Clinical Medicine*, 1967, 70:80–93.

66. Ziai M et al. Malaria prophylaxis and treatment in G-6-PD deficiency. An observation on the toxicity of primaquine and chloroquine. *Clinical Pediatrics (Philadelphia)*, 1967, 6:242–243.
67. Pannacciulli I et al. Hemolytic effects of standard single dosages of primaquine and chloroquine on G-6-PD-deficient Caucasians. *Journal of Laboratory and Clinical Medicine*, 1969, 74:653–661.
68. Fisher GU et al. Malaria in soldiers returning from Vietnam. Epidemiologic, therapeutic, and clinical studies. *American Journal of Tropical Medicine and Hygiene*, 1970, 19:27–39.
69. Everett WD, Yoshida A, Pearlman E. Hemoglobin E and glucose-6-phosphate deficiency in the Khmer Air Force (Cambodia). *American Journal of Tropical Medicine and Hygiene*, 1977; 26:597–601.
70. Khoo K-K. The treatment of malaria in glucose-6-phosphate dehydrogenase patients in Sabah. *Annals of Tropical Medicine and Parasitology*, 1981, 75:591–195.
71. Bangchang KN et al. Pharmacokinetics of primaquine in G6PD deficient and G6PD normal patients with vivax malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1994, 88:220–222.
72. Myat Phone K et al. The use of primaquine in malaria infected patients with red cell glucose-6-phosphate dehydrogenase (G6PD) deficiency in Myanmar. *Southeast Asian Journal of Tropical Medicine and Public Health*, 1994, 25:710–713.
73. Buchachart K et al. Effect of primaquine standard dose (15 mg/day for 14 days) in the treatment of vivax malaria patients in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, 2001, 32:720–726.
74. Silachamroon U et al. Clinical trial of oral artesunate with or without high-dose primaquine for the treatment of vivax malaria in Thailand. *American Journal of Tropical Medicine and Hygiene*, 2003, 69:14–18.
75. Krudsood S et al. Safety and tolerability of elubaquine (bulaquine, CDRI 80/53) for treatment of *Plasmodium vivax* malaria in Thailand. *Korean Journal of Parasitology*, 2006, 44:221–228.
76. Shekalaghe S et al. Primaquine clears submicroscopic *Plasmodium falciparum* gametocytes that persist after treatment with sulphadoxine–pyrimethamine and artesunate. *PLoS One*, 2007, 2:e1023.
77. Leslie T et al. A randomised trial of an eight-week, once weekly primaquine regimen to prevent relapse of *Plasmodium vivax* in Northwest Frontier Province, Pakistan. *PLoS One*, 2008, 3:e2861.
78. Shekalaghe SA et al. In Tanzania, hemolysis after a single dose of primaquine coadministered with an artemisinin is not restricted to glucose-6-phosphate dehydrogenase-deficient (G6PD A-) individuals. *Antimicrobial Agents and Chemotherapy*, 2010, 54:1762–1768.
79. Song J et al. Rapid and effective malaria control in Cambodia through mass administration of artemisinin-piperaquine. *Malaria Journal*, 2010, 9:57.

80. Takeuchi R et al. Directly-observed therapy (DOT) for the radical 14-day primaquine treatment of *Plasmodium vivax* malaria on the Thai–Myanmar border. *Malaria Journal*, 2010, 9:308.

81. Kondrashin AV, Baranova AM, Sergiev VM. *Large scale use of primaquine in population with the G6PD deficiency (review of experiences)*. Moscow, Martzinovski Institute of Medical Parasitology and Tropical Medicine, Moscow Medical Academy. 2010.

82. Kondrachine AV. *Malaria control in the Democratic People's Republic of Korea*. New Delhi, World Health Organization Regional Office for South-East Asia, 2001 (SEA-MAL-224).

83. Kondrachine AV. *Outcome and impact of mass chemoprophylaxis with primaquine on vivax malaria in the Democratic People's Republic of Korea*. New Delhi, World Health Organization Regional Office for South-East Asia, 2008 (SEA-MAL-251).

84. Phu NH. *Malaria mass chemoprophylaxis with primaquine in the Democratic People's Republic of Korea*. New Delhi, World Health Organization Regional Office for South-East Asia, 2008 (SEA-MAL-232).

Annex 3. Studies on the safety of pamaquine

Published studies of pamaquine, including safety evaluations

Reference and location: Vad and Mohile, 1927 (*1*), India

Study question: Pamaquine for treatment of *P. falciparum, P. vivax, P. ovale* or *P. malariae* infections given to patients with parasites in blood (five patients had mixed infections)

Pamaquine dose or regimen: 60 mg daily for vivax, ovale or malariae malaria with no quinine, and 30 mg with quinine (combined in one tablet as 10 mg pamaquine–125 mg quinine) daily for falciparum

Other antimalarial agents given: Quinine at 375 mg daily with pamaquine (one tablet: 10 mg pamaquine + 125 mg quinine) for falciparum infections

Adverse events surveillance: Daily counts of parasites in blood, clinical notes and urine analyses

Severe adverse events reported: None among 15 people who received pamaquine. The authors reported "no untoward effects of pamaquine on any system or organs"; no paleness or cyanosis observed. The first patients complained of epigastric pain; those who received pamaquine after meals did not complain. One patient with a mixed infection developed pneumonia and died on day 5; no autopsy was performed.

Reference and location: Green, 1929 (*2*), Federation of Malay States (now part of Malaysia)

Study question: Pamaquine as gametocytocidal in patients with crescents in blood (56 men: 35 Tamils, 15 Chinese, 6 Sikhs)

Primaquine dose or regimen: Eight tablets of pamaquine compound given as directly observed therapy for a total daily dose of 40 mg pamaquine (and 500 mg quinine) for 10–14 days

Other antimalarial agents given: 64.3% individuals received an average of 2.4 days of quinine before pamaquine; the rest had no quinine before receiving combined pamaquine–quinine

Adverse events surveillance: Parasitaemia assessed daily from thin and thick films; percentage Hb estimated every second day; a control group of 10 people (six Tamils, one Chinese, three Sikhs) received only quinine.

Severe adverse events reported: None among 56 people who received pamaquine. Three people (5.4%) had abdominal pain, but not when large amounts of liquid were given with the tablet; no cyanosis; Hb changes were no different in the group receiving pamaquine than that receiving only quinine.

