TREATING BURULI ULCER

A REVIEW OF PROSPECTS FOR EXISTING ANTIBIOTICS AND NEW THERAPEUTICS

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1. EXECUTIVE SUMMARY

Buruli ulcer disease is a serious necrotizing skin infection caused by the environmental pathogen *Mycobacterium ulcerans* and represents the third most common mycobacterial infection worldwide after tuberculosis and leprosy. This disease, if left untreated, can lead to disfiguring and disabling lesions, particularly affecting young populations in resource-poor settings. Antibiotic treatment recommended by the World Health Organization (WHO) since 2004 has simplified the delivery of care for Buruli ulcer and provided solid evidence that early and limited lesions can be effectively treated with antibiotics alone. However the current recommendations still present significant challenges including the use of aminoglycosides requiring intramuscular injection. These drugs are difficult and costly to administer in resource-poor settings and have significant side effects that present major obstacles to implementation. Therefore, there is an urgent need to identify alternative oral regimens that can be effective, short-course, all-oral, compatible for use in pediatric population and have few drug-drug interactions with antiretrovirals (ARVs).

The results of a literature review identified several drugs and drug candidates that demonstrated potential efficacy against Buruli ulcer with more attractive administration and toxicity profiles. However, data are fragmented, limiting the rigorous conclusions that can be drawn. Although recent studies indicate that a fully orally-administered treatment regimen such as clarithromycin-rifampicin may be equally effective as regimens containing aminoglycosides, further research is critically needed to identify and evaluate other treatment options. The anti-tuberculosis drug research and development pipeline also represents an important and potentially rich source of novel compounds for Buruli ulcer treatment.

In order to optimize therapy for patients with Buruli ulcer in low-resource settings,

Researchers and pharmaceutical companies should:

1. *Systematically evaluate potential drugs for efficacy against* M. ulcerans*, including drugs developed for other purposes and those that have been abandoned for other indications.*
2. *Support clinical trials to evaluate the most promising candidate drug combinations, especially those that are all-oral, have minimal side effect profiles and offer shorter duration of therapy.*
3. *Ensure that drugs for Buruli ulcer are both available and affordable for those living in resource-poor environments.*

WHO should:

1. *Rapidly evaluate the changing clinical evidence base and create evidence-based guidelines to enable countries and health workers to continually refine the treatment of Buruli ulcer.*
2. *Ensure that components of new and optimal therapies are included in the Essential Medicines List.*

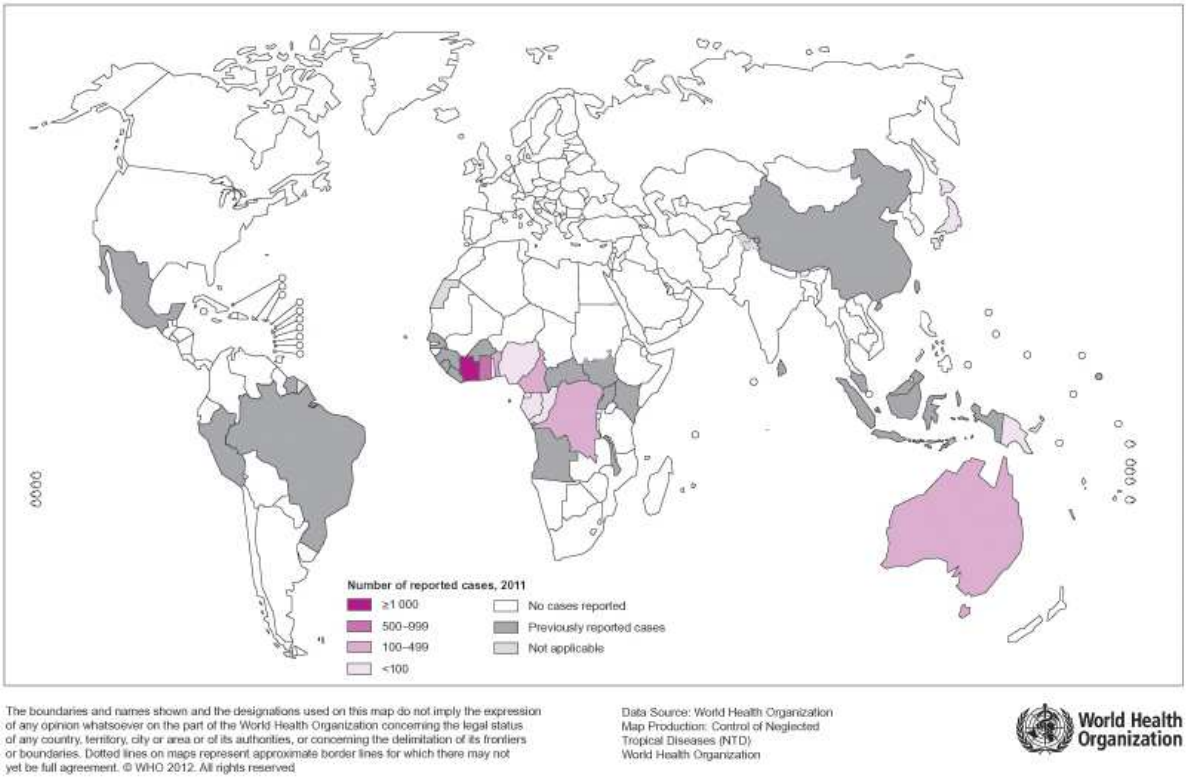
Endemic Countries should:

