

## Clinical implications of *Plasmodium* resistance to atovaquone/proguanil: a systematic review and meta-analysis

Henry M. Staines<sup>1,2†</sup>, Rebekah Burrow<sup>2†</sup>, Beatrix Huei-Yi Teo<sup>2</sup>, Irina Chis Ster<sup>2</sup>, Peter G. Kremsner<sup>3,4</sup> and Sanjeev Krishna<sup>1–5\*</sup>

<sup>1</sup>Centre for Diagnostics and Antimicrobial Resistance, Institute for Infection & Immunity, St George's University of London, London, UK;

<sup>2</sup>Institute for Infection & Immunity, St George's University of London, London, UK; <sup>3</sup>Institut für Tropenmedizin Universitätsklinikum Tübingen, Tübingen, Germany; <sup>4</sup>Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon; <sup>5</sup>St George's University Hospitals NHS Foundation Trust, London, UK

\*Corresponding author. Institute for Infection & Immunity, St George's University of London, Cranmer Terrace, London SW17 0RE, UK.  
Tel: +44-208-725-5836; Fax: +44-208-725-3487; E-mail: s.krishna@sgul.ac.uk

†Contributed equally.

Received 21 May 2017; returned 26 September 2017; revised 21 October 2017; accepted 23 October 2017

**Background:** Atovaquone/proguanil, registered as Malarone<sup>®</sup>, is a fixed-dose combination recommended for first-line treatment of uncomplicated *Plasmodium falciparum* malaria in non-endemic countries and its prevention in travellers. Mutations in the cytochrome *bc*<sub>1</sub> complex are causally associated with atovaquone resistance.

**Methods:** This systematic review assesses the clinical efficacy of atovaquone/proguanil treatment of uncomplicated malaria and examines the extent to which codon 268 mutation in cytochrome *b* influences treatment failure and recrudescence based on published information.

**Results:** Data suggest that atovaquone/proguanil treatment efficacy is 89%–98% for *P. falciparum* malaria (from 27 studies including between 18 and 253 patients in each case) and 20%–26% for *Plasmodium vivax* malaria (from 1 study including 25 patients). The *in vitro* *P. falciparum* phenotype of atovaquone resistance is an IC<sub>50</sub> value >28 nM. Case report analyses predict that recrudescence in a patient presenting with parasites carrying cytochrome *b* codon 268 mutation will occur on average at day 29 (95% CI: 22, 35), 19 (95% CI: 7, 30) days longer than if the mutation is absent.

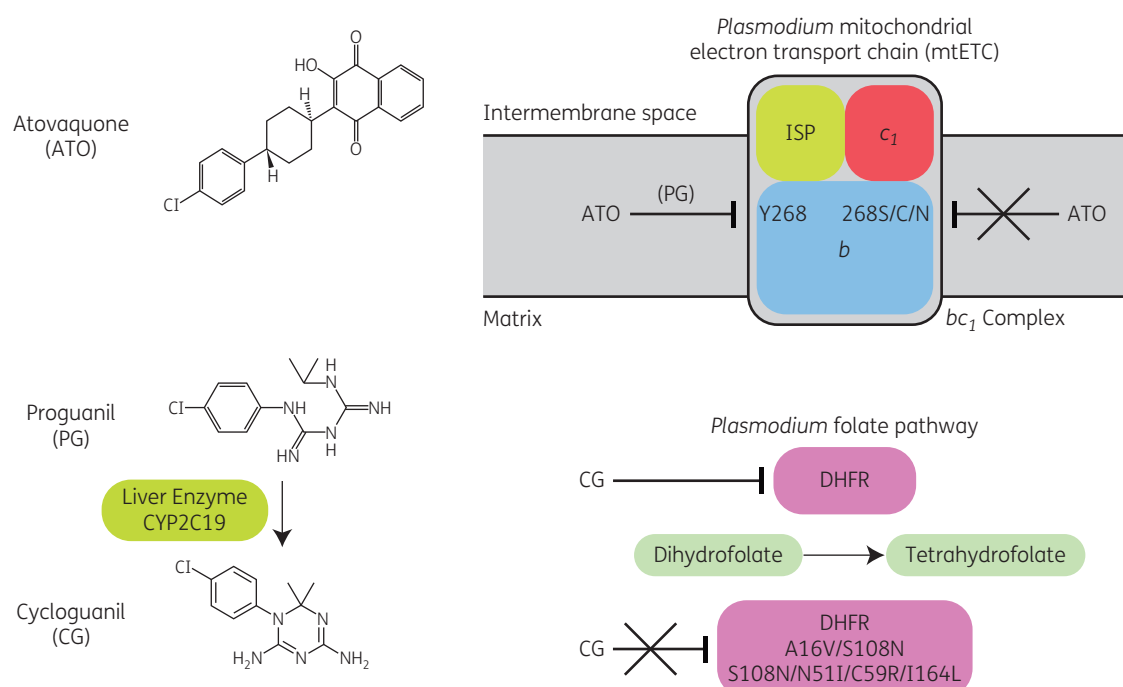
**Conclusions:** Evidence suggests atovaquone/proguanil treatment for *P. falciparum* malaria is effective. Late treatment failure is likely to be associated with a codon 268 mutation in cytochrome *b*, though recent evidence from animal models suggests these mutations may not spread within the population. However, early treatment failure is likely to arise through alternative mechanisms, requiring further investigation.

## Introduction

Infection with *Plasmodium* spp. is a major cause of mortality worldwide, causing 235 000–639 000 deaths in 2015 and 148 000 000–304 000 000 clinical cases of malaria. Most cases are in endemic countries, although malaria is also one of the most frequent causes of morbidity in travellers returning to non-endemic countries. Atovaquone/proguanil (Malarone<sup>®</sup>) is a fixed-dose combination often used as a first-line treatment for uncomplicated *Plasmodium falciparum* infections in non-endemic countries.<sup>1,2</sup> It has been used on a large scale as a treatment in areas where treatment failures of artemisinin combination therapies (TFACT)<sup>3</sup> are problematic.<sup>4</sup> It is now considered a first-line prophylaxis against malaria for travellers<sup>5</sup> and particularly military

personnel whose experience of adverse events with mefloquine prophylaxis is becoming increasingly recognized.<sup>6</sup> Atovaquone/proguanil is also being studied in a new chemo-vaccination strategy where individuals are exposed to *P. falciparum* sporozoites and then take atovaquone/proguanil to treat pre-symptomatic infections and generate antimalarial immunity (P. G. Kremsner, unpublished). Taken together with the recent expiry of patent protection for Malarone<sup>®</sup>, usage of atovaquone/proguanil is likely to rise in the future.

Atovaquone is a hydroxynaphthoquinone that selectively inhibits the mitochondrial electron transport chain at the cytochrome *bc*<sub>1</sub> complex of malaria parasites (Figure 1).<sup>7</sup> This mechanism of antiparasitic activity is complemented by the individual actions of proguanil and its metabolite, cycloguanil (Figure 1). Proguanil itself



**Figure 1.** Mechanisms of action and resistance to atovaquone/proguanil. Structures of atovaquone, proguanil and cycloguanil are shown. Atovaquone targets cytochrome *b* in the *bc*<sub>1</sub> complex [formed by cytochromes *b* and *c*<sub>1</sub> and the Rieske iron-sulphur protein (ISP)] of the *Plasmodium* mitochondrial electron transport chain. The mitochondrial electron transport chain is located on the inner membrane of mitochondria, separating the intermembrane space (the space between the outer and inner membranes) from the centrally located matrix. Atovaquone works in synergy with proguanil, but its activity is reduced by mutations in cytochrome *b* (and in particular Y268S/C/N). Proguanil is metabolized to cycloguanil by the liver enzyme CYP2C19. Cycloguanil targets the enzyme DHFR in the *Plasmodium* folate pathway. Activity of cycloguanil is reduced by mutations in DHFR, including A16V/S108N and S108N/N51I/C59R/I164L. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

has no direct effects on the parasite, but it enhances atovaquone's ability to collapse the membrane potential of malaria parasites by sensitizing mitochondria to atovaquone.<sup>8</sup> Proguanil is converted into cycloguanil by the hepatic CYP2C19 system and cycloguanil inhibits parasite dihydrofolate reductase (DHFR), which is essential for folate production and parasite replication.<sup>9</sup>

Several mechanisms can potentially influence the efficacy of atovaquone/proguanil for treatment. Mutations in *P. falciparum* cytochrome *b* (*PfCYTb*) (in particular leading to Y268S/C/N) cause atovaquone resistance both *in vitro* and *in vivo*.<sup>10–12</sup> Interestingly, a recent report, using a rodent model of malaria infection, describes that mutations in *Plasmodium berghei* *CYTb* are lethal during transmission of the parasite in the mosquito vector.<sup>13</sup> This suggests that these mutations may not be able to spread within a population, although this hypothesis has yet to be demonstrated for *P. falciparum* in the field. Cycloguanil resistance in parasites is conferred by multiple mutations in *DHFR*. Polymorphisms in host CYP2C19 also affect proguanil metabolism and can lower cycloguanil concentrations.<sup>14</sup>

Reports of frequencies of treatment failure associated with atovaquone/proguanil vary, although the risk of failure has not been systematically examined particularly with respect to mutations at codon 268 of *PfCYTb*. In this systematic review, we examine all original *in vivo* data where atovaquone/proguanil was used exclusively to treat malaria and relate findings on risk of recrudescence to mutations in *PfCYTb* and available results from *in vitro* assays.

We also estimate clinical efficacy of atovaquone/proguanil treatment of uncomplicated malaria. Results may impact on existing guidelines for the treatment of uncomplicated malaria.

