Antimicrobial treatment for early, limited *Mycobacterium ulcerans* infection: a randomised controlled trial

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Summary

Background Surgical debridement was the standard treatment for *Mycobacterium ulcerans* infection (Buruli ulcer disease) until WHO issued provisional guidelines in 2004 recommending treatment with antimicrobial drugs (streptomycin and rifampicin) in addition to surgery. These recommendations were based on observational studies and a small pilot study with microbiological endpoints. We investigated the efficacy of two regimens of antimicrobial treatment in early-stage *M. ulcerans* infection.

Methods In this parallel, open-label, randomised trial undertaken in two sites in Ghana, patients were eligible for enrolment if they were aged 5 years or older and had early (duration <6 months), limited (cross-sectional diameter <10 cm), *M. ulcerans* infection confirmed by dry-reagent-based PCR. Eligible patients were randomly assigned to receive intramuscular streptomycin (15 mg/kg once daily) and oral rifampicin (10 mg/kg once daily) for 8 weeks (8-week streptomycin group; n=76) or streptomycin and rifampicin for 4 weeks followed by rifampicin and clarithromycin for 4 weeks (4-week streptomycin plus 4-week clarithromycin group; n=75). Randomisation was done by computer-generated minimisation for study site and type of lesion (ulceration or no ulceration). The randomly assigned allocation was sent from a central site by cell-phone text message to the study coordinator. The primary endpoint was lesion healing at 1 year after the start of treatment without lesion recurrence or extensive surgical debridement. Analysis was by intention-to-treat. This trial is registered with ClinicalTrials.gov, number NCT00321178.

Findings Four patients were lost to follow-up (8-week streptomycin, one; 4-week streptomycin plus 4-week clarithromycin, three). Since these four participants had healed lesions at their last assessment, they were included in the analysis for the primary endpoint. 73 (96%) participants in the 8-week streptomycin group and 68 (91%) in the 4-week streptomycin plus 4-week clarithromycin group had healed lesions at 1 year (odds ratio 2·49, 95% CI 0·66 to infinity; p=0·16, one-sided Fisher’s exact test). No participants had lesion recurrence at 1 year. Three participants had vestibulotoxic events (8-week streptomycin, one; 4-week streptomycin plus 4-week clarithromycin, two). One participant developed an injection abscess and two participants developed an abscess close to the initial lesion, which was incised and drained (all three participants were in the 4-week streptomycin plus 4-week clarithromycin group).

Interpretation Antimycobacterial treatment for *M. ulcerans* infection is effective in early, limited disease. 4 weeks of streptomycin and rifampicin followed by 4 weeks of rifampicin and clarithromycin has similar efficacy to 8 weeks of streptomycin and rifampicin; however, the number of injections of streptomycin can be reduced by switching to oral clarithromycin after 4 weeks.

Funding European Union (EU FP6 2003-INCO-Dev2-015476) and Buruli Ulcer Groningen Foundation.

Introduction

Buruli ulcer is a necrotising infection of subcutaneous tissue caused by *Mycobacterium ulcerans*. The name Buruli ulcer comes from a region near the Nile River delta in Uganda, named Buruli County, where the disease was highly endemic in the 1960s. Today, the disease is emerging in west African countries with thousands of cases every year, mainly in children. A plasmid of *M. ulcerans* encodes the production of mycolactone, an immunomodulatory macrolide toxin that causes tissue necrosis. *M. ulcerans* is acquired near slow-flowing and stagnant water in tropical and subtropical environments. The natural reservoir and mode of transmission of the infection remain largely obscure and might differ between endemic foci around the world. However, skin injury and insect bites have been proposed as modes of transmission.

*M. ulcerans* infection usually starts as a nodule, papule, plaque, or oedema. When left alone, the lesion breaks open and a typical painless ulcer with undermined edges appears, which can progress to a large necrotic lesion. WHO has defined lesions with a cross-sectional diameter of less than 5 cm as category I, 5–15 cm as category II, and more than 15 cm, lesions on important sites (eye, breast, and genitalia), or multiple lesions as category III. *M. ulcerans* infection can be self-limiting, but scar tissue and contractures in joints leave patients...
with functional limitations and can result in social stigma.\textsuperscript{11,12} The diagnosis can be made clinically but culture is the gold standard. However, this method is difficult and has low sensitivity.\textsuperscript{1,3,13} Since the development of PCR targeting insertion sequence 2404 (IS2404)—a repetitive oligonucleotide unit with more than 200 copies in the genome of \textit{M ulcerans}\textsuperscript{14}—diagnostic confirmation has improved substantially.\textsuperscript{13,15,16}