Reference and location: Green, 1929 (*3*), Federation of Malay States (now part of Malaysia)

Study question: Activity of pamaquine against trophozoites and gametocytes in quartan malaria (40 men: 20 Tamils, 16 Chinese, 4 Sikhs)

Pamaquine dose or regimen: 60 mg daily

Other antimalarial agents given: A control group of 10 men (seven Tamils, three Chinese) received only quinine (amount not specified).

Adverse events surveillance: None reported

Severe adverse events reported: None among 40 men who received pamaquine. No cyanosis or haemolytic anaemia; 10% experienced abdominal pain, which was prevented by taking pamaquine with fluids; 5% presented some cardiac irregularities

Reference and location: Biggam and Arafa, 1930 (*4*), Egypt

Study question: Efficacy of pamaquine in 50 intravenous heroin addicts presenting to hospital with subtertian malaria

Pamaquine dose or regimen: Group 1 (n = 33: 60 mg pamaquine daily for 7 days in three doses, then 5 weeks of 4 days' rest, 3 days' same treatment. Group 2 (n = 9): as group 1, but intravenous pamaquine. Group 3 (n = 6): 40 mg pamaquine daily (in three doses) for 7 days, then as group 1

Other antimalarial agents given: Group 1: 0.75 g quinine daily (in three doses) with pamaquine. Group 2: 0.75 g quinine with pamaquine in the 5 weeks after intravenous administration. Group 3: 1.2 g quinine daily (in three doses) with pamaquine

Adverse events surveillance: Daily thick and thin blood smears from all patients, and presence of rings and crescents recorded

Severe adverse events reported: None among 50 patients who received pamaquine. The authors observed no cyanosis, anaemia or cardiac irregularities. Occasional, slight epigastric and splenic discomfort towards the end of the first week did not recur when treatment resumed after 4 days' rest.

Reference and location: Kligler and Mer, 1930 (*5*), British Mandate Palestine (now in Israel)

Study question: Effect of low-dose pamaquine–quinine in patients with tertian (benign or malignant) or quartan malaria

Pamaquine dose or regimen: Three tablets daily of 10 mg pamaquine and quinine given for 5 days or for 5 days with 3 days' rest, then 5 days' treatment again. Daily adult dose, 30 mg pamaquine given to all patients >10 years; daily dose was 2 mg for children <2 years, 10 mg for children aged 2–5 years and 20 mg for children aged 6–10 years.

Other antimalarial agents given: 900 mg quinine daily for adults; 250 mg daily for children <2 years, 300 mg for children aged 2–5 and 600 mg for children aged 6–10 years

Adverse events surveillance: Treatment in the field, not hospital. Pilot treatment of 27 children 1–10 years old showed no side-effects, and parasites cleared from peripheral blood within 5 days; then, mass treatment in two districts, with blood and spleen examinations

Severe adverse events reported: None in >1000 people who received pamaquine. One case of serious gastric disturbance without jaundice or cyanosis was seen in a patient who had received one course of treatment with no effects; the second treatment showed effects on day 4; pain disappeared after treatment was suspended.

Reference and location: Kingsbury and Amies, 1931 (*6*), Federation of Malay States (now part of Malaysia)

Study question: Value of pamaquine as a malaria prophylactic on rubber estates, including children and infants (no numbers provided, total $N = 330$)

Pamaquine dose or regimen: Adult dose, 40 mg given twice weekly for 12 months 30 min before the afternoon meal; children received doses proportional to body weight and infants an emulsion. Two estates ($n = 405$ and 362) contiguous to the experimental estate were used as controls, receiving no pamaquine.

Other antimalarial agents given: None

Adverse events surveillance: Before pamaquine, parasitaemia, splenic enlargement and Hb measured; after pamaquine, surveys carried out every 3 months

Severe adverse events reported: None in 330 individuals who received pamaquine. Some toxic reactions seen during the first 2 weeks, all in individuals with low Hb and a chronically enlarged spleen; all recovered once pamaquine was suspended (no details given)

Reference and location: Kliger and Mer, 1931 (*7*), British Mandate Palestine (now in Israel)

Study question: Relapse rates in heavily infected children aged 6 months–6 years in two locations (n = 18 and 21)

Pamaquine dose or regimen: 2 mg for infants aged 0–2 years and 7.5 mg for children aged 2–6 years daily for 5 days; daily dose given in two parts, in the morning and the afternoon

Other antimalarial agents given: Quinine at 250 mg to infants aged 0–2 years, 450 mg to children aged 2–6 years, daily for 5 days

Adverse events surveillance: Children followed up weekly for parasitaemia

Severe adverse events reported: None in 49 children who received pamaquine–quinine. A group that had received previous treatment showed higher relapse rates, mostly among children <4 years of age

Reference and location: Sinton, 1931 (*8*), India

Study question: Determination of safe doses of pamaquine and quinine in the treatment of benign tertian malaria

Pamaquine dose or regimen: Either 20 mg in the morning and 10 mg at night (total daily dose, 30 mg) (n = 75) or 30 mg daily in three doses of 10 mg each (n = 4), both for 21 days

Other antimalarial agents given: 1.3 g quinine in two doses (0.65 g in the morning and evening) or 1.22 g in three doses (620 mg in the morning, 300 mg at noon and in the evening), both treatments daily with pamaquine for 21 days

Adverse events surveillance: Some patients appear to have been hospital inpatients.

Severe adverse events reported: None among 79 patients who received pamaquine. A few instances of epigastric pain

Reference and location: Jerace and Giovanolla, 1933 (*9*), Italy

Study question: Gametocytocidal and prophylactic action of pamaquine against falciparum malaria in mosquitoes feeding on falciparum malaria patients (n = 31) before and after administration of pamaquine

Pamaquine dose or regimen: 20 mg single dose

Other antimalarial agents given: 20 patients received quinine only, nine received mepacrine and two both quinine and mepacrine (treatments were given before the study and not always at the same dose or same number of days)

Adverse events surveillance: Gametocytes in peripheral blood, flagellation and infectivity to mosquitoes all assessed before exposure to mosquitoes, and then each day after administration of pamaquine for up to 6 days in some cases

Severe adverse events reported: None among 31 patients who received pamaquine

Reference and location: Carman, 1935 (*10*), Kenya

Study question: Mepacrine, pamaquine and quinine in the treatment of tertian malaria assessed in four groups: mepacrine (n = 35), mepacrine–pamaquine–quinine (n = 31), mepacrine–pamaquine (n = 22) or quinine (n = 25)

Pamaquine dose or regimen: 10 mg alone or 10 mg + 125 mg quinine three times a day

Other antimalarial agents given: 300 mg quinine daily; controls received 600 mg daily

Adverse events surveillance: Patients with clinical symptoms of malaria admitted to hospital after confirmed parasitaemia on slides; treatment was commenced and patients were kept in hospital until their health improved

Severe adverse events reported: None among 53 patients who received pamaquine. Patients given mepacrine reverted more quickly to normal health (average time in hospital, 7.2 days) than those on quinine (8–9 days); addition of pamaquine did not give rise to toxic symptoms but retarded the return to normal health.