1. *Invest in educational campaigns to promote early diagnosis along with treatment programs for Buruli ulcer.*
2. *Ensure that components of Buruli ulcer therapy are included in national Essential Medicines Lists.*
3. *Support research efforts to test the efficacy and safety of new drug candidates and combination drug regimens.*

2. INTRODUCTION

Buruli ulcer disease, a cutaneous infection caused by the environmental pathogen *Mycobacterium ulcerans,* is associated with significant morbidity and functional disability worldwide [1-3]. Reported in over 30 countries, mainly in the tropical and subtropical regions of Africa, Latin America, Asia and Western Pacific, it represents the third most common mycobacterial infection after tuberculosis and leprosy (Figure 2.1). It is endemic primarily to rural and remote regions where populations have limited access to medical care [3].

Figure 2.1 – Distribution of Buruli ulcer, worldwide, 2011. Figure courtesy of WHO.



Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization

Buruli ulcer disease largely affects children, with the median age of patients around 15 years. Although it initially starts as a painless nodule in the skin, the disease can progress if untreated to form extensive, ulcerative lesions, often involving the extremities and leading to significant functional limitations [4]. These lesions are classified according to their size and appearance, with implications for their management (Table 2.1) [5].

Until recently the mainstay of treatment of Buruli ulcer disease had been wide surgical excision of the cutaneous lesion(s) [1,2,6]. However, there are significant challenges to the implementation and effectiveness of this approach in the primarily resource-poor settings where Buruli ulcer disease is endemic. Indeed surgery is accessible to only a small fraction of the population at risk for Buruli ulcer. Moreover, surgical management has limited efficacy with recurrence rates ranging from 6 to 17%, in part due to the difficulty of completely eradicating the infection with excision alone [7-11].

Lastly, ulcer involvement can include the face, eyes or other structures, where excision may be challenging or associated with disfiguring or disabling consequences.

Table 2.1 – WHO Classification of Buruli ulcer disease [5].