Buruli ulcer is one of 19 neglected tropical diseases addressed by WHO in its \textit{Global plan to combat neglected tropical diseases 2008–2015}.\textsuperscript{5} In this plan, the organisation describes Buruli ulcer as a disease for which there are no cost-effective control methods. Since the disease’s first description in 1948,\textsuperscript{8} different treatments have been investigated. Extensive surgical debridement, with or without subsequent skin grafting, is standard treatment. However, surgery cannot completely remove all bacilli\textsuperscript{8} and recurrence is common, with reported rates varying between 6% and 47%.\textsuperscript{20–22} Although larger excisions might be more effective, they can increase chances of residual

**Methods**

**Participants**

The study design was partly based on discussions within a WHO expert group on Buruli ulcer that took place between 2001, and 2003. Between April, 2006, and January, 2008, patients were recruited at two sites (Nkawie-Toase Government Hospital, Nkawie, and Agogo Presbyterian Hospital, Agogo) in Ghana. Patients clinically diagnosed with \textit{M ulcerans} disease were recruited by active case finding. Patients were eligible for enrolment
if they were aged 5 years or older, had a reported disease duration of less than 6 months, and had lesions with a cross-sectional diameter (indurated area) of 10 cm or less. *M* *ulcerans* infection was confirmed by IS2404 dry-reagent-based PCR.²⁹ Exclusion criteria were pregnancy in female participants aged 10 years or older, and hearing tests in all participants (AS208 portable equipment; Interacoustics, Assens, Denmark) to obtain baseline audiometric data. HIV antibody testing was done with cold-stored sera after completion of the study.

Lesions were photographed and traced onto acetate sheets. Three 3 mm punch biopsy samples were taken under local anaesthesia; two swabs of ulcerated lesions were also taken. All samples were transported to the Kumasi Centre for Collaborative Research in Tropical Medicine laboratory in Kumasi, Ghana, for IS2404 dry-reagent-based PCR and Ziehl-Neelsen staining to detect acid-fast bacilli; mycobacterial culture was done on Löwenstein-Jensen slopes at 32°C.³⁰ One punch biopsy was reserved for histopathological examination.

Participants started streptomycin (15 mg/kg once daily intramuscularly) and rifampicin (10 mg/kg once daily orally) after the diagnostic procedures. After assessments and start of treatment at the hospital, most participants were treated as outpatients. Once a week, participants were given study drugs to take to the nearest health facility to receive directly observed treatment (DOT) for the subsequent days, with daily wound care. Only participants that had extensive oedema or lesions at difficult sites (joints, eye, or genitalia), or lesions with suspected secondary infection were admitted to hospital; participants who could not receive DOT or wound care at home were also admitted to hospital. DOT was recorded on forms by the health-care worker or helper who was observing the treatment. Participants were followed up at weekly intervals during the first 8 weeks. At these visits, clinical assessments and digital photographs were taken, DOT forms were checked, and participants were invited to report any adverse events. Once every 2 weeks, the size of the lesion was traced onto an acetate sheet and blood cell counts were taken; we also undertook liver and kidney function tests and hearing tests in all participants, and pregnancy tests in female participants aged 10 years or older.

### Randomisation and masking

Before the end of week 4, participants with *M* *ulcerans* infection confirmed by PCR were randomly assigned to receive streptomycin intramuscularly and rifampicin orally for 4 more weeks (8-week streptomycin group) or rifampicin and clarithromycin (7·5 mg/kg once daily), both orally, for another 4 weeks (4-week clarithromycin group). Randomisation was done with minimisation for study site and type of lesion (ulceration or no ulceration). The study coordinator (WAN) forwarded the information of every enrolled participant by cell-phone text messaging to a statistician (JPS) at the Department of Epidemiology, University Medical Centre Groningen, Netherlands. There, a computer-generated randomisation program was used, and the randomly assigned allocation was then sent by text message to the study coordinator. Individuals who were clinically diagnosed with *M* *ulcerans* disease but