Reference and location: Dimson and McMartin, 1946 (*11*), Burma (now Myanmar)

Study question: Haemoglobinuria in Indian and British troops receiving standard Army treatment or "blanket treatment" (about 10 000 men in each group, mostly Indian)

Pamaquine dose or regimen: 10 mg for 5 days (thrice daily for British and twice daily for Indian troops). Blanket treatment: 10 mg for 3 days; most Indians received 5 days' treatment

Other antimalarial agents given: Quinine at 10 grains (0.65 g) thrice daily for 2 days, mepacrine at 100 mg thrice daily for 5 days, rest for 2 days, then pamaquine. Blanket treatment: mepacrine at 100 mg thrice daily for 5 days, rest for 2 days, then pamaquine

Adverse events surveillance: Urine, blood, spleen and liver of all patients admitted to hospital with haemoglobinuria were examined.

Severe adverse events reported: 13 Indian (mostly Punjabi) troops given blanket treatment developed haemoglobinuria while taking pamaquine and were admitted to hospital; three died. Another five cases of haemoglobinuria occurred among Indian troops given standard treatment. The urine of haemoglobinuria patients was red–black. The authors noted that, as most of the patients with haemoglobinuria were not having an attack of malaria and had not had chronic malaria previously, the haemoglobinuria was due to pamaquine and not blackwater fever, although the clinical signs and post-mortem findings are identical for the two conditions. Mepacrine might enhance the toxic effects of pamaquine, as when blanket treatment and pamaquine alone were repeated in 12 haemoglobinuric patients in hospital, intravascular haemolysis occurred in nine patients.

Reference and location: Hardgrove and Applebaum, 1946 (*12*), Panama

Study question: Symptoms of pamaquine toxicity in adult male patients admitted to hospital with suspected pamaquine poisoning (n = 258)

Pamaquine dose or regimen: 10 mg thrice daily for 5 days after mepacrine; 61 of the 258 patients did not finish the 5 days of pamaquine.

Other antimalarial agents given: 100 mg mepacrine thrice daily for 5 days, then 2 days' rest, followed by pamaquine

Adverse events surveillance: Patients were asked about any symptoms and were examined physically, and blood was examined in the laboratory.

Severe adverse events reported: All 258 patients showed some degree of toxicity: 136 mild, 63 moderate and 59 severe; all recovered with no complications after treatment. The symptoms found were: abdominal pain (69%), dark urine (58%), anorexia (45%), jaundice (45%), headache (39%), nausea and vomiting (34%), fever (25%), weakness and malaise (23%), backache (22%) and less frequently vertigo, chest pain, diarrhoea, chills, nasal congestion and cyanosis. Symptoms began after 4 days of pamaquine and lasted 3–4 days; 75% patients had an RBC count of <4 million/µL, and 50% had Hb <70% of baseline levels; 22% had an RBC count <2 million/µL, and 16% patients had Hb as low as 40% of initial values. Mild anaemia improved within 3–4 days, while severe cases lasted 10 days to 2 weeks; 60 patients required blood transfusions.

Reference and location: Most et al., 1946 (*13*), USA

Study question: Effect on subsequent relapse of vivax malaria of Pacific origin of combined quinine–pamaquine treatment for 14 days

Pamaquine dose or regimen: 10 mg with quinine every 8 h for 14 days (N = 72)

Other antimalarial agents given: 1 g quinine given with pamaquine at 8-h intervals on day 1; on days 2–14, 650 mg with pamaquine every 8 h; control group received only quinine

Adverse events surveillance: Parasite counts twice daily until negative for 3 days; Hb and methaemoglobin daily. Patients examined daily for toxic effects; relapses followed up for a minimum of 120 days, with smears twice weekly (if parasitaemia was observed, then daily). An oral temperature ≥ 100 °F (37.8 °C) with a positive smear was considered to represent a clinical relapse.

Severe adverse events reported: None among 72 patients who received pamaquine. No major toxic manifestations observed, and all patients were able to complete therapy. 40% patients had some complaint of the gastrointestinal tract, probably related to pamaquine; these were mostly severe abdominal cramps or abdominal soreness, which usually began on day 3–6 and lasted 1–7 days. Cyanosis was observed in one patient with methaemoglobin of 12% on day 11 of treatment; 90% of patients had methaemoglobinaemia above normal at some time during treatment, varying from 1% to 12% (average, 2.3%) of total Hb; 16% patients had a fall in Hb of 11–20% in the second week of treatment, and 16% had a 15.3% fall during the first 5 days, which was apparently due to active malaria rather than pamaquine, as it occurred during the first few days of the acute attack and reversed with continued treatment. No severe anaemia or haemolytic crisis was observed.

Reference and location: Craige et al., 1947 (*14*), USA

Study question: Clinical standardization of pamaquine in mosquito-induced (Chesson strain) vivax malaria in white male prison inmate volunteers

Pamaquine dose or regimen: 15 mg for 14 days, 15 mg for 28 days, 31 mg for 14 days, 45 mg for 14 days or 63 mg for 14 days, either with or followed by 8 days of quinine

Other antimalarial agents given: Quinine at 2 g daily in six equally divided doses at 4-h intervals with pamaquine or for 8 days after pamaquine

Adverse events surveillance: Plasma drug concentrations and evidence of toxicity were determined. Relapse rate and latent period duration were determined by examining thick blood smears made regularly until relapse occurred.