## Methods

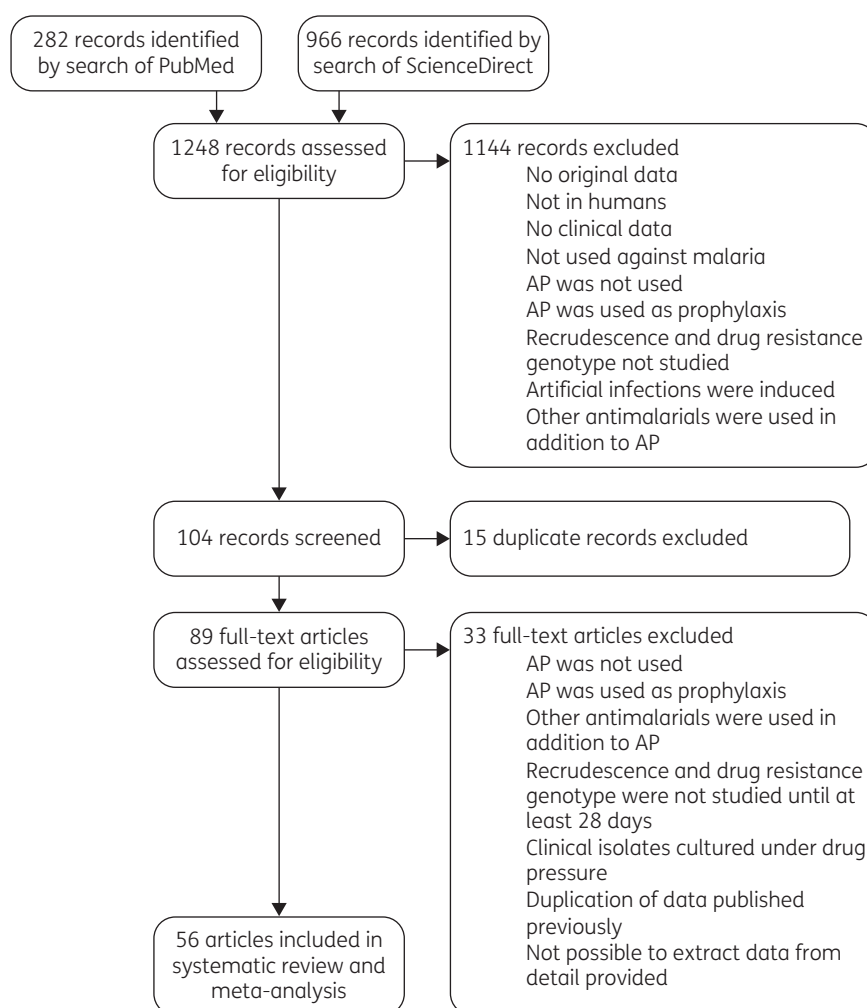
### Search strategy and selection criteria

This systematic review was registered at PROSPERO (number CRD42015020757) on 25 February 2015 and updated on 13 October 2017.

PubMed (1966–present) and ScienceDirect (1823–present) were interrogated on the 19 May 2015 with the following search strategy {(Atovaquone AND Proguanil) OR (Malarone)} AND (falciparum OR vivax OR ovale OR malariae OR knowlesi)}. Records were assessed for eligibility using title, or title and abstract. Eligible records were screened for duplicates and full-text obtained for the remaining records that were then reassessed for eligibility. Data were extracted from these articles by two reviewers and tabulated. Inclusion and exclusion criteria and extracted data variables are summarized in the [Supplementary Methods](#) (available as [Supplementary data](#) at JAC Online).

### Group studies

Two reviewers assessed group study eligibility and the risk of bias in the trials using the modified Cochrane risk of bias tool.<sup>15</sup> Six domains of bias were assessed with regard to selection, performance, detection, attrition, reporting and other, and the risk of bias deemed as low, medium, high or unclear. The information was not used to exclude studies from this review, but the assessment fed into the interpretation of results.



**Figure 2.** Study selection. AP, atovaquone/proguanil.

For all group studies, the total numbers of patients enrolled into each treatment arm, those followed up to 28 days and those with treatment failure or recrudescence were extracted and combined to obtain the proportion of patients for whom treatment had been successful in the ITT and PP populations. For randomized controlled trials (RCT), this information was also extracted for the comparator antimalarial arm(s) to allow meta-analyses (pooled ORs of the alternative intervention versus atovaquone/proguanil).

A random effects model to derive a pooled OR of treatment success for atovaquone/proguanil versus comparator treatments, if appropriate, was applied and interpreted in conjunction with a corresponding heterogeneity  $\chi^2$  test and additional sensitivity analyses undertaken (Supplementary Methods). Data were analysed with Stata version 14, with forest plots generated in Review Manager version 5.3.

### ***In vitro/ex vivo* studies**

For *in vitro/ex vivo* studies, no mathematical synthesis was carried out.

### **Case reports**

Preliminary exploratory analyses examined all the variables using graphs and statistical tests for comparisons according to the nature of the data. Regression techniques were implemented to understand potential

associations between pretreatment parasitaemia and (i) minimum days to recrudescence (defined as the length of time in days since treatment to the occurrence of clinical signs or parasitological diagnosis, whichever came first), and (ii) parasitaemia at recrudescence with presence of mutation in *PfCYTb* codon 268 in both cases (Supplementary Methods).

## **Results**

A total of 282 records were returned using PubMed and 966 using ScienceDirect (Figure 2). The 1248 records were assessed for eligibility, using title, or title and abstract, and 1144 records were excluded at this point, as they did not meet the inclusion criteria. Of the remaining 104 records, 15 duplicate records were excluded. Full text was obtained for the remaining 89 records and assessed for eligibility. Of these, 33 were excluded as they did not meet the inclusion criteria. Thus, 56 articles met the inclusion criteria for this systematic review; within these, 20 included case reports, 29 included group studies and 15 included *in vitro/ex vivo* data. The case reports and group studies were included in the meta-analysis.

The 29 group studies (Table 1) consisted of 27 with eligible data for atovaquone/proguanil treatment of *P. falciparum* infection and single studies with eligible data for atovaquone/proguanil

Table 1. Characteristics of group studies

| Paper                                    | Species of <i>Plasmodium</i> | Country of infection | Country of diagnosis/ treatment | Period of study | Type of study    | Number of patients with ITT with atovaquone/ proguanil | Number of patients assessed at day 28 | Number of patients cured at day 28 | Percentage attendance | Percentage treatment success (ITT population) | Percentage treatment success (PP population) |
|--|------------------------------|----------------------|---------------------------------|-----------------|------------------|--|---------------------------------------|------------------------------------|-----------------------|---|--|
| Anbwani et al. 1999 <sup>30</sup>        | <i>P. falciparum</i>         | Kenya                | Kenya                           | 1994            | RCT              | 84   | 81                                    | 76                                 | 96.4                  | 90.5  | 93.8   |
| Borrmann et al. 2003 <sup>31</sup>       | <i>P. falciparum</i>         | Gabon                | Gabon                           | 1999–2000       | RCT              | 100  | 92                                    | 87                                 | 92                    | 87  | 94.6   |
| Bouchard et al. 2000 <sup>32</sup>       | <i>P. falciparum</i>         | Worldwide            | France                          | 1994–95         | RCT              | 25   | 21                                    | 21                                 | 84                    | 84  | 100  |
| Bustos et al. 1999 <sup>33a</sup>        | <i>P. falciparum</i>         | Philippines          | Philippines                     | 1994–95         | RCT              | 55   | 54                                    | 54                                 | 98                    | 98.2  | 100  |
| Carrasquilla et al. 2012 <sup>17</sup>   | <i>P. falciparum</i>         | Columbia             | Columbia                        | 2007–08         | RCT              | 53   | 53                                    | 52                                 | 100                   | 98.1  | 98.1   |
| de Alencar et al. 1997 <sup>34</sup>     | <i>P. falciparum</i>         | Brazil               | Brazil                          | 1995–96         | RCT              | 88   | 73                                    | 72                                 | 83                    | 81.8  | 98.6   |
| Gürkuv et al. 2008 <sup>35</sup>         | <i>P. falciparum</i>         | Ethiopia             | Ethiopia                        | 2006            | RCT              | 32   | 30                                    | 28                                 | 93.8                  | 87.5  | 93.3   |
| Giao et al. 2004 <sup>36</sup>           | <i>P. falciparum</i>         | Vietnam              | Vietnam                         | 2001–02         | RCT              | 81   | 77                                    | 73                                 | 95.1                  | 90.1  | 94.8   |
| Llanos-Cuentas et al. 2001 <sup>37</sup> | <i>P. falciparum</i>         | Peru                 | Peru                            | 1995–96         | RCT              | 20   | 19                                    | 19                                 | 95                    | 95  | 100  |
| Loareesuwan et al. 1999 <sup>38</sup>    | <i>P. falciparum</i>         | Thailand             | Thailand                        | 1993–94         | RCT              | 91   | 79                                    | 79                                 | 86.8                  | 86.8  | 100  |
| Mulenga et al. 1999 <sup>39</sup>        | <i>P. falciparum</i>         | Zambia               | Zambia                          | 1993–94         | RCT              | 82   | 80                                    | 80                                 | 97.6                  | 97.6  | 100  |
| Mulenga et al. 2006 <sup>21</sup>        | <i>P. falciparum</i>         | Zambia               | Zambia                          | 2000–02         | RCT              | 128  | 97                                    | 92                                 | 75.8                  | 71.9  | 94.8   |
| Radloff et al. 1996 <sup>40</sup>        | <i>P. falciparum</i>         | Gabon                | Gabon                           | 1994–95         | RCT              | 71   | 63                                    | 62                                 | 88.7                  | 87.3  | 98.4   |
| Tahar et al. 2014 <sup>41</sup>          | <i>P. falciparum</i>         | Cameroon             | Cameroon                        | 2008–09         | RCT              | 168  | 156                                   | 140                                | 92.9                  | 83.3  | 89.7   |
| total RCT                                |                              |                      |                                 |                 |                  |  |                                       |                                    |                       |   |  |
|  |                              |                      |                                 |                 |                  | 1078   | 975                                   | 935                                | 92.5 (88.4, 95.8)     | 89.2 (84.7, 93)                               | 97.6 (95.4, 99.2)                            |
|  |                              |                      |                                 |                 |                  | weighted average (95% CI) <sup>b</sup>                 |                                       |                                    |                       |   |  |
| Blonde et al. 2007 <sup>42</sup>         | <i>P. falciparum</i>         | Africa               | France                          | 2004–05         | Obs              | 48   | 15 <sup>d</sup>                       | 15                                 | 31.3                  | 31.3  | 100  |
| Boggild et al. 2009 <sup>43</sup>        | <i>P. falciparum</i>         | Thailand             | Thailand                        | 2004–05         | Obs <sup>c</sup> | 70   | 68                                    | 67                                 | 97.1                  | 95.7  | 98.5   |
| Bouchard et al. 2012 <sup>44</sup>       | <i>P. falciparum</i>         | Worldwide            | Europe                          | 2003–09         | Obs              | 253  | 194                                   | 191                                | 76.7                  | 75.5  | 98.5   |
| Chih et al. 2006 <sup>45</sup>           | <i>P. falciparum</i>         | Africa               | Australia                       | 2003–05         | Obs              | 52   | 19 <sup>d</sup>                       | 19                                 | 36.5                  | 36.5  | 100  |
| Gay et al. 1997 <sup>46</sup>            | <i>P. falciparum</i>         | Worldwide            | Philippines France              | 1993–95         | Obs <sup>c</sup> | 18   | 18                                    | 18                                 | 100                   | 100   | 100  |