<table>
<thead>
<tr>
<th>Study site</th>
<th>Group (n=76)</th>
<th>Group (n=75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agogo</td>
<td>27 (36%)</td>
<td>19 (25%)</td>
</tr>
<tr>
<td>Nkawie</td>
<td>54 (71%)</td>
<td>53 (71%)</td>
</tr>
<tr>
<td>Akan</td>
<td>20 (26%)</td>
<td>18 (24%)</td>
</tr>
<tr>
<td>Other</td>
<td>56 (74%)</td>
<td>57 (76%)</td>
</tr>
<tr>
<td>Duration of disease (weeks)</td>
<td>4 (2–6)</td>
<td>3 (2–4)</td>
</tr>
<tr>
<td>Lesion surface area (cm²)</td>
<td>29 (9–55)</td>
<td>26 (10–46)</td>
</tr>
<tr>
<td>Category of lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>29 (38%)</td>
<td>29 (39%)</td>
</tr>
<tr>
<td>II or III</td>
<td>47 (62%)</td>
<td>46 (61%)</td>
</tr>
<tr>
<td>Type of lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ulceration</td>
<td>49 (64%)</td>
<td>43 (57%)</td>
</tr>
<tr>
<td>Ulceration</td>
<td>27 (36%)</td>
<td>32 (43%)</td>
</tr>
<tr>
<td>Lesion distribution (side of body)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>27 (36%)</td>
<td>28 (37%)</td>
</tr>
<tr>
<td>Right</td>
<td>49 (64%)</td>
<td>47 (63%)</td>
</tr>
<tr>
<td>HIV infection</td>
<td>0</td>
<td>3 (4%)</td>
</tr>
</tbody>
</table>

Table 1: Patient baseline characteristics
who did not have confirmation by PCR continued treatment with streptomycin plus rifampicin and were not randomised; these individuals were followed up and analysed separately. This was an open-label trial.

Follow-up and study outcomes
After 8 weeks of antimicrobial treatment, missed doses were not supplemented. Participants were followed up at week 10 and week 12 after start of treatment, and then monthly to week 36, and bimonthly to week 52. Study visits included clinical assessment with reporting of adverse effects, measurement of lesion size (if not healed) by tracing onto an acetate sheet, and photography of the lesion. Participants’ travel costs were reimbursed and small monthly incentives (sugar, condensed milk, and cocoa powder) were offered for time spent in the study.

Treatment failure was recorded if a participant’s lesion had not healed by week 52, lesion recurrence occurred within 1 year, or lesion size increased to 150% or more at any timepoint compared with baseline with surgical debridement undertaken as deemed necessary by the attending doctor in the hospital. *Weeks after start of treatment. †Results for insertion sequence 2404 dry-reagent-based PCR, Ziehl-Neelsen (ZN) staining to identify acid-fast bacilli, and M. ulcerans culture. Patients in the 8-week streptomycin group were assigned to receive intramuscular streptomycin and oral rifampicin for 8 weeks. Patients in the 4-week streptomycin plus 4-week clarithromycin group were assigned to receive streptomycin and rifampicin for 4 weeks followed by rifampicin and clarithromycin, both orally, for 4 weeks.

Table 2: Characteristics of ten participants with treatment failure
but does not affect bacterial load. These interventions were therefore not regarded as evidence of treatment failure.

The primary clinical endpoint was lesion healing (complete re-epithelialisation) at 1 year after the start of treatment without recurrence or extensive surgical debridement. Secondary outcomes were time to wound healing and time to complete wound coverage by a crust. Daily sterile dressings were only applied at the health facility if lesions were open and discharging.

Before final healing occurs, lesions might turn dry with a crust. At this stage, participants could cover the lesions for protection at home, without visiting the health facility to receive wound care and sterile dressings. Since participants reported this stage of wound healing as beneficial, we also measured time to complete wound coverage by a crust without complete re-epithelialisation as a secondary endpoint. The safety outcome measure was occurrence of adverse events.

### Statistical analysis

When the study was designed, there was no information available about healing rates for the proposed regimens; therefore, we assumed a healing rate of 80% in the 8-week streptomycin group. We calculated that a sample size of 148 randomised and fully assessable participants (74 in each group) would be needed to detect a difference in healing rate of 20% or more (<60% in the 4-week streptomycin plus 4-week clarithromycin group) with a one-sided alpha of 0.05 and a power of 80%.