Category	Description
I	Small, early lesion – e.g. nodule, papule, plaque, ulcer < 5 cm in diameter
II	Nonulcerative or ulcerative plaques and edematous forms Large ulcerative lesions (between 5 and 15 cm in diameter) Lesions in the head and neck
III	Lesions > 15 cm in diameter Disseminated/mixed forms (e.g. osteitis, osteomyelitis, joint involvement)

Early attempts to explore the use of antibiotic treatment in the management of Buruli ulcer led to inconclusive and disappointing results, discouraging the use of chemotherapeutic approaches [12-18]. The apparent lack of efficacy of antibiotics in these earlier studies may have been compromised by the paradoxical development of new lesions or increased size of existing lesions during antibiotic treatment. This phenomenon has recently been recognized as an immune-mediated paradoxical reaction due to mycobacterial killing and is not believed to represent worsening disease or relapse [19-21]. In addition, several *in vitro* and *in vivo* studies have shown that antibiotics do have significant bacteriostatic or bactericidal activity against the causative agent, *M. ulcerans* [14,15,22-36]. The combination of rifampicin with an aminoglycoside, such as streptomycin or amikacin, consistently emerged as the most effective against *M. ulcerans* infection in murine models [24,37,38].

Based on the available data, the 6th World Health Organization (WHO) Advisory Committee on *M. ulcerans* disease in 2004 recommended for the first time an antimicrobial regimen for Buruli ulcer treatment, comprised of 8 weeks of rifampicin and streptomycin [5]. While subsequent studies have demonstrated the clinical efficacy of this regimen in treating early stages of disease, larger lesions have continued to require surgical excision [39-41]. There are additional limitations to the current treatment recommendations, including the challenges of intramuscular drug administration in endemic areas and the potential toxicities associated with aminoglycosides. Thus, despite recent advances in the management of Buruli ulcer disease, the identification of alternative antimicrobials or novel agents with simplified delivery of care remains a high priority. Ideally, the treatment regimen for Buruli ulcer should be fully orally administered, appropriate for pediatric or pregnant patients, and well-tolerated with minimal side effects. Further characteristics of the ideal and acceptable end-user requirements for Buruli ulcer treatment are detailed in Table 2.2.

With these goals in mind, we sought to summarize the available information about potential chemotherapeutic agents for Buruli ulcer as well as propose a research agenda aimed at identifying improved treatment regimens for Buruli Ulcer.

Table 2.2 –Proposed ideal and acceptable end-user requirements for Buruli ulcer chemotherapy.

Profile	Ideal	Acceptable
Target population	Acceptable for special populations: ▪ Pregnant and lactating women, ▪ Pediatric patients, ▪ Immunocompromised patients	Acceptable for special populations: ▪ Pediatric patients, ▪ Immunocompromised patients
Efficacy*	▪ For lesions with cross-sectional diameter of ≤10 cm: Relapse-free cure rate without excision surgery >95% ▪ For lesions with cross-sectional diameter of >10 cm: Reduction in need for and extent of surgical interventions. Relapse-free cure rate >95%	▪ For lesions with cross-sectional diameter of ≤10 cm: Relapse-free cure rate without excision surgery >90% ▪ For lesions with cross-sectional diameter of >10 cm: Reduction in need for and extent of surgical interventions. Relapse-free cure rate >90%
Drug-drug interaction	▪ No drug-drug interactions with ARVs (e.g. no interaction in addition to existing interactions between rifampicin and ARVs)	▪ Some drug-drug interactions, but can be used with dose adjustments
Formulation	▪ Oral administration ▪ Small-sized or dispersible tablets (for pediatric use) ▪ Different fixed dosages (i.e. 25mg/50mg/100mg) ▪ FDC or blister-packs	▪ Oral administration ▪ Small-sized or dispersible tablets (for pediatric use) ▪ Possible scoring for weight-based dosing (i.e. double-scored 100mg tablet)
Dosing frequency and duration	▪ Once daily ▪ Duration <8 weeks	▪ Once daily ▪ Duration ≤ 8 weeks
Safety/Tolerability	▪ Well-tolerated (minimal GI distress). ▪ Minimal dosing instructions / specifications (e.g. take with food, take on empty stomach, etc…) ▪ No laboratory monitoring	▪ Well-tolerated (minimal GI distress). ▪ No laboratory monitoring
Stability	▪ No cold chain requirement ▪ Minimum 4-year shelf life at room	▪ No cold chain requirement ▪ Minimum 2-year shelf life at room

	temperature in tropical settings	temperature in tropical settings
Price	▪ Half the current price of antimicrobial treatment (including syringes) or less: < US$50 for an adult patient	▪ Equal to or less than the current price of antimicrobial treatment (including syringes): < US$100 for an adult patient