|  |                      |           |                    |           |     |  |            |            |                     |                     |                      |
|--|----------------------|-----------|--------------------|-----------|-----|--|------------|------------|---------------------|---------------------|----------------------|
| Grynberg et al.<br>2015 <sup>47</sup>  | <i>P. falciparum</i> | Worldwide | Israel             | 2001–13   | Obs | 44   | 44         | 38         | 100                 | 86.4                | 86.4                 |
| Krudoosod et al.<br>2007 <sup>48</sup> | <i>P. falciparum</i> | Thailand  | Thailand           | 2004–05   | Obs | 140  | 137        | 134        | 97.9                | 95.7                | 97.8                 |
| Lacy et al.<br>2002 <sup>49</sup>      | <i>P. falciparum</i> | Indonesia | Indonesia          | 1999–2000 | Obs | 19   | 19         | 18         | 100                 | 94.7                | 94.7                 |
| Malvy et al.<br>2002 <sup>50</sup>     | <i>P. falciparum</i> | Worldwide | France             | 1999–2001 | Obs | 112  | 112        | 112        | 100                 | 100                 | 100                  |
| Na-Bangchang et al. 2005 <sup>51</sup> | <i>P. falciparum</i> | Thailand  | Thailand           | 2000–01   | Obs | 26   | 22         | 22         | 84.6                | 84.6                | 100                  |
| Sabchareon et al. 1998 <sup>52</sup>   | <i>P. falciparum</i> | Thailand  | Zambia<br>Thailand | 1994–95   | Obs | 32   | 26         | 26         | 81.3                | 81.3                | 100                  |
| Tahar et al.<br>2013 <sup>53</sup>     | <i>P. falciparum</i> | Cameroon  | Cameroon           | 2008–09   | Obs | 18   | 18         | 17         | 100                 | 94.4                | 94.4                 |
| Thybo et al.<br>2004 <sup>54</sup>     | <i>P. falciparum</i> | Africa    | Denmark            | 1999–2000 | Obs | 50   | 28         | 28         | 56                  | 56                  | 100                  |
| <b>total Obs</b>                       |                      |           |                    |           |     |  |            |            |                     |                     |                      |
|  |                      |           |                    |           |     | <b>882</b>                                   | <b>720</b> | <b>705</b> |                     |                     |                      |
|  |                      |           |                    |           |     | <b>weighted average (95% CI)<sup>b</sup></b> |            |            | <b>87.6</b>         | <b>83.4</b>         | <b>99.1</b>          |
|  |                      |           |                    |           |     |  |            |            | <b>(73.8, 97.1)</b> | <b>(69.7, 93.8)</b> | <b>(97.4, 99.97)</b> |
| Looreesuwan et al. 1996 <sup>55</sup>  | <i>P. vivax</i>      | Thailand  | Thailand           | 1990–93   | Obs | 25   | 19         | 5          | 76                  | 20                  | 26.3                 |
| Radloff et al.<br>1996 <sup>56</sup>   | <i>P. ovale</i> spp. | Gabon     | Gabon              | 1995      | Obs | 3  | 3          | 3          | 100                 | 100                 | 100                  |
| <i>P. malariae</i>                     |                      |           |                    |           |     | 3  | 3          | 3          | 100                 | 100                 | 100                  |

Obs, observational study.

<sup>a</sup>Atovaquone/proguanil data from this paper are included in the RCT section, but further analysis including data for the comparator antimalarial treatments was not undertaken for the following reason. Participants were originally randomized to atovaquone/proguanil and chloroquine, but a low cure rate for the latter resulted in a protocol amendment to include sulfadoxine/pyrimethamine. However, at the time of this change, participants in the atovaquone/proguanil arm were not separated to allow direct comparison.

<sup>b</sup>Weighted averages were calculated taking into account both population size and heterogeneity.

<sup>c</sup>Data are from an RCT, but either the study was not designed to test the efficacy of atovaquone/proguanil (or another antimalarial with atovaquone/proguanil as the control) or the trial data are not described.

<sup>d</sup>Denominator excludes patients with mixed infections or those receiving non-atovaquone/proguanil treatments (<15% of the total for each study). Denominator would increase if these patients were included, but the overall cure rates would remain unchanged at 100%.

**Table 2.** Risk of bias in RCT

| Paper   | Type of bias |         |             |           |           |           |         |
|---|--------------|---------|-------------|-----------|-----------|-----------|---------|
|   | selection    |         | performance | detection | attrition | reporting | other   |
|   | RSG          | AC      |             |           |           |           |         |
| Anabwani <i>et al.</i> 1999 <sup>30</sup>       | unclear      | unclear | high        | unclear   | low       | low       | unclear |
| Borrmann <i>et al.</i> 2003 <sup>31</sup>       | low          | low     | high        | unclear   | medium    | low       | unclear |
| Bouchard <i>et al.</i> 2000 <sup>32</sup>       | unclear      | unclear | high        | unclear   | medium    | low       | unclear |
| Bustos <i>et al.</i> 1999 <sup>33</sup>         | unclear      | unclear | high        | unclear   | low       | low       | unclear |
| Carrasquilla <i>et al.</i> 2012 <sup>17</sup>   | unclear      | unclear | high        | low       | low       | medium    | low     |
| de Alencar <i>et al.</i> 1997 <sup>34</sup>     | unclear      | unclear | high        | unclear   | medium    | medium    | unclear |
| Gürkuv <i>et al.</i> 2008 <sup>35</sup>         | unclear      | unclear | high        | unclear   | low       | medium    | low     |
| Giao <i>et al.</i> 2004 <sup>36</sup>           | low          | low     | high        | unclear   | low       | medium    | unclear |
| Llanos-Cuentas <i>et al.</i> 2001 <sup>37</sup> | unclear      | unclear | high        | unclear   | low       | low       | unclear |
| Looareesuwan <i>et al.</i> 1999 <sup>38</sup>   | unclear      | unclear | high        | unclear   | medium    | low       | unclear |
| Mulenga <i>et al.</i> 1999 <sup>39</sup>        | unclear      | unclear | high        | unclear   | low       | low       | unclear |
| Mulenga <i>et al.</i> 2006 <sup>21</sup>        | low          | unclear | low         | unclear   | high      | low       | unclear |
| Radloff <i>et al.</i> 1996 <sup>40</sup>        | low          | unclear | high        | unclear   | medium    | medium    | unclear |
| Tahar <i>et al.</i> 2014 <sup>41</sup>          | unclear      | unclear | high        | unclear   | low       | medium    | low     |

RSG, random sequence generation; AC, allocation concealment.

treatment of *Plasmodium vivax* infection and *Plasmodium ovale* spp. and *Plasmodium malariae* infection. Together, the 27 *P. falciparum* studies began with 1960 patients, of whom 1695 were treated and followed up to 28 days (86.5%). A total of 1640 patients were successfully treated up to 28 days, 83.7% of the 1960 original patients and 96.8% of the 1695 treated and followed-up patients. The one *P. vivax* study began with 25 patients, of whom 19 were treated and followed up to 28 days (76%). Five patients were successfully treated up to 28 days, 20% of the original 25 patients, and 26.3% of the treated and followed up patients. The one study of *P. ovale* spp. and *P. malariae* began with six patients and all were successfully treated up to 28 days.

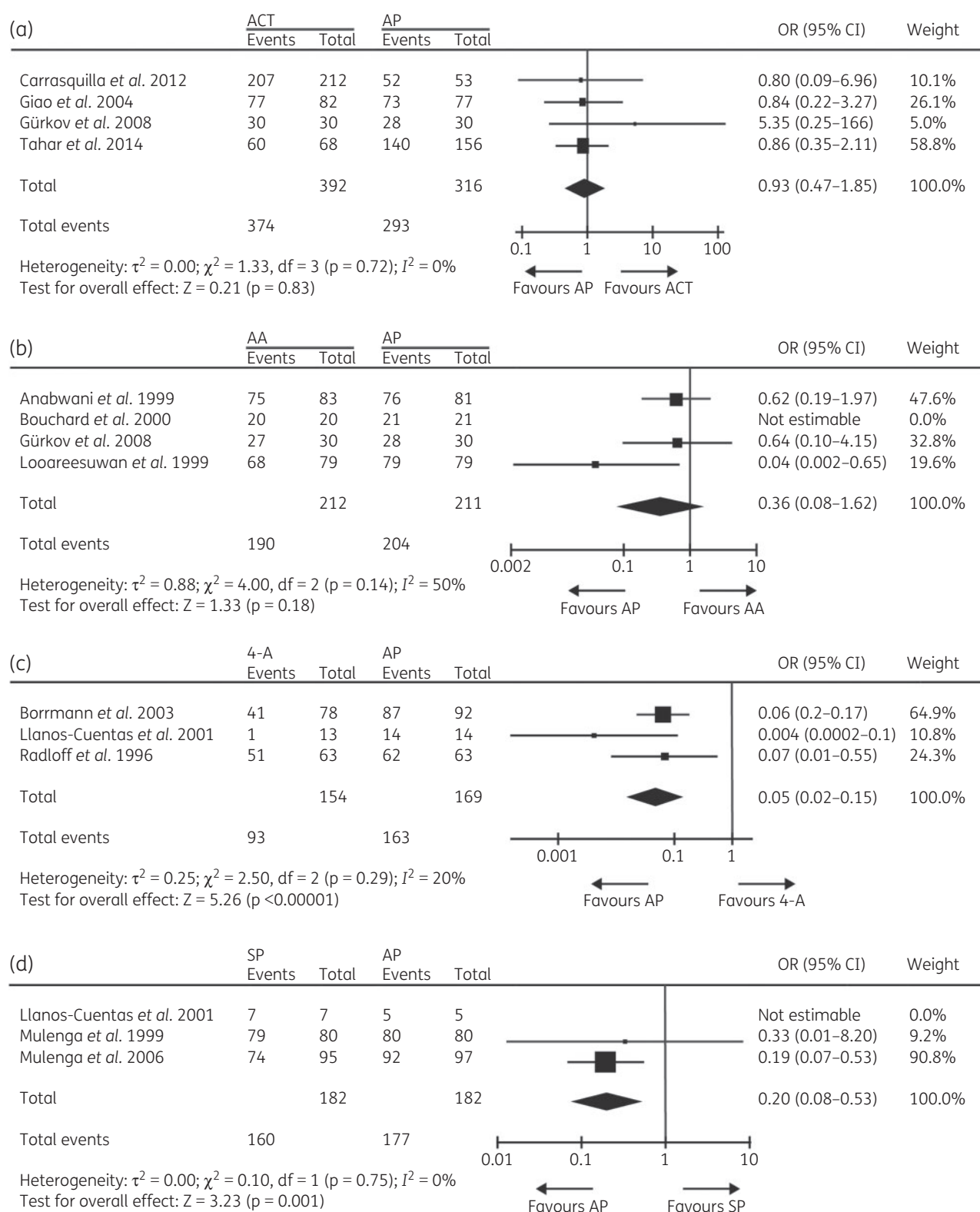
Of note, only 14 of the studies were RCT designed to test the efficacy of atovaquone/proguanil or used atovaquone/proguanil as a control treatment and participants of these made up only 55% of the total participants included here. Most of the studies from which these data were gathered, including the RCT, were of low methodological quality, being small and having between 18 and 253 participants receiving atovaquone/proguanil. Risk of bias during selection was determined to be unclear in 10 of 14 RCT group studies, as methods for randomization and concealment of allocation were unclear (Table 2). Risk of bias during performance was determined to be high in 13 of 14 studies, as blinding of participants and researchers was used in only one study. Risk of detection bias was determined to be unclear in all but one RCT study, as allocated interventions were not blinded. Risk of bias due to a high rate of attrition (<10%, low; between 10% and 20%, medium; >20% high) or patients withdrawn from the trial without explanation was high in only one RCT study. Risk of bias due to selective reporting was low to medium in all studies as 28 day cure rate was defined as either a primary (low) or secondary (medium) outcome in all cases. Another potential bias was that 11 of the 14 RCT studies were carried out by, funded by or supported by GlaxoSmithKline

or its preceding companies Glaxo Wellcome and Wellcome Research Laboratories.