We calculated an odds ratio for the primary clinical endpoint by use of Fisher’s exact test. Because secondary outcome data were interval-censored, we analysed the cumulative incidence of healing by use of actuarial life table analysis and weighted log-rank tests for interval-censored data, in particular the group proportional hazards model and a generalised Wilcoxon-Mann-Whitney test, which emphasises early events. We calculated the exact permutation p value for the scores of the group proportional hazards model and Wilcoxon-Mann-Whitney tests, and the non-parametric maximum likelihood estimate of the survival distribution function. Other secondary outcome measures were assessed by actuarial life table analysis. All analyses were by intention to treat. Statistical analysis was done with SPSS version 16.0, R version 2.9.2, and Stata version 10.1.

### Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, writing

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### Table 3: Actuarial life table for cumulative proportion of healing for both treatment groups, by time interval

<table>
<thead>
<tr>
<th>Week</th>
<th>8-week streptomycin group</th>
<th>4-week streptomycin plus 4-week clarithromycin group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n)</td>
<td>Healed (n)</td>
</tr>
<tr>
<td>Week 1</td>
<td>76</td>
<td>1</td>
</tr>
<tr>
<td>Week 2</td>
<td>75</td>
<td>3</td>
</tr>
<tr>
<td>Week 4</td>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td>Week 5</td>
<td>71</td>
<td>1</td>
</tr>
<tr>
<td>Week 6</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>Week 7</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>Week 8</td>
<td>69</td>
<td>2</td>
</tr>
<tr>
<td>Weeks 8–10</td>
<td>67</td>
<td>1</td>
</tr>
<tr>
<td>Weeks 10–12</td>
<td>66</td>
<td>3</td>
</tr>
<tr>
<td>Weeks 12–16</td>
<td>63</td>
<td>4</td>
</tr>
<tr>
<td>Weeks 16–20</td>
<td>59</td>
<td>12</td>
</tr>
<tr>
<td>Weeks 20–24</td>
<td>46</td>
<td>9</td>
</tr>
<tr>
<td>Weeks 24–28</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Weeks 28–32</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>Weeks 32–36</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Weeks 36–44</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Weeks 44–52</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Patients in the 8-week streptomycin group were assigned to receive intramuscular streptomycin and oral rifampicin for 8 weeks. Patients in the 4-week streptomycin plus 4-week clarithromycin group were assigned to receive streptomycin and rifampicin for 4 weeks followed by rifampicin and clarithromycin, both orally, for 4 weeks.
of the report, or in decisions about submission of results for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. 180 eligible patients started treatment. 26 patients with suspected but unconfirmed *M. ulcerans* infection received streptomycin and rifampicin for 8 weeks. Of 151 participants who were enrolled and randomised, eight had a clinical diagnosis without confirmation of *M. ulcerans* infection by PCR. Five of these eight participants had infection later confirmed by one or more diagnostic tests (Ziehl-Neelsen staining, two; culture, one; histopathology, two). Three randomised participants did not have diagnosis confirmed by any test. Table 1 shows baseline characteristics of study participants. Lesions were more frequently seen on the right side of the body (64%) than on the left side (36%; p<0·0001). Three (2%) participants were HIV positive; these individuals had initial lesions and clinical presentations that were indiscernible from those of HIV-negative participants.

One participant in the 8-week streptomycin group withdrew from the study at week 6. In the 4-week streptomycin plus 4-week clarithromycin group, two participants moved out of the study area and were lost to follow-up (week 32 and 36) and one participant, who later tested positive for HIV infection, died in week 16 of urosepsis. Since these four participants had healed lesions at their last assessment, they were included in the analysis for the primary clinical endpoint and in the analyses for time to healing.

Compliance to study treatment was assessed by use of DOT forms, signed by health personnel at the health facilities. Adherence to treatment protocol was 98% in the 8-week streptomycin group and 99% in the 4-week streptomycin plus 4-week clarithromycin group.

Treatment failure was recorded in ten participants, three in the 8-week streptomycin group and seven in the 4-week streptomycin plus 4-week clarithromycin group. Table 2 shows the characteristics of these individuals. Five participants were not healed at week 52, all of whom had a substantial decrease in lesion size. One participant had several lesions, and four had large lesions at the start of treatment (one of whom had HIV infection). Of the five participants with treatment failure before week 52, two had large lesions, one had a pre-ulcerative lesion that ulcerated later, one had a progressive lesion, and one had a lesion that...
almost healed, but opened up again. No participants with healed lesions had a recurrence at week 52.