Severe adverse events reported: Some patients given 63 mg pamaquine had methaemoglobinaemia. Adverse events observed at this dose were: anorexia, nausea, vomiting, epigastric distress or pain; some patients had to discontinue treatment before 14 days. Methaemoglobinaemia was common, and, when it exceeded 6–7%, cyanosis was clinically evident. Granulocytopenia was less common, and no haemolytic anaemia occurred. At 15 mg, pamaquine caused only minimal methaemoglobinaemia (3.0% of total Hb); 30 mg caused mild toxicity (4.9%), 45 mg moderate toxicity (5.6%) and 63 mg severe toxicity (12%).

Reference and location: Dick and Bowles, 1947 (*15*), British Somaliland (now Somalia)

Study question: Gametocyte survival in two groups of unselected Somalis with malaria (enlisted soldiers and people of all ages living in an isolated village) treated with quinine, mepacrine and pamaquine

Pamaquine dose or regimen: 10 mg three times a day for 3 days after quinine and mepacrine; children aged 6–12 years received 5 mg three times a day, 2–6-year-olds received 5 mg twice daily, and infants aged 6 months to 2 years received 5 mg daily.

Other antimalarial agents given: Quinine at 10 grains (0.14 g) three times a day, followed by mepacrine at 100 mg three times a day for 5 days, followed by pamaquine. Quinine was given to children at a dose of 5 grains (0.35 g), three times a day to those aged 6–12 years, twice daily to those aged 2–6 years and once daily for infants aged 6 months to 2 years. Mepacrine was given to children at a dose of 50 mg, three times a day to those aged 6–12 years, twice daily to those aged 2–6 years and once a day to infants aged 6 months to 2 years.

Adverse events surveillance: Thick blood films and microscopy for evaluation of crescents

Severe adverse events reported: None in 86 people who received pamaquine. No toxic symptoms observed and no complaints by patients. These results are not surprising, as the 10-mg dose is low. The gametocyte incidence in people with sub-tertian malaria was relatively high, and crescents were found in 49% of 215 consecutive adult hospital admissions; 59 (54%) showed crescents in peripheral blood at some time.

Reference and location: Jones et al., 1948 (*16*), USA

Study question: Prophylactic effectiveness of several 8-aminoquinoline against mosquito-transmitted *P. vivax* (Chesson strain) in prison inmate volunteers

Pamaquine dose or regimen: 90 mg daily in divided doses at 4-h intervals for 7 days, starting the day before exposure to sporozoite infection by mosquito bites ($N = 5$)

Other antimalarial agents given: Three other 6-methoxy 8-aminoquinolines tested in parallel: SN-l,452, SN-ll,191 and SN-13,276. All four drugs were given at high doses (close to the maximum tolerated) in divided doses at 4-h intervals.

Adverse events surveillance: Thick films examined for parasites at frequent intervals; Hb and methaemoglobin measured daily in blood samples

Severe adverse events reported: All the drugs were highly toxic: common abdominal epigastric discomfort or pain, anorexia, nausea and vomiting; cyanosis when methaemoglobin >6–7% of total Hb (for pamaquine, the highest level was 11.7%); total Hb fell slowly, on average to 1.75 g/dL blood, the greatest loss occurring on days 12–14 after the start of medication

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Reference and location: Monk, 1948 (*17*), England

Study question: Therapeutic effect of concurrent paludrine and pamaquine on acute attacks of benign tertian malaria in soldiers returning from India, Burma and the Malay Peninsula

Pamaquine dose or regimen: 10 mg primaquine (every 8 h for 10 days) for cases of relapsing malaria and primary attacks; all had received mepacrine, which was discontinued 4–5 weeks before admission to hospital

Other antimalarial agents given: Paludrine at 250 mg or quinine at 10 grains (0.7 g) every 8 h for 10 days with pamaquine

Adverse events surveillance: Parasitaemia was measured from thick slides; all patients were hospitalized, and urinary examinations were conducted for all patients with toxic reactions.

Severe adverse events reported: None in 179 men given paludrine–pamaquine or 168 given quinine–pamaquine. Minimal toxicity was observed in 7% of patients given quinine–pamaquine and in 37% given paludrine–pamaquine, including cyanosis, anorexia and gastric discomfort; no haemoglobinuria or methaemoglobinuria was detected. Therapy was discontinued for one patient.

Reference and location: Ruhe et al., 1949 (*18*), USA

Study question: Therapeutic action of pamaquine against Saint Elizabeth strain of vivax malaria in white prison inmates infected by mosquito bites and presenting with initial late attacks

Pamaquine dose or regimen: (1) pamaquine at 60 mg daily (10 mg every 4 h) for 6 days after 6 days of quinine (n = 4), (2) 30 mg daily (5 mg every 4 h) for 12 days (n = 6), (3) 60 mg daily (10 mg every 4 h) for 12 days (n = 7) or (4) 90 mg daily (10 and 20 mg alternately every 4 h) for 12 days (n = 6)

Other antimalarial agents given: Quinine at 2 g daily (0.5 g every 6 h) for 12 days with pamaquine

Adverse events surveillance: Blood smears for parasitaemia daily during and immediately after therapy and at least once weekly until 18 months after exposure; methaemoglobin measured every other day and Hb daily; white and RBC counts and urinalyses carried out frequently

Severe adverse events reported: None in 23 volunteers who received various regimens of quinine–pamaquine. The adverse events observed were slight abdominal cramps and cyanosis. One volunteer who received 30 mg pamaquine had marked cyanosis associated with a methaemoglobin value of 12.1% total Hb; with 60 mg, methaemoglobin was 5–14.3% of total Hb, and the dose had to be reduced for one volunteer with abdominal cramps; with 90 mg, all but one patient developed marked cyanosis, methaemoglobin during the last 5 days of treatment was 5.2–19.5%, and one patient experienced such severe cramps and a reduction in total white blood cell count to 3000/μL that treatment was discontinued on day 9.