High-quality data for the efficacy of atovaquone/proguanil are scarce, but provide estimates of treatment success in RCT group studies of between 89% and 98% for *P. falciparum* malaria (Table 1; weighted averages based on population size and heterogeneity), between 20% and 26.3% for *P. vivax* malaria (from one study) and 100% (in three patients each) for *P. malariae* and *P. ovale* spp. malaria.

Comparator antimalarial treatments (with number of times trialled in parentheses) were chloroquine (two), amodiaquine (two), sulfadoxine/pyrimethamine (three), chloroquine/sulfadoxine/pyrimethamine (one), quinine (one), quinine/tetracycline (one), halofantrine (two), mefloquine (one), and the artemisinin-based combination therapies (ACT), artemether/lumefantrine (two), artesunate/mefloquine (one), artesunate/amodiaquine (one) and dihydroartemisinin/piperaquine/trimethoprim/primaquine (one). Nine of the 14 RCT presented here were analysed in a previous Cochrane Library systematic review from 2005.<sup>16</sup> Subsequent RCT involving atovaquone/proguanil have used ACT predominantly as the comparator treatment(s). Given the diversity of treatments used in the trials and to allow results to be generalized to a larger population, trial data involving ACT, 4-aminoquinolines (chloroquine and amodiaquine) and amino alcohols (mefloquine, halofantrine and quinine), were grouped for a meta-analysis (Table S1). Sulfadoxine/pyrimethamine was analysed alone. The analysis indicates that there is no significant difference ( $P = 0.83$ ) in treatment success between the use of atovaquone/proguanil and ACT (Figure 3a). Sensitivity analysis was consistent with this outcome (Table S2). Given the grouped ACT in this analysis, we combined the data for two different ACT in one three-arm study.<sup>17</sup> However, analysing each arm separately did not change the outcome of the analysis (Table S2). Analysis of atovaquone/proguanil versus the amino alcohols group (Figure 3b) indicates that





**Figure 3.** Forest plots for the relative treatment successes at day 28 of patients treated with atovaquone/proguanil (AP) or (a) ACT, (b) amino alcohols (AA), (c) 4-aminoquinolines (4-A) or (d) sulfadoxine/pyrimethamine (SP).

treatment success with atovaquone/proguanil is not significantly more effective ( $P = 0.18$ ) and statistical significance was maintained for the majority of scenarios during sensitivity analysis (Table S2). As previously reported individually for amodiaquine and chloroquine,<sup>16</sup> meta-analysis of the three trials that used atovaquone/proguanil versus 4-aminoquinolines (Figure 3c) suggested that atovaquone/proguanil is more effective than 4-aminoquinolines ( $P < 0.00001$ ) and the sensitivity analysis was predominantly consistent with this outcome (Table S2). This can be explained by the prevalence of mutations in *pfprt* and *pfmdr1* conferring resistance to chloroquine and amodiaquine in the regions of study.<sup>18–20</sup> Similar findings ( $P = 0.001$ ) emerged when analysing atovaquone/proguanil versus sulfadoxine/pyrimethamine (Figure 3d and Table S2). This can be explained by the increasing development of sulfadoxine/pyrimethamine resistance over time between the two studies undertaken in Zambia.<sup>21,39</sup>

Eligible data on *in vitro/ex vivo* clinical isolates exposed to atovaquone were available in 15 papers (Table 3). The amount of data and the level of detail available did not allow further mathematical syntheses, but the data can be used to hypothesize about what the *in vitro/ex vivo* phenotype of atovaquone resistance might be. All *P. falciparum* isolates with the WT Y amino acid at codon 268 have an atovaquone  $IC_{50} \leq 28$  nM, with the majority  $< 10$  nM. All single isolates with N, C or S at 268 have  $IC_{50}$  values between 20.5 and 17000 nM. A further four isolates with S at 268 were reported to have a median (IQR)  $IC_{50}$  value of 5.7 nM (1.7–1216).<sup>22</sup> Isolates with mixed genotypes were susceptible to atovaquone *in vitro*, with median  $IC_{50}$  values between 4.7 and 5 nM. Isolates of unknown genotype ranged in  $IC_{50}$  values from low nanomolar to low micromolar. The 38 *P. vivax* isolates had a pooled mean  $IC_{50}$  value of 29.4 nM.<sup>23</sup>

Data for case reports were available from 20 papers for 36 individuals (Table 4). Thirty-three of the cases were of *P. falciparum* infection and there was one case each of *P. malariae*, *P. ovale* spp. and *P. vivax* infection. Variables have been summarized, with means, standard deviations (SD), medians and IQR for continuous or count data and proportions for categorical or binary data types (Table S3). Data for pretreatment parasitaemia (baseline), parasitaemia at treatment failure/recrudescence and genotype were not available for non-*falciparum* infections and so these species were not included in subsequent analyses.

A raw data plot, Figure 4(a), presents the minimum number of days to recrudescence of infection after atovaquone/proguanil treatment, which takes into account the onset of symptoms if prior to parasitological diagnosis, versus the absence or presence of mutation (Y268S/C/N) in *PfCYTb* at the time of recrudescence. This suggests that distributions may differ across groups by mutation (confirmed by a preliminary Kruskal–Wallis test;  $P < 0.001$ ). In a subset of parasite isolates it was possible to define if there had been a change in codon 268 following treatment. A raw data plot of the minimum number of days to recrudescence versus this dataset suggested distributions may differ by codon 268 change ( $P = 0.009$ ; Kruskal–Wallis test; Figure 4b).

Figure 5 presents the relationship between pretreatment parasitaemia and minimum days until recrudescence in the absence or presence of a mutation in *PfCYTb*, using an interaction model (Figure 5a and b). Analyses of the complete and observed (by multiple imputation) datasets suggest that pretreatment parasitaemia does not appear to influence the minimum days until

recrudescence in general and that there is evidence that this effect is not modified by the presence of mutation in *PfCYTb* ( $P = 0.62$  and  $0.87$ , respectively; Table S4). However, according to complete data analysis, there is evidence ( $P < 0.001$ ; Table S4) that grouping (the codon 268 present post-treatment) is a statistically significant predictor of the minimum days until recrudescence and the evidence is further supported by the observed data analysis ( $P = 0.002$ ; Table S4). The model predicts that patients presenting with a baseline parasitaemia of 1% will have an average minimum number of days until recrudescence of 29 (95% CI: 22, 35) days if mutation in codon 268 in *PfCYTb* is present, whilst this is 19 (95% CI: 7.3, 30) days shorter in duration if the mutation is absent. Note that although a slight departure from normality for the standardized residuals ( $P = 0.02$ ) was calculated, we opted for model simplicity rather than introducing another quadratic term.

Figure 5 also presents the relationship between baseline pretreatment parasitaemia and parasitaemia at recrudescence (post-treatment parasitaemia) in the absence or presence of a mutation in *PfCYTb*, using an interaction model (Figure 5c and d). Analyses of the complete and observed datasets suggest that baseline parasitaemia (on a log scale) increases slightly and linearly with parasitaemia at recrudescence of infection ( $P = 0.004$  and  $0.029$ , respectively; Table S5). Furthermore, analysis of the complete dataset suggests that the level of increase differs by grouping using codon 268 presence post-treatment, although this effect no longer holds when observed data analysis has been implemented ( $P = 0.04$  versus  $P = 0.217$ ; Table S5). Note that the two settings do not exhibit massive differences in estimates and their precisions. Here, the model predicts that patients presenting with a baseline parasitaemia of 1% (geometric mean, which coincides with the median; Table S5) will have an average post-treatment parasitaemia of 2.0% (95% CI: 1.2%, 2.8%) if a mutation in codon 268 in *PfCYTb* is present.

Additional analyses to incorporate pretreatment parasitaemia interval values as  $< 0.01$  and  $< 5$  (Table 4), using scenarios in which these values were '1', their upper limit, '2', half the interval values and '3', a 10th of the value, provided no substantial quantitative changes in the above estimates presented and their precision and no qualitative changes to the conclusion (Table S6 and Table S7).

## Discussion

Atovaquone/proguanil was developed as a combination therapy when early clinical studies showed that atovaquone as a single agent was associated with recrudescence of highly atovaquone-resistant infections in ~30% of patients.<sup>24</sup> *In vitro* evidence of synergy with proguanil prompted development of this combination, whose initial high cost precluded widespread use. As generic formulations of atovaquone/proguanil reduce costs, and as TFACT emerge, atovaquone/proguanil is one of the few non-ACT combinations registered for management of malaria. Determining its overall efficacy and identifying markers that predict treatment failures is important for policymakers in public health.