73 (96%) participants in the 8-week streptomycin group and 68 (91%) participants in the 4-week streptomycin plus 4-week clarithromycin group had healed lesions at week 52 (odds ratio for failure in healing for 4-week streptomycin plus 4-week clarithromycin vs 8-week streptomycin 2.49, 95% CI 0.66 to infinity, p=0.16). We obtained consistent findings when the four participants who were not followed up to week 52 were excluded from the analysis and when two wound experts masked to treatment assignment assessed the primary endpoint by use of photographs available at the different timepoints (data not shown).

Table 3 shows the actuarial life table for cumulative proportion of healing. The estimated cumulative proportion of patients healed at week 52 was 0.99 (95% CI 0.94–1.00) in the 8-week streptomycin group and 0.96 (95% CI 0.88–0.99) in the 4-week streptomycin plus 4-week clarithromycin group; a difference of 0.034 (95% CI -0.024 to 0.091) between groups. Figure 2 shows the non-parametric maximum likelihood estimates for healing in the intention-to-treat population. Neither the group proportional hazards model (p=0.26; 99% CI 0.22–0.29) nor the generalised Wilcoxon-Mann-Whitney test (p=0.60; 99% CI 0.56–0.64) showed a significant difference in time to healing between groups. The group proportional hazards model suggested a shorter time to healing in the 8-week streptomycin group whereas the Wilcoxon-Mann-Whitney test suggested that time to healing was shorter in the 4-week streptomycin plus 4-week clarithromycin group. Adjustment for study site and type of lesion (ulceration or no ulceration) did not affect the results (data not shown).

Five participants received skin grafts, four in the 8-week streptomycin group (at week 16, 24, 24, and 28), and one in the 4-week streptomycin plus 4-week clarithromycin group (at week 20). Time to healing of category I lesions (median 18 weeks, 95% CI 14–22) was significantly shorter than that for category II and III lesions (30 weeks, 95% CI 26–34, p=0.002; data pooled for the two treatment groups; five participants with skin grafts not included). Time to complete wound coverage by a crust was also significantly shorter for category I lesions than category II and III lesions (14 weeks, 95% CI 11–18, vs 22 weeks, 95% CI 22–26; p=0.002).

Three participants had vestibulotoxic events, one in the 8-week streptomycin group (aged 49 years, starting after 7 weeks of treatment) and two in the 4-week streptomycin plus 4-week clarithromycin group (aged 24 years and 38 years, starting after 4 weeks and 3 weeks of treatment, respectively). Analysis of digital photographs showed that three participants had mild to moderate functional limitations at the end of the study: one had a contracture with substantial decrease in range of movement of the thumb and index finger (4-week streptomycin plus 4-week clarithromycin group); two had ulcers on the back of the hand and wrist that resulted in claw-hands (one in each group). No liver or kidney function test abnormalities or audiological deterioration occurred that necessitated termination of streptomycin treatment. One participant developed an injection abscess (4-week streptomycin plus 4-week clarithromycin group) and two participants (both in the 4-week streptomycin plus 4-week clarithromycin group) developed an abscess close to the initial lesion which was incised and drained. One participant in the 8-week streptomycin group and two participants in the 4-week streptomycin plus 4-week clarithromycin group reported abdominal discomfort.

Some participants had additional diagnostic tests not specified in the protocol. Table 4 shows the characteristics of the five participants in whom M ulcerans was isolated by culture after treatment; all were in the 4-week streptomycin plus 4-week clarithromycin group. Three of these five participants had treatment failure: in two, surgical debridement was done; in the third, multiple nodules ulcerated successively over 52 weeks before final healing. Two participants had lesion healing without further intervention within the study period.