Reference and location: Clayman et al., 1952 (*19*), USA

Study question: Comparison of toxicity of pamaquine and primaquine in white adult male volunteer prison inmates

Pamaquine dose or regimen: 31.5 or 63 mg daily or 15, 31.5 or 63 mg with quinine for 14 days

Other antimalarial agents given: Quinine at 2 g daily with pamaquine

Adverse events surveillance: Hb and methaemoglobin measured before drug administration, every other day during clinical malaria, then daily; Hb, methaemoglobin and white blood cell counts usually measured on days 1, 7, 10 and 14 of drug administration; urinalyses when toxicity was anticipated

Severe adverse events reported: None among 131 volunteers who received pamaquine. Three men receiving 63 mg pamaquine daily could not complete the course because of severe abdominal adverse effects. More methaemoglobinaemia was seen with primaquine than with pamaquine, and quinine did not diminish the methaemoglobinaemia seen with pamaquine. Pamaquine was slightly more haemolytic than primaquine: two men taking 15 mg and four taking 63 mg (pamaquine with quinine) lost 2–3 g/dL Hb. No similar loss was observed with primaquine alone or in combination. White blood cell abnormalities occurred less frequently with pamaquine than with primaquine.

Reference and location: Garrison et al., 1952 (*20*) (preliminary results at 11 months); Alving et al., 1953 (*21*) (results at 18 months); USA

Study question: Comparison of curative effectiveness of primaquine and pamaquine given with chloroquine to US Army veterans with vivax malaria who had served in Korea

Pamaquine dose or regimen: Single dose of 27 mg daily for 14 consecutive days with chloroquine

Other antimalarial agents given: Chloroquine at a total dose of 1.5 g, in three doses of 300 mg each during the first 24 h, followed by single doses of 300 mg daily for 2 days

Adverse events surveillance: All patients hospitalized during treatment and observed daily for toxicity. White blood count, Hb and routine urinalysis conducted on admission, after 1 week and at the end of therapy; follow-up every 6 weeks with interviews and a thick film for parasites. Patients with relapse were treated as originally.

Severe adverse events reported: None among 272 patients who received pamaquine + chloroquine. No significant toxicity was observed: no evidence of haemolytic anaemia or cyanosis; 15% patients had mild-to-moderate anaemia (Hb, 6.4–12 g/dL) at baseline, and their Hb increased during therapy as the acute attack was controlled. A few patients who received chloroquine + pamaquine complained of mild crampy abdominal pain at some time during therapy, but this was not of sufficient severity to warrant withdrawal of the drug.

Reference and location: Cooper et al., 1953 (*22*), USA

Study question: Comparison of efficacy of 8-aminoquinolines (primaquine, isopentaquine, SN-3883 and pamaquine [pamaquine]) in inmate volunteers infected with *P. vivax* Chesson strain by mosquito bites

Pamaquine dose or regimen: 60 mg daily with quinine given in four equal doses at 06:00, 12:00, 18:00 and 24:00 for 14 days

Other antimalarial agents given: Quinine at 1 g daily starting on day 3–5 of patent parasitaemia

Adverse events surveillance: Blood smears every other day until 72 days after bites, twice weekly until day 180, once weekly until day 270. 102 volunteers (half of each group) were observed clinically for 505 days and the remaining 102 for 350 days. Hb, methaemoglobin and haematocrit were estimated immediately before treatment and on days 7 and 14.

Severe adverse events reported: None among 34 volunteers who received pamaquine. No haemolysis was observed; methaemoglobin was highest with pamaquine and lowest with primaquine; cyanosis occurred in 9/34 volunteers given pamaquine. One man who received pamaquine had such severe nausea and vomiting that treatment was stopped on day 9.

References

1. Vad BG, Mohile GB. The place of plasmochin on the treatment of malaria. *Indian Medical Gazette*, 1927, 62:430–434.
2. Green R. *The treatment of "crescent carriers" with plasmoquine compound*. Kuala Lumpur, Government Printing Office, 1929:1–20 (Bulletins Institute for Medical Research (Malaysia), Issue 3).
3. Green R. *The treatment of quartan malaria with plasmoquine*. Kuala Lumpur, Government Printing Office, 1929 (Bulletins Institute for Medical Research (Malaysia), Issue 3).
4. Biggam AG, Arafa MA. Observations on a series of cases of atificially induced subtertian malaria with special reference to the effect of treatment by plasmoquine compound. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1930, 23:591–607.
5. Kligler IJ, Mer G. Studies on malaria: V. Therapeutic value of mixtures of plasmochin and quinine. *Rivista di Malariologia*, 1930, 9:272–283.
6. Kingsbury AN, Amies CR. A field experiment on the value of plasmoquine in the prophylaxis of malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1931, 25:159–172.
7. Kliger IJ, Mer G. Studies on malaria. VII. Relapse rate after quinine–plasmoquine treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1931, 25:121–127.
8. Sinton JA. *Note on the treatment of chronic benign tertian malaria with plasmoquine and quinine*. Geneva: World Health Organization, 1931.
9. Jerace F, Giovannola A. L'azione sterilizzante della plasmochina sui gameti dei parassiti malarigeni a sua importanza profilattica [The sterilizing action of plasmoquine on gametocytes of malaria parasites and its prophylactic importance]. *Rivista di Malariologia*, 1933, 12:457.
10. Carman JA. Atebrin, plasmoquine and quinine in the treatment of malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1935, 29:191–202.
11. Dimson SB, McMartin RB. Pamaquin haemoglobinuria. *QJM*, 1946, 15:25–46.
12. Hardgrove M, Applebaum IL. Plasmochin toxicity; analysis of 258 cases. *Annals of Internal Medicine*, 1946, 25:103–112.
13. Most H et al. Combined quinine–plasmochin treatment of vivax malaria; effect of relapse rate. *American Journal of the Medical Sciences*, 1946, 212:550–560.
14. Craige B et al. Clinical standardization of Pamaquin (plasmochin) in mosquito-induced vivax malaria, Chesson strain: a preliminary report. *American Journal of Tropical Medicine and Hygiene*, 1947, s1-27:309–315.
15. Dick GW, Bowles RV. The value of plasmoquine as a gametocide in sub-tertian malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1947, 40:447–450.

16. Jones R et al. A study of the prophylactic effectiveness of several 8-aminoquinolines in sporozoite-induced vivax malaria (Chesson strain). *Journal of Clinical Investigation*, 1948, 27:6–11.
17. Monk JF. Results of an investigation of the therapeutic action of paludrine and pamaquin on acute attacks of benign tertian malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1948, 41:657–662.
18. Ruhe DS et al. Studies in human malaria; the therapeutic action of pamaquine against St Elizabeth strain vivax malaria. *American Journal of Hygiene*, 1949, 49:367–373.
19. Clayman CB et al. Toxicity of primaquine in Caucasians. *Journal of the American Medical Association*, 1952, 149:1563–1568.
20. Garrison PL et al. Cure of Korean vivax malaria with pamaquine and primaquine. *Journal of the American Medical Association*, 1952, 149:1562–1563.
21. Alving AS et al. Korean vivax malaria. II. Curative treatment with pamaquine and primaquine. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:970–976.
22. Cooper WC et al. Studies in human malaria. XXXI. Comparison of primaquine, isopentaquine, SN-3883, and pamaquine as curative agents against Chesson strain vivax malaria. *American Journal of Tropical Medicine and Hygiene*, 1953, 2:949–957.