To carry out the widest scrutiny of evidence on the efficacy of atovaquone/proguanil, we included two broad types of studies. The first type (summarized in Table 1) describes efficacy of atovaquone/proguanil in the treatment of malaria often (in just over 50% of cases) in the context of an RCT. The quality of these types



**Table 3.** Characteristics of *in vitro/ex vivo* studies

| Paper   | Species of <i>Plasmodium</i> | Country of infection | Country of diagnosis/ treatment | Period of study <sup>a</sup> | Number of isolates | Atovaquone IC <sub>50</sub> (nM)           | Dispersion (nM)                 | Codon 268 |
|---|------------------------------|----------------------|---------------------------------|------------------------------|--------------------|--|---------------------------------|-----------|
| Basco 2003 <sup>57</sup>                        | <i>P. falciparum</i>         | Cameroon             | Cameroon                        | 2001–02                      | 37                 | 0.58 geometric mean                        | 0.27–2.2 range                  | Y         |
| Durand <i>et al.</i> 2008 <sup>58</sup>         | <i>P. falciparum</i>         | DRC                  | France                          | 2007                         | 1 <sup>b</sup>     | 10   | not stated                      | Y         |
| Fivelman <i>et al.</i> 2002 <sup>11</sup>       | <i>P. falciparum</i>         | Nigeria              | UK                              | 2002                         | 1 <sup>c</sup>     | 1888 mean                                  | 107 SD                          | N         |
| Gay <i>et al.</i> 1997 <sup>46</sup>            | <i>P. falciparum</i>         | worldwide            | The Philippines, France         | 1993–95                      | 96                 | 1.4 median                                 | 5.5 90 <sup>th</sup> percentile | –         |
| Ingasia <i>et al.</i> 2015 <sup>22</sup>        | <i>P. falciparum</i>         | Kenya                | Kenya                           | 2008–12                      | 143                | 3 median                                   | 1–6.9 IQR                       | Y         |
|   |                              |                      |                                 |                              | 4                  | 5.7 median                                 | 1.7–1216 IQR                    | S         |
|   |                              |                      |                                 |                              | 74                 | 4.7 median                                 | 2.2–11.1 IQR                    | Y/S       |
|   |                              |                      |                                 |                              | 6                  | 5 median                                   | 2–11.8 IQR                      | Y/S/N     |
|   |                              |                      |                                 |                              | 83                 | 3.4 mean                                   | 1.6 SD                          | Y         |
| Khositruthikul <i>et al.</i> 2008 <sup>59</sup> | <i>P. falciparum</i>         | Thailand             | Thailand                        | 1998–2005                    | 83                 | 3.4 mean                                   | 0.83–6.81 range                 | –         |
| Legrand <i>et al.</i> 2007 <sup>60</sup>        | <i>P. falciparum</i>         | French Guiana        | French Guiana                   | 2005                         | 1 <sup>b</sup>     | 1.6  | not stated                      | Y         |
|   |                              |                      |                                 |                              | 1 <sup>c</sup>     | 20.5                                       | not stated                      | S         |
| Looareesuwan <i>et al.</i> 1996 <sup>55</sup>   | <i>P. falciparum</i>         | Thailand             | Thailand                        | 1990–93                      | 12 <sup>b</sup>    | 9 mean                                     | not stated                      | –         |
|   |                              |                      |                                 |                              | NS                 | 13486 mean                                 | not stated                      | –         |
|   |                              |                      |                                 |                              | 3 <sup>c</sup>     | 10.4 mean                                  | not stated                      | –         |
|   |                              |                      |                                 |                              | 3 <sup>d</sup>     | 3.3 mean                                   | not stated                      | –         |
|   |                              |                      |                                 |                              | 37 <sup>b</sup>    | 3.2  | not stated                      | –         |
| Lüttendorf <i>et al.</i> 2006 <sup>61</sup>     | <i>P. falciparum</i>         | Thailand             | Thailand                        | 2000                         | 37 <sup>b</sup>    | 3.2  | not stated                      | –         |
| Musset <i>et al.</i> 2006 <sup>62</sup>         | <i>P. falciparum</i>         | worldwide            | France                          | 1999–2004                    | 477                | 1.79 geometric mean, 2 median <sup>e</sup> | 0.1–28 range                    | Y         |
|   |                              |                      |                                 |                              | 1 <sup>c</sup>     | 8230                                       | not stated                      | S         |
| Musset <i>et al.</i> 2006 <sup>12</sup>         | <i>P. falciparum</i>         | W. Africa            | France                          | 2003–05                      | 1 <sup>c</sup>     | 9.89                                       | not stated                      | Y         |
|   |                              |                      |                                 |                              | 1 <sup>c</sup>     | 1.49                                       | not stated                      | Y         |
|   |                              |                      |                                 |                              | 1 <sup>c</sup>     | 7.87                                       | not stated                      | Y         |
|   |                              |                      |                                 |                              | 1 <sup>c</sup>     | 17000                                      | not stated                      | C         |
|   |                              |                      |                                 |                              | 1 <sup>c</sup>     | 8230                                       | not stated                      | S         |
|   |                              |                      |                                 |                              | 1 <sup>c</sup>     | 10400                                      | not stated                      | S         |
|   |                              |                      |                                 |                              | 1 <sup>b</sup>     | 2.9  | not stated                      | Y         |
| Savini <i>et al.</i> 2008 <sup>63</sup>         | <i>P. falciparum</i>         | Comoros              | France                          | 2008                         | 1 <sup>c</sup>     | 390  | not stated                      | S         |
|   |                              |                      |                                 |                              | 1 <sup>c</sup>     | 390  | not stated                      | S         |
| Tahar <i>et al.</i> 2014 <sup>41</sup>          | <i>P. falciparum</i>         | Cameroon             | Cameroon                        | 2008–09                      | 55 <sup>b</sup>    | 1.32 geometric mean                        | 1.06–1.65 95% CI                | Y         |
| Treiber <i>et al.</i> 2011 <sup>23</sup>        | <i>P. vivax</i>              | Thailand             | Thailand                        | 2008                         | 38                 | 29.4 mean                                  | 0.184–5.30 range                | –         |
| van Vugt <i>et al.</i> 2002 <sup>64</sup>       | <i>P. falciparum</i>         | Thailand             | Thailand                        | 1998–2000                    | 39 <sup>b</sup>    | 2.21 median                                | 0.11–17.8 range                 | –         |
|   |                              |                      |                                 |                              | 10 <sup>c</sup>    | 2.86 median                                | 0.84–38.9 range                 | –         |

NS, recurrence after atovaquone treatment alone – although number not stated.

<sup>a</sup>Where not given, the year of publication is given in italics.

<sup>b</sup>Pretreatment.

<sup>c</sup>Recurrence after atovaquone/proguanil treatment.

<sup>d</sup>Pretreatment isolates from <sup>c</sup>.

<sup>e</sup>Means include the data from the isolate taken after recurrence after atovaquone/proguanil treatment.

of studies is relatively low for several reasons associated with potentials for bias (Table 2). The second more mechanistic analysis of atovaquone/proguanil's efficacy (summarized in Tables 3 and 4) included review of *in vitro* susceptibility analysis of parasites, where available, and detailed analysis of individual case reports of treatment failures and their association with parasitaemia and

mutation in *PfCYTb*. These latter reports are often richer in data and provide insights that complement findings from larger studies.

While datasets were small and associated with potential bias (and thus requiring cautious interpretation), the overall efficacy of atovaquone/proguanil expressed as a weighted average based on study population sizes and heterogeneity is 89% and 83% in ITT

Table 4. Characteristics of case reports

| Paper                                   | Species of <i>Plasmodium</i> | Country of infection | Country of diagnosis/treatment | Period of study <sup>a</sup> | Pretreatment parasitaemia (%)                                  | Codon 268 pretreatment <sup>b</sup>                       | Days until symptomatic                         | Days until parasitological diagnosis            | Minimum days until recrudescence                      | Parasitaemia at recrudescence (%)                             | Codon 268 post-treatment <sup>b</sup>                          |
|---|------------------------------|----------------------|--------------------------------|------------------------------|--|---|--|---|---|---|--|
| Blossom et al. 2005 <sup>65</sup>       | <i>P. vivax</i>              | Zambia               | USA                            | 2002                         | -  | -   | 21   | 21  | 21  | -   | -  |
| Contentin et al. 2011 <sup>66</sup>     | <i>P. falciparum</i>         | Guinea               | France                         | 2011                         | 7  | -   | 20   | 20  | 20  | 1.7   | -  |
| David et al. 2003 <sup>67</sup>         | <i>P. falciparum</i>         | Cameroon             | Denmark                        | 2002                         | 1  | -   | 21   | 21  | 21  | 2.5   | -  |
| Durand et al. 2008 <sup>68</sup>        | <i>P. falciparum</i>         | DRC                  | France                         | 2007                         | 1.6  | Y**   | -  | 28  | 28  | 0.001   | Y**  |
| Färnert et al. 2003 <sup>10</sup>       | <i>P. falciparum</i>         | Ivory Coast          | Sweden                         | 2000                         | 1  | Y*  | 2  | 2   | 2   | 4   | Y*   |
| Fivelman et al. 2002 <sup>11</sup>      | <i>P. falciparum</i>         | Nigeria              | UK                             | 2002                         | 0.5<br>1.5   | S*<br>-   | 28<br>28                                       | 28<br>33  | 28<br>28  | 1.6<br><1   | S**<br>N   |
| Forestier et al. 2011 <sup>68</sup>     | <i>P. falciparum</i>         | Cameroon             | France                         | 2009                         | 2  | -   | 21   | 21  | 21  | 3   | -  |
| Koch et al. 2007 <sup>69</sup>          | <i>P. falciparum</i>         | Ghana                | Germany                        | 2007                         | 1  | -   | 4  | 4   | 4   | <1  | -  |
| Kuhn et al. 2005 <sup>70</sup>          | <i>P. falciparum</i>         | Sierra Leone         | Canada                         | 2005                         | -  | Y**   | 19   | -   | 19  | -   | S**  |
| Legrand et al. 2007 <sup>60</sup>       | <i>P. falciparum</i>         | French Guiana        | French Guiana                  | 2005                         | -  | Y**   | -  | 24  | 24  | -   | S**  |
| Müller-Stöver et al. 2007 <sup>71</sup> | <i>P. malariae</i>           | Nigeria              | Germany                        | 2007                         | -  | -   | 98   | 98  | 98  | -   | -  |
| Musset et al. 2006 <sup>12</sup>        | <i>P. falciparum</i>         | W. Africa            | France                         | 2003-05                      | 0.002<br>0.3<br>0.007<br>0.35<br>13<br>4<br>0.15<br>0.2<br>2.8 | Y*<br>Y**<br>Y**<br>Y**<br>Y**<br>Y**<br>Y**<br>Y*<br>Y** | 3<br>-<br>11<br>22<br>26<br>26<br>39<br>3<br>- | 3<br>7<br>11<br>22<br>26<br>26<br>39<br>3<br>31 | 3<br>7<br>11<br>22<br>26<br>26<br>39<br>3<br>28<br>31 | 0.5<br>1<br>0.75<br>0.47<br>5<br>5<br>0.25<br>1.1<br>1.5<br>- | Y*<br>Y**<br>Y**<br>C**<br>S**<br>C**<br>S**<br>Y*<br>S**<br>- |
| Oswald et al. 2007 <sup>72</sup>        | <i>P. ovale</i> spp.         | Mozambique           | USA                            | 2007                         | -  | -   | 18   | 34  | 18  | 2   | C  |
| Perry et al. 2009 <sup>73</sup>         | <i>P. falciparum</i>         | India, Nepal         | Canada                         | 2007                         | 3.4  | -   | 31   | 34  | 31  | 3   | S**  |
| Plucinski et al. 2014 <sup>74</sup>     | <i>P. falciparum</i>         | Nigeria              | USA                            | 2012-13                      | <5   | Y**   | -  | 33  | 33  | 3.2   | S  |
| Rose et al. 2008 <sup>75</sup>          | <i>P. falciparum</i>         | Mozambique           | Canada                         | 2006                         | 1.2  | -   | 23   | 23  | 23  | 1.3   | S**  |
| Savini et al. 2008 <sup>63</sup>        | <i>P. falciparum</i>         | Comoros              | France                         | 2008                         | 0.5  | Y**   | 30   | 30  | 30  | -   | S**  |
| Schwartz et al. 2003 <sup>76</sup>      | <i>P. falciparum</i>         | Kenya                | Israel                         | 2002                         | 3  | Y**   | -  | -   | -   | -   | -  |