Discussion

Our study has shown that early, limited M ulcerans infection can be safely and effectively managed by antimicrobial treatment alone, without surgical debridement. The drug regimen proposed by WHO,
consisting of 8 weeks of streptomycin and rifampicin, seemed effective and was not associated with deterioration requiring subsequent surgical debridement. Treatment with oral clarithromycin plus rifampicin during the second 4-week period resulted in similar outcomes to continuation of treatment with streptomycin and rifampicin. Our findings are important for patients with \textit{M} \textit{ulcerans} infection who live in remote, resource-poor areas in west Africa, where people often need to walk for several hours to reach health-care facilities, skilled personnel are scarce, and patients tend to refrain from treatment because of fear of surgery. Our results also support the use of antimicrobial treatment in individuals who are unable to receive streptomycin—eg, pregnant women or those who cannot tolerate aminoglycosides. With few reported side-effects, the treatment regimens used in this trial seemed well tolerated, although vestibulotoxicity remains a concern. The rate of lesion recurrence in our study at 52 weeks was lower than that reported in retrospective studies assessing the effect of surgery, in which rates of between 6% and 47% were reported.\textsuperscript{20–22}

Time to healing was a median of 18 weeks for category I lesions and 30 weeks for larger lesions. The length of this healing period might have obscured the potential of antimicrobial treatment in earlier studies that either looked at healing after 2 months,\textsuperscript{23} or assigned participants to surgery when early healing was not seen during follow-up.\textsuperscript{23} HIV was not an important confounder; most case-control studies from west Africa report a low incidence of HIV in patients with \textit{M} \textit{ulcerans} infection.\textsuperscript{34,35}

One strength of this study is the large proportion of participants (147 of 151) who were followed up to week 52. Second, most participants (148 of 151) had laboratory-confirmed \textit{M} \textit{ulcerans} infection, which contrasts with previous trials that were partly undertaken before PCR-based diagnostic confirmation tests were available.\textsuperscript{21,24} Finally, the sample size in our study was substantially larger than that in earlier studies.

A potential weakness of our study is the open-label design. However, masking would have substantially increased costs, and a trial in which children can be assigned to placebo injections is not justified for safety reasons. Moreover, although only one injection abscess was recorded, intramuscular injections in rural Africa are not the preferred option. Another limitation of our study is that no formal external monitoring was done. However, limited auditing was organised. Additionally, consistent results were obtained when two wound experts who were masked to treatment assignment reviewed all digital photographs available at the different timepoints. We therefore believe that the study was robust.

One concern is that healing took a fairly long time. Additionally, we could not address the issue of prevention of disabilities in a formal way, although our analysis of digital photographs combined with clinical assessment showed that only three participants had mild to moderate functional limitations at week 52 (all three involving hand function). Contractures and functional limitations are common in ulcers that are close to joints.\textsuperscript{25} Future studies should assess prevention of disabilities, include all categories of lesions, and investigate oral drug regimens.

Thus, antimicrobial treatment is highly effective for treatment of early, limited \textit{M} \textit{ulcerans} infection, and the number of intramuscular injections of streptomycin can be reduced without compromising efficacy.

\begin{footnotesize}

\textbf{Contributors}

TSvdW and YS designed and supervised the study. WAN coordinated the study. WAN, WAT, PCA, and EOA were responsible for patient screening and enrolment. KMA, WT, and WAN provided patient care and requested informed consent from participants, participants’ parents, or legal representatives, and collected the clinical and laboratory data. GB, VS, NYA-B, and OA were responsible for the laboratory confirmation. JPS, WAN, and YS did the statistical analyses. TSvdW, WAN, and YS contributed to the interpretation of the results and the writing and critical review of the report. All authors have seen and approved the final version of the report.

\textbf{Conflicts of interest}

We declare that we have no conflicts of interest.

\textbf{Acknowledgments}

We thank all study participants and health-care workers involved in the trial. We thank Richard O Phillips for invaluable help during the start of the trial, Carin J Stek for occasional monitoring activities, the “de Sprong” outpatient pharmacy team, University Medical Centre Groningen for drug supply, Marieke M de Waard and Janneke Huizinga for undertaking masked assessment of digital images of the participants’ lesions, William R Faber, Paul D R Johnson, and Alan J Knell for serving on the data safety monitoring board, and Kingsley Asiedu, Global Buruli Ulcer Initiati, WHO, Geneva, Switzerland. This study was supported by the European Union (EU FP6 2003-INCO-Dev2-015476) and Buruli Ulcer Groningen Foundation. YS received a ZonMW AGIKO grant (Netherlands Foundation for Research). WAN is currently at the Department of Internal Medicine, Medical Center Leeuwarden, Leeuwarden, Netherlands. YS is currently at Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands.

\textbf{References}


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