Annex 4. Reports to the Uppsala Monitoring Centre on adverse reactions to primaquine

On 10 August 2012, we extracted all case reports submitted to the Uppsala Monitoring Centre, the WHO collaborating centre for international drug monitoring (http://www.who-umc.org), between 1969 and 30 July 2012 in which primaquine was suspected to be a causative or interacting factor for the reaction. A total of 1429 reports on 4560 reactions or events were submitted to the Centre from all WHO regions.

Some reports include multiple reactions or events. Diseases present at the time of a reaction were reported as events if there was uncertainty and if they were suspected as possible contributors to the reaction. The Monitoring Centre enters all reactions and events in each report into the database, for analyses aggregated by country, year and reaction classification. Reactions are considered individually and not grouped by report type. Demographic data on patients, such as age and gender, were available in the case reports but not the database.

The *Medical Dictionary for Regulatory Activities* (*1*) was used to classify the adverse reactions and events associated with primaquine into "system–organ class", resulting in 25 categories (Table A4.1). The cases included two deaths.

As pharmacovigilance relies on passive reporting of events, the pattern in different countries is inconsistent, as many—particularly in tropical areas—do not have a pharmacovigilance system. There is therefore reporting bias by year, country and individual reporter, all of which are evident from this dataset.

Of the 4560 reactions, 4062 (89%) were reported from Thailand, and of these 3925 were reported in 1997–1998. There was only one report (three reactions) from Africa, which was from South Africa. The other countries that reported reactions were Argentina (1), Australia (102), Canada (25), Colombia (7), Cuba (3), Denmark (1), Germany (3), India (15), Indonesia (9), Ireland (3), Italy (1), Malaysia (16), New Zealand (3), Peru (46), Republic of Korea (4), Romania (3), Singapore (6), Spain (4), Sweden (5), Switzerland (9), the United Kingdom (13) and the USA (216).

Table A4.1. *Medical Dictionary for Regulatory Activities* classification of reactions or events possibly related to primaquine administration reported to the Uppsala Monitoring Centre

Category of reaction or event	N	Details (no. of cases)
Blood and lymphatic system disorders	107	Haemolytic anaemia or haemolysis (25)
Cardiac disorders	184	Cyanosis (13) Arrhythmia (2) Palpitations (166)
Congenital, familial and genetic disorders	3	G6PD deficiency (3)
Ear and labyrinth disorders	13	
Eye disorders	99	Visual impairment (88)
Gastrointestinal disorders	1372	Nausea and/or vomiting (1086)
General disorders and administration site conditions	52	Death (2)
Hepatobiliary disorders	42	Hepatic failure (2) Jaundice (36)
Immune system disorders	2	Anaphylactoid reaction (1) Hypersensitivity reaction (1)
Infections and infestations	7	Malaria (1) *Pneumocystis jiroveci* pneumonia
Injury, poisoning and procedural complications	7	Accidental overdose or drug administration error (6)
Investigations	21	Changes in standard laboratory parameters, e.g. blood methaemoglobin (3)
Metabolism and nutrition disorders	328	Decreased appetite (327)
Musculoskeletal and connective tissue disorders	8	
Neoplasms benign, malignant and unspecified	4	
Nervous system disorders	1518	Dizziness (838) Headache (657) Anoxic seizure (1)
Not classifiable	4	
Pregnancy, puerperium and perinatal conditions	1	Missed abortion (intrauterine pregnancy present but no longer developing normally)
Psychiatric disorders	510	Agitation (260) Confusional state (125)
Renal and urinary disorders	21	Haematuria (10)
Reproductive system and breast disorders	2	
Respiratory, thoracic and mediastinal disorders	25	Dyspnoea (11)
Skin and subcutaneous tissue disorders	178	Stevens–Johnson syndrome (2) Toxic epidermal necrolysis (1) Erythema multiforme (3) Urticaria (12)
Surgical and medical procedures	1	Malaria prophylaxis
Vascular disorders	53	Pallor (49)

The remarks refer to reactions that are usually serious, accounted for most reactions in that category or are particularly associated with primaquine administration, e.g. haemolytic anaemia, methaemoglobinaemia

In several reports, other drugs were implicated as possible contributors to the reaction. For example, many of the reports from Thailand described dizziness in patients who also received artesunate and mefloquine, which were listed as possible causes. Mefloquine is well known as a cause of dizziness, sleep disturbance and other neuropsychiatric reactions, and it is therefore likely that primaquine did not contribute to the observed reaction.

Deaths

We reviewed in more detail events that resulted in death or are considered to be life threatening. Two deaths were reported, one in the United Kingdom and one in the USA. The death in the United Kingdom was notified through the Medicines and Healthcare Products Regulatory Agency and is described in section 3.3.2. The second death was reported in the USA in 1997 in a male patient (no age given). Three reactions were reported: death, thrombocytopenia and diarrhoea; cefipime (2 g daily dose) and primaquine were notified as possible causes; drugs given concomitantly, but considered not to be implicated, were zidovudine and clindamycin. No doses and no other information about the events leading to death were given. It may be inferred from the concomitant medications that the patient had HIV infection and possibly *P. jiroveci* pneumonia, which is sometimes treated with primaquine–clindamycin. The G6PD status of the patient was not stated.

Reproductive toxicity

A missed abortion (intrauterine pregnancy present but no longer developing normally) was reported in Switzerland in 2002. The mother was also being treated with ciprofloxacin for a bacterial infection and artemether–lumefantrine for malaria. These drugs were reported as potential causative agents. Malaria is known to be an independent risk factor for fetal loss (*2*).

Hypersensitivity and serious cutaneous reactions

Anaphylaxis

An anaphylactoid reaction was reported by a general practitioner in Indonesia in 1996. The patient was a 35-year-old man who also received SP, terbutaline and acetylsalicylic acid, caffeine and phenacetin. All the drugs were reported as possible contributors.