|  |                      |        |                            |         |      |    |    |    |    |      |   |
|--|----------------------|--------|----------------------------|---------|------|----|----|----|----|------|---|
| Sutherland <i>et al.</i><br>2008 <sup>27</sup> | <i>P. falciparum</i> | Africa | Africa, UK,<br>Switzerland | 2004–08 | –    | –  | –  | 42 | 42 | 1.1  | C |
|  |                      |        |                            |         | 1    | 2  | 2  | 2  | 2  | 4    | Y |
|  |                      |        |                            |         | 2.5  | 3  | 3  | 3  | 3  | 0.1  | Y |
|  |                      |        |                            |         | 0.1  | 23 | 25 | 23 | 23 | 0.3  | S |
|  |                      |        |                            |         | –    | –  | 4  | 4  | 4  | 1    | Y |
|  |                      |        |                            |         | –    | –  | 21 | 21 | 21 | 0.2  | C |
|  |                      |        |                            |         | <0.1 | –  | 26 | 26 | 26 | <0.1 | C |
|  |                      |        |                            |         | –    | 32 | 32 | 32 | 32 | 3    | C |
|  |                      |        |                            |         | 0.1  | 19 | 19 | 19 | 19 | 0.01 | Y |
| Wichmann <i>et al.</i><br>2004 <sup>77</sup>   | <i>P. falciparum</i> | DRC    | Germany                    | 2004    | –    | –  | –  | –  | –  | –    | – |

<sup>a</sup>Where not given, the year of publication is given in italics.

<sup>b</sup>Where given,

\*PfDHR S108, N51, C59 and

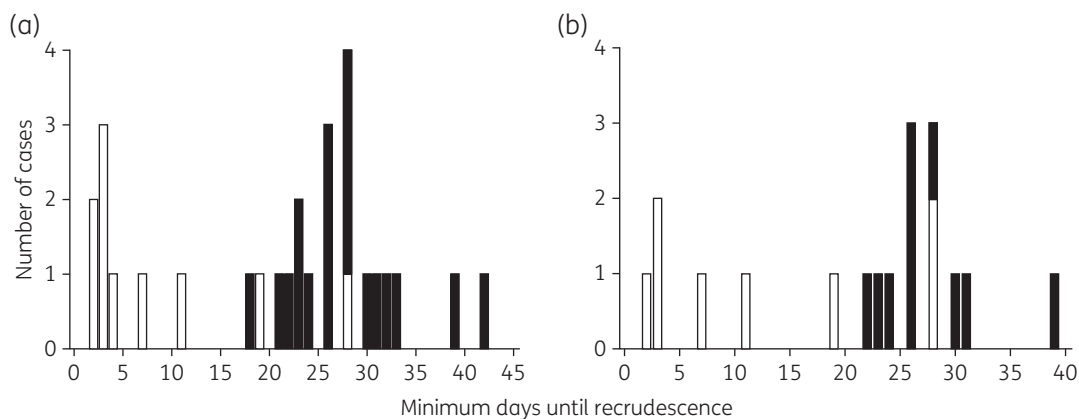
\*\*PfDHR S108N, N51I, C59R.

analyses of RCT and observational studies, respectively, and is 98% and 99% in PP analyses. This is a reassuringly acceptable level of efficacy and to date there are no indications of treatment failures becoming associated with particular geographical areas that would preclude atovaquone/proguanil use to treat travellers or prevent infections from such areas. Furthermore, meta-analysis suggests that atovaquone/proguanil treatment success is equivalent to the use of ACT and amino alcohols and better than 4-aminoquinolines and sulfadoxine/pyrimethamine, although caution is required in some cases due to the grouping of different antimalarials within a class. This extends findings from a prior meta-analysis that concluded that atovaquone/proguanil is more effective than chloroquine, amodiaquine and mefloquine.<sup>16</sup> This general reassurance is important particularly in light of complications that are being associated with the use of mefloquine and that have been reviewed recently in a UK House of Commons Defence Committee report on mefloquine's use in military personnel.<sup>25</sup> Doxycycline and atovaquone/proguanil remain as the only alternatives to mefloquine recommended for antimalarial prophylaxis.<sup>5</sup> While atovaquone/proguanil is considered safe, it has been reported that safety data are relatively sparse and would benefit from further large trials.<sup>16</sup> The safety of atovaquone/proguanil was not studied here.

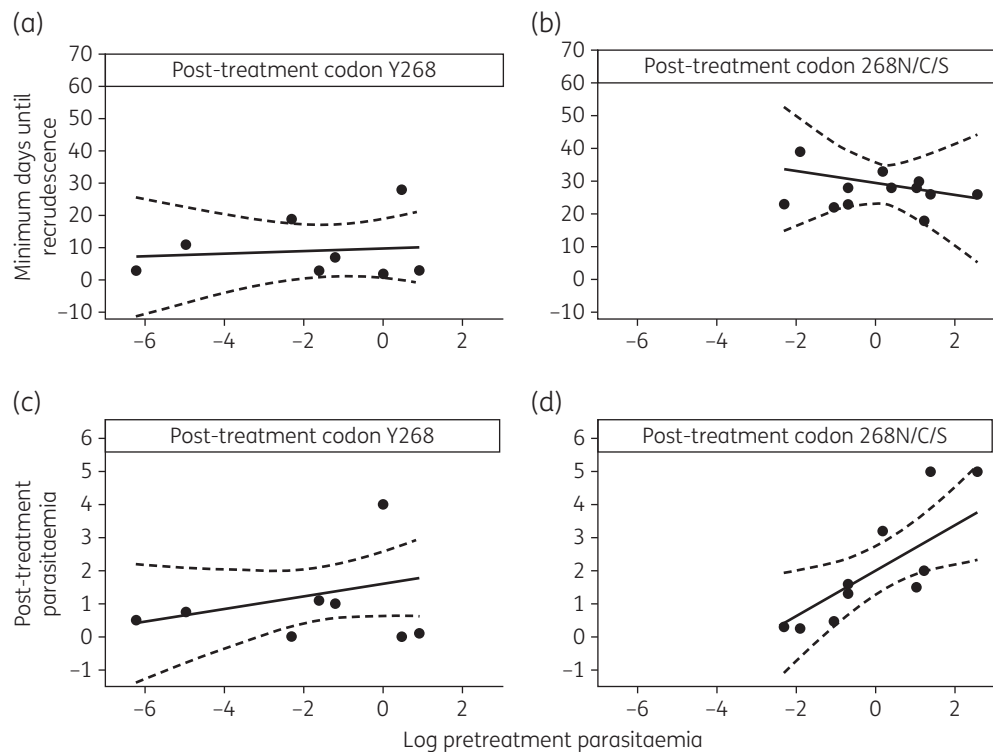
The *in vitro* phenotypic assays for atovaquone susceptibility and its relationship to target genotype suggest that WT amino acid (Y268) is uniformly associated with susceptibility. The threshold for defining susceptibility is an IC<sub>50</sub> value ≤28 nM, with most isolates in different studies having IC<sub>50</sub> values <10 nM. Although the aggregated IC<sub>50</sub> values for *P. vivax* were 29 nM, it is unlikely that this slightly higher value compared with *P. falciparum* susceptibility contributed to the higher treatment failure rates as these are most likely due to relapse because of the non-susceptibility of hypnozoite stages found in the liver to atovaquone/proguanil.<sup>26</sup>

Analysis of individual case reports and the dynamics of recrudescing infection highlight further interesting findings. The presence or appearance of mutation (Y268S/C/N) in *PfCYTb* is strongly associated with a late recrudescing infection (Figures 4 and 5) where late onset of symptoms or parasitological recrudescence (whichever is earlier, which we have defined as minimum days to recrudescence here) is on average 29 days (95% CI: 22, 35) after treatment has commenced. This is in accord with a previous estimate of the mean time to recrudescence of parasites carrying the Y268C mutation of 28 days (95% CI: 23.0, 33.0).<sup>27</sup> Understanding the mechanisms that account for the length of time until recrudescence is worthy of further investigation. One possible factor underlying this phenotype is a loss of parasite fitness due to mutation. This has been reported previously, using *in vitro* growth assays, for atovaquone-resistant parasites carrying *PfCYTb* mutations, though not at position 268.<sup>28</sup> Our data suggest that patients should be monitored for up to 42 days. Late recrudescence in these cases should always be treated with an alternative antimalarial treatment regimen.

A recent report has demonstrated that mutations in *P. berghei* *CYTb* are invariably lethal to the parasite during transmission in the mosquito vector.<sup>13</sup> This finding lends weight to the hypothesis that *PfCYTb* mutations may not be able to spread within a population. If true, this would preclude the requirement to monitor for these mutations in endemic areas. The available data are in general



**Figure 4.** Relationship between the number of days until recrudescence of malaria infection and the status of codon 268 in *PfCYTb*. Numbers of cases of patients infected with *P. falciparum* parasites (a) with (white bars) or without (black bars) mutation at codon 268 in *PfCYTb* at the time of recrudescence and (b) with (white bars) or without (black bars) a change at codon 268 in *PfCYTb* between the initial infection and the time of recrudescence.



**Figure 5.** Relationship between pretreatment parasitaemia and (a and b) minimum days until recrudescence and (c and d) post-treatment parasitaemia in the absence or presence of mutation at codon 268 in *PfCYTb*. Complete data sets (filled circles) are shown with predicted lines of fit by multiple imputation (continuous lines) and their 95% CI (broken lines).

agreement with this, as codon 268 mutations are very rarely observed in parasites from patients that suffer later recrudescence, prior to drug pressure (Table 4) and no geographical foci of atovaquone/proguanil treatment failure or *PfCYTb* mutations have been reported. However, this does not preclude the spread of *PfCYTb* mutations carried by parasite sub-populations, where the mutation cannot be detected by conventional means, or the spread of parasites with permissive genetic backgrounds that favour *PfCYTb* mutation following drug pressure. Our findings also

identify the need for further characterization of the genetic backgrounds of parasites in patients experiencing early recrudescence. These studies should aim to determine the mechanism of this high-grade resistance as well as identifying associated markers, although other factors that may cause or contribute to the phenotype of early treatment failure will need to be considered carefully (e.g. non-compliance to treatment, use of substandard or counterfeit medications, poor absorption or metabolism of the medication by the patient).