Stevens–Johnson syndrome

A case was reported in Thailand in 2002 in a 34-year-old woman who also received mefloquine. Another case, notified from India in 2011, was in a 12-year-old girl who was also given sulfadoxine for malaria. Other drugs notified as possible causes were clindamycin and paracetamol. As SP has been associated with Stevens–Johnson syndrome frequently, the sulfa drug would appear to be the likely cause. Stevens–Johnson syndrome has also been associated with mefloquine but less commonly.

Toxic epidermal necrolysis

One case of toxic epidermal necrolysis was reported in Malaysia in 2011 in an 18-year-old man who had received chloroquine.

Serious cardiac reactions

In 1992, in Germany, a 35-year-old man being treated for falciparum malaria with mefloquine and SP and primaquine was reported to have had ventricular arrhythmia (type not specified). An association with primaquine was considered to be unlikely.

Hepatic failure

Two cases of hepatic failure were reported, both notified by general practitioners in the USA and both in 45-year-old women. The first, in 1997, was a woman who had received primaquine and mefloquine. The reported adverse reactions included abdominal pain, hallucinations, hepatic failure (severity unclear), nausea and jaundice. In 2000, a second woman received an accidental overdose of primaquine, and hepatic failure, thrombocytopenia, abdominal pain and asthenia were reported. Mefloquine was listed as a concomitant drug. It would have been important to exclude haemolytic jaundice in these two cases.

Serious neurological or psychiatric reactions

Anoxic seizure

Anoxic seizure was reported in a 40-year-old woman in Thailand in 2006 who had received chloroquine and primaquine for *P. falciparum* malaria. Both drugs were considered to be possible causes; however, the report states that the patient received 250 mg primaquine but also states that she received 30 mg. She may have received a drug such as mefloquine, which comes in a 250-mg tablet strength and is associated with seizures. This would have been more appropriate treatment for her falciparum malaria.

Psychosis

There were three reports of psychosis. One in Malaysia in 2008 was in a 27-year-old woman who received primaquine at 15 mg daily for 5 days with chloroquine. Both drugs were assessed as possible causes of her psychosis and anaemia; quinine was listed as a concomitant cause. Chloroquine is known to cause psychosis rarely. The second report was from the USA of a 35-year-old man who received primaquine and mefloquine daily for 41 days. (This would have been well above the recommended dosage.) The adverse reactions reported included psychosis, manic reaction, neurosis and aggressive behaviour. The third case, from the USA in 2010, was of an 11-year-old girl with psychotic disorder and mania who had received mefloquine, artesunate and primaquine; risperidone was listed as concomitant medication. In the last two cases, mefloquine was the more likely culprit, as it is known to be associated with neuropsychiatric reactions.

Adverse reactions associated with confirmed G6PD deficiency

One case of acute haemolytic anaemia probably triggered by primaquine was reported in Australia in 1984 in a 22-year-old G6PD-deficient man. The concomitant drugs listed were chloroquine and SP. The second report was from Malaysia in 1988 of a 28-year-old G6PD-deficient man, who had acute haemolytic anaemia after treatment with primaquine + SP for malaria. Both drugs were considered probable causes. The third case was reported in the USA in 2007, of a 21-year-old man given primaquine prophylactically. An acute haemolytic reaction requiring hospitalization was described after the first day of treatment (dose not stated), associated with vomiting, pyrexia, headache and diarrhoea, which resolved on withdrawal of primaquine. As the patient was confirmed to have G6PD deficiency, a causative relation is highly likely.

Anaemia

Acute haemolytic anaemia

Between 1968 and 2012, nine cases of haemolytic anaemia and 15 cases of haemolysis were reported from seven countries (Table A4.2): Australia (3), Germany (1), India (1), Malaysia (3), Sweden (1), Thailand (11) and the USA (5). Seventeen (71%) episodes occurred in males. As the severity of the anaemia was not reported, the clinical significance cannot be assessed. A causative association is highly likely.

Other anaemia

Anaemia without haemolysis was reported in four patients who had received primaquine in Malaysia, Peru, Thailand and the USA. The severity of the anaemia was not reported, although one case was aplastic.

Methaemoglobinaemia

Methaemoglobinaemia was reported as an adverse event 47 times between 1973 and 2011 (Table A4.3), in Australia (14), Canada (4), Ireland (1), Malaysia (1), Singapore (1), Spain (2), Switzerland (3), Thailand (2), the United Kingdom (2) and the USA (17). It was reported more commonly in males (67%) and associated with concomitant administration of dapsone in several cases. G6PD status was not reported for any of the cases, and the severity of methaemoglobinaemia was reported only infrequently.

Table A4.2. Reports to the Uppsala Monitoring Centre of acute haemolytic anaemia associated with treatment with primaquine

Country	Year	M/F	Age (years)	Causality assessment	Other drugs implicated	Concomitant medication	Other reactions or events
Australia	1968	M	26	–	–	Chloroquine, Proguanil, dapsone	Jaundice
Sweden	1981	F	51	Probable	–	–	*P. vivax* malaria
USA	1984	F	15	–	Chloroquine, SP	–	Antinuclear antibody-positive Agranulocytosis
Australia	1984	M	22	Probable	–	Chloroquine, SP	G6PD deficiency
Thailand	1986	M	36	Possible	–	–	–
Thailand	1986	F	11	Possible	SP	–	–
Thailand	1986	M	16	Possible	SP	Paracetamol	Haemoglobinaemia
Thailand	1987	M	–	Possible	–	–	–
Thailand	1987	M	31	Possible	–	Chloroquine	Malaria
Thailand	1987	M	14	Possible	–	Paracetamol	–
Germany	1993	M	21	–	–	Chloroquine	Bilirubinaemia, *P. vivax* malaria
USA	1993	F	19			Mefloquine	Abdominal pain, haematuria
Thailand	1994	M	22	Probable	SP, mefloquine	–	Haematuria
Thailand	1996	M	31	Possible	SP	–	Fever, nausea
Thailand	1997	M	32	Possible	–	Chloroquine	–
Australia	2000	F	21	Possible	Mebendazole		Jaundice
Thailand	2000	F	18	Unlikely	–	Chloroquine	–
Thailand	1998	M	12	Probable	–	Tetracycline, quinine, chloroquine, paracetamol	
Malaysia	2003	M	23	Probable	SP	–	G6PD deficiency
Malaysia	2003	M	13	Probable	SP	–	
USA	2004	F	42	–	Clindamycin		*P. jiroveci* pneumonia, vomiting, rash, *C. difficile* diarrhoea
USA	2007	M	21	–	–	–	Pyrexia, vomiting, headache, diarrhoea, G6PD deficiency
Malaysia	2007	M	20	Possible	–	–	Jaundice
India	2012	M	22	Probable	–	Ranitidine, paracetamol	G6PD deficiency

Table A4.3. Case reports of methaemoglobinaemia in patients who received primaquine

Country	Year	M/F	Age (years)	Causality assessment	Other drugs implicated	Concomitant medication[a]	Other reactions or events
Australia	1973	F	37	–	–	Chloroquine	*P. vivax* malaria
Australia	1975	F	14		–	Chloroquine	
Australia	1986	M	23	Possible	Pyrimethamine–dapsone	Chloroquine	
Australia	1986	M	40	Possible	Pyrimethamine–dapsone	Chloroquine	
Australia	1986	M	21	Possible	Pyrimethamine–dapsone	–	
Australia	1986	M	30	Possible	Pyrimethamine–dapsone	–	
Australia	1987	F	43	Probable	–	–	
Australia	1987	F	16	Possible	–	–	*P. vivax* malaria, abdominal pain, pallor, cyanosis, headache, fatigue
Australia	1988	M	4	Probable	–	–	*P. vivax* malaria
USA	1995	M	31	–	Dapsone	–	
USA	1995	F	38	–	–	–	Skin discoloration
USA	1996	M	–	–	–	–	
USA	1996	M	34	–	–	Clindamycin	Nystagmus, headache, fever
Canada	1997	M	31	Possible	–	Clindamycin, pentamidine, erythromycin, naproxen	Died (unrelated)
USA	1997	M	29	–	–	Clindamycin, fluconazole, clarithromycin, Aciclovir	Abdominal pain, dyspnoea, nausea
USA	1997	F	6	–	Mepacrine	–	Accidental overdose
United Kingdom	1998	F	–	–	–	Benzylpenicillin, ranitidine, clindamycin, heparin, sulfamethoxazole–trimethoprim	
USA	1998	M	46	–	–	–	
USA	1998	M	45	–	–	–	Fear, paresthesia, dizziness
USA	1998	M	36	–	Dapsone	–	Diarrhoea, dyspnoea, nausea, pyrexia
Canada	1999	M	32	–	Dapsone	Aciclovir, clindamycin, atovaquone, ciprofloxacin	
Australia	2000	M	43	Possible	–	–	

Table A4.3. Case reports of methaemoglobinaemia in patients who received primaquine (cont'd)

Country	Year	M/F	Age (years)	Causality assessment	Other drugs implicated	Concomitant medication[a]	Other reactions or events
Australia	2000	M	43	Possible	Dapsone	–	
USA	2000	M	–	–	Dapsone	–	Cyanosis, dyspnoea, hypoxia,fatigue
Canada	2001	M	42	–	–	Ganciclovir, fluconazole, clindamycin, dapsone	Cyanosis, dyspnoea
USA	2001	M	–	–	–	Clindamycin, calcium carbonate, amitryptiline, ranitidine, levetiracetam	Coughing, bronchospasm
Singa-pore	2004	M	21	Possible	–	–	
Switzer-land	2004	M	59	Probable	–	Clindamycin, cyclosporine, mycophenolic acid	Accidental overdose, *P. jiroveci* pneumonia, renal transplant
USA	2004	M	64	–	Sulfamethoxa-zole–trimetho-prim	–	*P. jiroveci* pneumonia
Switzer-land	2005	M	40	Probable	–	Insulin, morphine, midazolam, heparin, hydrocorti-sone, gentamicin, tazobactam, clindamycin	HIV infection, *P. jiroveci* pneumonia
USA	2005	M	41		Dapsone	–	*P. jiroveci* pneumonia
USA	2005	F	24	–	Dapsone	–	Chronic obstructive airways disease, *P. jiroveci* pneumonia
Australia	2006	F	25	Possible	Chloroquine	–	Cyanosis
USA	2006	M	57	–	Dapsone	–	
USA	2006	M	–	–	Metoclopra-mide, dapsone	Simvastatin, lisinopril, lorazepam, gabapentin, clindamycin, ceftriaxone, dexametha-sone, enoxaparin, furosemide, pantoprazole, metoprolol, dronabinol	
Thailand	2007	F	15	Probable	–	Clindamycin	Pneumocystosis

Table A4.3. Case reports of methaemoglobinaemia in patients who received primaquine (cont'd)

Country	Year	M/F	Age (years)	Causality assessment	Other drugs implicated	Concomitant medication[a]	Other reactions or events
United Kingdom	2007	M	36	–	–	–	*P. vivax* malaria, lethargy
Canada	2008	M	21	–	Mefloquine	–	Hypoxia, dyspnoea, cyanosis
Spain	2008	F	38	Probable	–	–	Malaria
Switzerland	2008	F	66	Probable	Sulfamethoxazole–trimethoprim	Tacrolimus, mycophenolic acid, aspirin, prednisolone, metoprolol	*P. jiroveci* pneumonia
Australia	2009	F	39	Possible	–	–	
Spain	2009	F	54	Probable	–	–	–
Thailand	2009	M	25	Possible	Chloroquine	–	*P. knowlesi* malaria
USA	2009	M	44	–	Dapsone	–	–
Australia	2011	F	50	Possible	Doxycycline, albendazole	Magnesium, fish oil, cholecalciferol, furosemide	Splenomegaly, pancytopenia
Ireland	2011	F	68	Could not be assessed	Paclitaxel, doxorubicin, cyclophosphamide	Pegfilgrastim	*P. jiroveci* pneumonia, febrile neutropenia
Malaysia	2011	F	9	Possible	–	Chloroquine	–

[a] Medicine prescribed simultaneously that was not suspected to be a cause of the event

References

1. International Federation of Pharmaceutical Manufacturers and Associations. *Medical dictionary for regulatory activities*. Geneva.
2. McGready R et al. Adverse effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population-based study. *Lancet Infectious Diseases*, 2012, 12:388–396.

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