While not considered in detail, it is worth noting that there are 17 case reports that provide molecular markers for cycloguanil resistance, the triple *PfDHFR* mutation S108N, N51I, C59R (Table 4). Only 4 of 17 infections carried parasites with sensitive genotypes at first presentation. One of these four infections recrudescenced with parasites carrying a resistant genotype, leaving three infections caused by parasites with *PfDHFR*-inhibitor sensitive genotypes post-treatment. Interestingly, all parasites defined as recrudescing by day 3 (Table 4) carried *PfDHFR* sensitive genotypes, suggesting that cycloguanil did not contribute to failure. All later treatment failures (from day 7) were caused by parasites carrying genotypes associated with resistance to cycloguanil. Therefore, atovaquone/proguanil treatment failures from day 7 onwards are most likely to be caused by parasites that are already resistant to cycloguanil.

After our database search was closed, an additional series of case reports that was not picked up was identified independently.<sup>29</sup> These six cases were of patients who had recrudescenced more than once after atovaquone/proguanil treatment and in all cases time to recrudescence was  $\geq 19$  days. In five cases where the post-treatment genotype of *PfCYTb* was available, it was of the 268C/S mutation. In four of six patients with second recrudescences, the time to recrudescence was  $\geq 20$  days and all four genotypes bore mutant variants at position 268. These observations suggest that the proguanil component of atovaquone/proguanil has sufficient antimalarial efficacy to suppress parasitaemias for 2–3 weeks and that the dynamics of late treatment failure are consistent with absence of atovaquone efficacy. These cases were incorporated into a secondary analysis of the case reports. Findings with regard to the relationship between pretreatment parasitaemia and minimum days until recrudescence in the absence or presence of a mutation in *PfCYTb* are consistent with those presented in Table S8.

Overall, atovaquone/proguanil therapy is comparable in efficacy to ACT used in treating uncomplicated malaria. Detailed genotype-phenotype analysis in this systematic review has illustrated several new findings. There are differences between early and late treatment failures because mutations in the target conferring resistance to atovaquone are identified most commonly in late and not early treatment failures. The mechanism of early treatment failure after atovaquone/proguanil treatment needs further investigation. Recent evidence is also reassuring that spread of the 268 mutations conferring atovaquone resistance may be limited by poor transmissibility in the insect stages of *P. falciparum* infections.

## Funding

This work was supported by the European Union Seventh Framework Programme under grant agreement n° 304948—NanoMal (to S. K. and H. M. S.). H. M. S. is supported by the Wellcome Trust Institutional Strategic Support Fund (204809/Z/16/Z) awarded to St George's University of London.

## Transparency declarations

None to declare.

## Author contributions

S. K., together with P. G. K., designed the systematic review and meta-analysis protocol. B. H.-Y. T. and S. K. created the search strategy. B. H.-Y.

T., H. M. S. and R. B. searched for publications. B. H.-Y. T., H. M. S. and R. B. did the review and data extraction. I. C. S. conducted all the statistical aspects of the study. I. C. S., H. M. S. and R. B. performed the analysis and all authors critically interpreted the results. H. M. S., R. B. and S. K. wrote the first draft of the article and all authors provided critical revisions to writing thereafter.

## Supplementary data

Supplementary Methods and Tables S1 to S8 are available as Supplementary data at JAC Online.

## References

- WHO. *Guidelines for the Treatment of Malaria, Third Edition*. 2015. <http://www.who.int/malaria/publications/atoz/9789241549127/en/>.
- WHO. *World Malaria Report 2016*. 2016. <http://www.who.int/malaria/publications/world-malaria-report-2016/en/>.
- Krishna S, Kremsner PG. Antidogmatic approaches to artemisinin resistance: reappraisal as treatment failure with artemisinin combination therapy. *Trends Parasitol* 2013; **29**: 313.
- WHO. *Update on Artemisinin Resistance—April 2012*. 2012. <http://www.who.int/malaria/publications/atoz/updateartemisininresistanceapr2012/en/>.
- PHE. *Guidelines for Malaria Prevention in Travellers from the UK*. 2017. <https://www.gov.uk/government/publications/malaria-prevention-guidelines-for-travellers-from-the-uk>.
- Nevin RL. Rational risk-benefit decision-making in the setting of military mefloquine policy. *J Parasitol Res* 2015; **2015**: 260106.
- Barton V, Fisher N, Biagini GA et al. Inhibiting *Plasmodium* cytochrome bc1: a complex issue. *Curr Opin Chem Biol* 2010; **14**: 440–6.
- Srivastava IK, Vaidya AB. A mechanism for the synergistic antimalarial action of atovaquone and proguanil. *Antimicrob Agents Chemother* 1999; **43**: 1334–9.
- Foote SJ, Galatis D, Cowman AF. Amino acids in the dihydrofolate reductase-thymidylate synthase gene of *Plasmodium falciparum* involved in cycloguanil resistance differ from those involved in pyrimethamine resistance. *Proc Natl Acad Sci USA* 1990; **87**: 3014–7.
- Färnert A, Lindberg J, Gil P et al. Evidence of *Plasmodium falciparum* malaria resistant to atovaquone and proguanil hydrochloride: case reports. *BMJ* 2003; **326**: 628–9.
- Fivelman QL, Butcher GA, Adagu IS et al. Malarone treatment failure and in vitro confirmation of resistance of *Plasmodium falciparum* isolate from Lagos, Nigeria. *Malar J* 2002; **1**: 1.
- Musset L, Bouchaud O, Matheron S et al. Clinical atovaquone-proguanil resistance of *Plasmodium falciparum* associated with cytochrome b codon 268 mutations. *Microbes Infect* 2006; **8**: 2599–604.
- Goodman CD, Siregar JE, Mollard V et al. Parasites resistant to the antimalarial atovaquone fail to transmit by mosquitoes. *Science* 2016; **352**: 349–53.
- Kaneko A, Bergqvist Y, Taleo G et al. Proguanil disposition and toxicity in malaria patients from Vanuatu with high frequencies of CYP2C19 mutations. *Pharmacogenetics* 1999; **9**: 317–26.
- Higgins JP, Altman DG, Gøtzsche PC et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011; **343**: d5928.
- Osei-Akoto A, Orton L, Owusu-Ofori SP. Atovaquone-proguanil for treating uncomplicated malaria. *Cochrane Database Syst Rev* 2005; issue **4**: CD004529.
- Carrasquilla G, Barón C, Monsell EM et al. Randomized, prospective, three-arm study to confirm the auditory safety and efficacy of artemether-lumefantrine in Colombian patients with uncomplicated *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 2012; **86**: 75–83.



- 18 Bacon DJ, McCollum AM, Griffing SM *et al.* Dynamics of malaria drug resistance patterns in the Amazon basin region following changes in Peruvian national treatment policy for uncomplicated malaria. *Antimicrob Agents Chemother* 2009; **53**: 2042–51.
- 19 Frank M, Lehnert N, Mayengue PI *et al.* A thirteen-year analysis of *Plasmodium falciparum* populations reveals high conservation of the mutant pfcr1 haplotype despite the withdrawal of chloroquine from national treatment guidelines in Gabon. *Malar J* 2011; **10**: 304.
- 20 Mayengue PI, Kalmbach Y, Issifou S *et al.* No variation in the prevalence of point mutations in the Pfcr1 and Pfmdr1 genes in isolates from Gabonese patients with uncomplicated or severe *Plasmodium falciparum* malaria. *Parasitol Res* 2007; **100**: 487–93.
- 21 Mulenga M, Malunga P, Bennett S *et al.* Folic acid treatment of Zambian children with moderate to severe malaria anemia. *Am J Trop Med Hyg* 2006; **74**: 986–90.
- 22 Ingasia LA, Akala HM, Imbuga MO *et al.* Molecular characterization of the cytochrome *b* gene and *in vitro* atovaquone susceptibility of *Plasmodium falciparum* isolates from Kenya. *Antimicrob Agents Chemother* 2015; **59**: 1818–21.
- 23 Treiber M, Wernsdorfer G, Wiedermann U *et al.* Sensitivity of *Plasmodium vivax* to chloroquine, mefloquine, artemisinin and atovaquone in north-western Thailand. *Wien Klin Wochenschr* 2011; **123** Suppl 1: 20–5.
- 24 Canfield CJ, Pudney M, Gutteridge WE. Interactions of atovaquone with other antimalarial drugs against *Plasmodium falciparum* *in vitro*. *Exp Parasitol* 1995; **80**: 373–81.
- 25 House of Commons Defence Committee. *An Acceptable Risk? The Use of Larium for Military Personnel: Government Response to the Committee's Fourth Report of Session 2015–16*. 2016. <https://www.parliament.uk/business/committees/committees-a-z/commons-select/defence-committee/inquiries/parliament-2015/publications/>.
- 26 Dembele L, Gego A, Zeeman AM *et al.* Towards an *in vitro* model of *Plasmodium* hypnozoites suitable for drug discovery. *PLoS One* 2011; **6**: e18162.
- 27 Sutherland CJ, Laundry M, Price N *et al.* Mutations in the *Plasmodium falciparum* cytochrome *b* gene are associated with delayed parasite recrudescence in malaria patients treated with atovaquone-proguanil. *Malar J* 2008; **7**: 240.
- 28 Peters JM, Chen N, Gatton M *et al.* Mutations in cytochrome *b* resulting in atovaquone resistance are associated with loss of fitness in *Plasmodium falciparum*. *Antimicrob Agents Chemother* 2002; **46**: 2435–41.
- 29 Cottrell G, Musset L, Hubert V *et al.* Emergence of resistance to atovaquone-proguanil in malaria parasites: insights from computational modeling and clinical case reports. *Antimicrob Agents Chemother* 2014; **58**: 4504–14.
- 30 Anabwani G, Canfield CJ, Hutchinson DB. Combination atovaquone and proguanil hydrochloride vs. halofantrine for treatment of acute *Plasmodium falciparum* malaria in children. *Pediatr Infect Dis J* 1999; **18**: 456–61.
- 31 Borrmann S, Faucher JF, Bagathou T *et al.* Atovaquone and proguanil versus amodiaquine for the treatment of *Plasmodium falciparum* malaria in African infants and young children. *Clin Infect Dis* 2003; **37**: 1441–7.
- 32 Bouchaud O, Monlun E, Muanza K *et al.* Atovaquone plus proguanil versus halofantrine for the treatment of imported acute uncomplicated *Plasmodium falciparum* malaria in non-immune adults: a randomized comparative trial. *Am J Trop Med Hyg* 2000; **63**: 274–9.
- 33 Bustos DG, Canfield CJ, Canete-Miguel E *et al.* Atovaquone-proguanil compared with chloroquine and chloroquine-sulfadoxine-pyrimethamine for treatment of acute *Plasmodium falciparum* malaria in the Philippines. *J Infect Dis* 1999; **179**: 1587–90.
- 34 de Alencar FE, Cerutti C Jr, Durlacher RR *et al.* Atovaquone and proguanil for the treatment of malaria in Brazil. *J Infect Dis* 1997; **175**: 1544–7.
- 35 Gürkov R, Eshetu T, Miranda IB *et al.* Ototoxicity of artemether/lumefantrine in the treatment of falciparum malaria: a randomized trial. *Malar J* 2008; **7**: 179.
- 36 Giao PT, de Vries PJ, Hung LQ *et al.* CV8, a new combination of dihydroartemisinin, piperazine, trimethoprim and primaquine, compared with atovaquone-proguanil against falciparum malaria in Vietnam. *Trop Med Int Health* 2004; **9**: 209–16.
- 37 Llanos-Cuentas A, Campos P, Clendenes M *et al.* Atovaquone and proguanil hydrochloride compared with chloroquine or pyrimethamine/sulfadoxine for treatment of acute *Plasmodium falciparum* malaria in Peru. *Braz J Infect Dis* 2001; **5**: 67–72.
- 38 Looareesuwan S, Wilairatana P, Chalermarut K *et al.* Efficacy and safety of atovaquone/proguanil compared with mefloquine for treatment of acute *Plasmodium falciparum* malaria in Thailand. *Am J Trop Med Hyg* 1999; **60**: 526–32.
- 39 Mulenga M, Sukwa TY, Canfield CJ *et al.* Atovaquone and proguanil versus pyrimethamine/sulfadoxine for the treatment of acute falciparum malaria in Zambia. *Clin Ther* 1999; **21**: 841–52.
- 40 Radloff PD, Philipps J, Nkeyi M *et al.* Atovaquone and proguanil for *Plasmodium falciparum* malaria. *Lancet* 1996; **347**: 1511–4.
- 41 Tahar R, Almelli T, Debue C *et al.* Randomized trial of artesunate-amodiaquine, atovaquone-proguanil, and artesunate-atovaquone-proguanil for the treatment of uncomplicated falciparum malaria in children. *J Infect Dis* 2014; **210**: 1962–71.
- 42 Blonde R, Naudin J, Bigirimana Z *et al.* Tolerance and efficacy of atovaquone-proguanil for the treatment of paediatric imported *Plasmodium falciparum* malaria in France: clinical practice in a university hospital in Paris. *Arch Pediatr* 2008; **15**: 245–52.
- 43 Boggild AK, Krudsood S, Patel SN *et al.* Use of peroxisome proliferator-activated receptor  $\gamma$  agonists as adjunctive treatment for *Plasmodium falciparum* malaria: a randomized, double-blind, placebo-controlled trial. *Clin Infect Dis* 2009; **49**: 841–9.
- 44 Bouchaud O, Mühlberger N, Parola P *et al.* Therapy of uncomplicated falciparum malaria in Europe: MALTHER—a prospective observational multicentre study. *Malar J* 2012; **11**: 212.
- 45 Chih DT, Heath CH, Murray RJ. Outpatient treatment of malaria in recently arrived African migrants. *Med J Aust* 2006; **185**: 598–601.
- 46 Gay F, Bustos D, Traore B *et al.* *In vitro* response of *Plasmodium falciparum* to atovaquone and correlation with other antimalarials: comparison between African and Asian strains. *Am J Trop Med Hyg* 1997; **56**: 315–7.
- 47 Grynberg S, Lachish T, Kopel E *et al.* Artemether-lumefantrine compared to atovaquone-proguanil as a treatment for uncomplicated *Plasmodium falciparum* malaria in travelers. *Am J Trop Med Hyg* 2015; **92**: 13–7.
- 48 Krudsood S, Patel SN, Tangpukdee N *et al.* Efficacy of atovaquone-proguanil for treatment of acute multidrug-resistant *Plasmodium falciparum* malaria in Thailand. *Am J Trop Med Hyg* 2007; **76**: 655–8.
- 49 Lacy MD, Maguire JD, Barcus MJ *et al.* Atovaquone/proguanil therapy for *Plasmodium falciparum* and *Plasmodium vivax* malaria in Indonesians who lack clinical immunity. *Clin Infect Dis* 2002; **35**: e92–5.
- 50 Malvy D, Djossou F, Vatan R *et al.* Experience with the combination atovaquone-proguanil in the treatment of uncomplicated *Plasmodium falciparum* malaria—report of 112 cases. *Med Trop (Mars)* 2002; **62**: 229–31.
- 51 Na-Bangchang K, Manyando C, Ruengweeraayut R *et al.* The pharmacokinetics and pharmacodynamics of atovaquone and proguanil for the treatment of uncomplicated falciparum malaria in third-trimester pregnant women. *Eur J Clin Pharmacol* 2005; **61**: 573–82.
- 52 Sabchareon A, Attanath P, Phanuksook P *et al.* Efficacy and pharmacokinetics of atovaquone and proguanil in children with multidrug-resistant *Plasmodium falciparum* malaria. *Trans R Soc Trop Med Hyg* 1998; **92**: 201–6.
- 53 Tahar R, Sayang C, Ngane Foumane V *et al.* Field evaluation of rapid diagnostic tests for malaria in Yaounde, Cameroon. *Acta Trop* 2013; **125**: 214–9.

- 54 Thybo S, Gjorup I, Ronn AM *et al.* Atovaquone-proguanil (malarone): an effective treatment for uncomplicated *Plasmodium falciparum* malaria in travelers from Denmark. *J Travel Med* 2004; **11**: 220–3.
- 55 Looareesuwan S, Viravan C, Webster HK *et al.* Clinical studies of atovaquone, alone or in combination with other antimalarial drugs, for treatment of acute uncomplicated malaria in Thailand. *Am J Trop Med Hyg* 1996; **54**: 62–6.
- 56 Radloff PD, Philipps J, Hutchinson D *et al.* Atovaquone plus proguanil is an effective treatment for *Plasmodium ovale* and *P. malariae* malaria. *Trans R Soc Trop Med Hyg* 1996; **90**: 682.
- 57 Basco LK. Molecular epidemiology of malaria in Cameroon. XVII. Baseline monitoring of atovaquone-resistant *Plasmodium falciparum* by *in vitro* drug assays and cytochrome b gene sequence analysis. *Am J Trop Med Hyg* 2003; **69**: 179–83.
- 58 Durand R, Prendki V, Cailhol J *et al.* *Plasmodium falciparum* malaria and atovaquone-proguanil treatment failure. *Emerg Infect Dis* 2008; **14**: 320.
- 59 Khositnithikul R, Tan-Ariya P, Mungthin M. *In vitro* atovaquone/proguanil susceptibility and characterization of the cytochrome b gene of *Plasmodium falciparum* from different endemic regions of Thailand. *Malar J* 2008; **7**: 23.
- 60 Legrand E, Demar M, Volney B *et al.* First case of emergence of atovaquone resistance in *Plasmodium falciparum* during second-line atovaquone-proguanil treatment in South America. *Antimicrob Agents Chemother* 2007; **51**: 2280–1.
- 61 Lütgendorf C, Rojanawatsirivet C, Wernsdorfer G *et al.* Pharmacodynamic interaction between atovaquone and other antimalarial compounds against *Plasmodium falciparum* *in vitro*. *Wien Klin Wochenschr* 2006; **118**: 70–6.
- 62 Musset L, Pradines B, Parzy D *et al.* Apparent absence of atovaquone/proguanil resistance in 477 *Plasmodium falciparum* isolates from untreated French travellers. *J Antimicrob Chemother* 2006; **57**: 110–5.
- 63 Savini H, Bogreau H, Bertaux L *et al.* First case of emergence of atovaquone-proguanil resistance in *Plasmodium falciparum* during treatment in a traveler in Comoros. *Antimicrob Agents Chemother* 2008; **52**: 2283–4.
- 64 van Vugt M, Leonardi E, Phaipun L *et al.* Treatment of uncomplicated multidrug-resistant falciparum malaria with artesunate-atovaquone-proguanil. *Clin Infect Dis* 2002; **35**: 1498–504.
- 65 Blossom DB, King CH, Armitage KB. Occult *Plasmodium vivax* infection diagnosed by a polymerase chain reaction-based detection system: a case report. *Am J Trop Med Hyg* 2005; **73**: 188–90.
- 66 Contentin L, Grammatico-Guillon L, Desoubeaux G *et al.* Atovaquone-proguanil treatment failure in *Plasmodium falciparum*. *Presse Med* 2011; **40**: 1081–3.
- 67 David KP, Alifrangis M, Salanti A *et al.* Atovaquone/proguanil resistance in Africa: a case report. *Scand J Infect Dis* 2003; **35**: 897–8.
- 68 Forestier E, Labe A, Raffenot D *et al.* Post-malaria neurological syndrome complicating a relapse of *Plasmodium falciparum* malaria after atovaquone-proguanil treatment. *Med Mal Infect* 2011; **41**: 41–3.
- 69 Koch S, Göbels K, Richter J *et al.* Cerebral malaria in spite of peripheral parasite clearance in a patient treated with atovaquone/proguanil. *Parasitol Res* 2007; **100**: 747–8.
- 70 Kuhn S, Gill MJ, Kain KC. Emergence of atovaquone-proguanil resistance during treatment of *Plasmodium falciparum* malaria acquired by a non-immune north American traveller to west Africa. *Am J Trop Med Hyg* 2005; **72**: 407–9.
- 71 Müller-Stöver I, Verweij JJ, Hoppenheit B *et al.* *Plasmodium malariae* infection in spite of previous anti-malarial medication. *Parasitol Res* 2008; **102**: 547–50.
- 72 Oswald CB, Summer AP, Fischer PR. Relapsing malaria infection in an adolescent following travel to Mozambique. *Travel Med Infect Dis* 2007; **5**: 254–5.
- 73 Perry TL, Pandey P, Grant JM *et al.* Severe atovaquone-resistant *Plasmodium falciparum* malaria in a Canadian traveller returned from the Indian subcontinent. *Open Med* 2009; **3**: e10–6.
- 74 Plucinski MM, Huber CS, Akinyi S *et al.* Novel mutation in cytochrome b of *Plasmodium falciparum* in one of two atovaquone-proguanil treatment failures in travelers returning from same site in Nigeria. *Open Forum Infect Dis* 2014; **1**: ofu059.
- 75 Rose GW, Suh KN, Kain KC *et al.* Atovaquone-proguanil resistance in imported falciparum malaria in a young child. *Pediatr Infect Dis J* 2008; **27**: 567–9.
- 76 Schwartz E, Bujanover S, Kain KC. Genetic confirmation of atovaquone-proguanil-resistant *Plasmodium falciparum* malaria acquired by a non-immune traveler to East Africa. *Clin Infect Dis* 2003; **37**: 450–1.
- 77 Wichmann O, Muehlen M, Gruss H *et al.* Malarone treatment failure not associated with previously described mutations in the cytochrome b gene. *Malar J* 2004; **3**: 14.