Abbreviations: GI, gastrointestinal; FDC, fixed dose combination; ART, antiretroviral therapy; US$, U.S. dollars
* Rigorous evaluation of treatment efficacy is currently challenging due to lack of reliable biomarkers for treatment response. Thus, efficacy has been described using the same criteria applied in recently completed and ongoing clinical trials on Buruli ulcer treatment.

3. METHODOLOGY

We searched the following databases using the search terms described in Appendix Table A.1: PubMed, EMBASE, Scopus, WHO Global Health Library, CAB Abstracts and Cochrane Library. As a complementary strategy, individual searches were performed to identify articles testing the activity against *Mycobacterium ulcerans* of specific drugs with known activity against other mycobacterial species. Using the ClinicalTrials.gov registry, relevant clinical trials focusing on Buruli ulcer disease management were identified. We also screened abstracts submitted to relevant international conferences and meeting proceedings. While there were no restrictions on publication date or type, only articles in English, French and Italian were included for review. Articles published or available as of December 31, 2012 were accessed. Both clinical and laboratory studies were reviewed, including randomized clinical trials, observational studies, case reports or series, as well as experimental studies of *in vitro* and *in vivo* activity against *Mycobacterium ulcerans*. Articles were excluded that did not establish microbiological diagnosis of *M. ulcerans* as the causative agent or specifically focus on antimicrobial interventions. References of all included articles were searched for additional studies. Data were extracted by two reviewers (M.C. and C.G.).

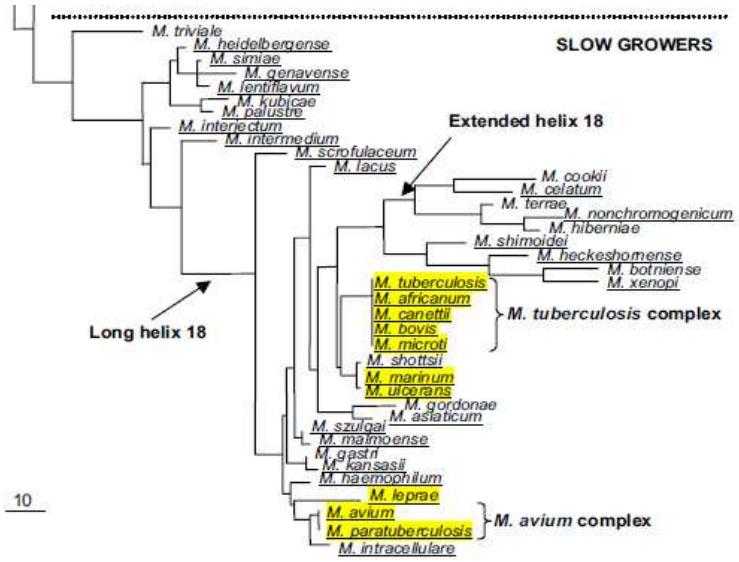
4. IDENTIFICATION OF POTENTIAL ANTIBIOTICS FOR BURULI ULCER

Due to the similarity of genomic sequences among members belonging to the same genus, there is a high probability that molecular targets of different drugs are conserved, such that the same drugs can be active against different members of the same genus. *Mycobacterium ulcerans* has been shown to be closely related taxonomically to *M. tuberculosis* and other mycobacterial species (Figure 4.1) [42], suggesting that drug molecular targets may be conserved among these mycobacterial species.

All drugs with reported anti-mycobacterial activity, including those not routinely used in the clinical management of mycobacterial infections, are listed in the Appendix (see Appendix Table A.2). In addition, new drug candidates in clinical development as anti-tuberculosis drugs are included

We explored the potential applicability of drugs currently used in the treatment of various mycobacterial infections in treating Buruli ulcer disease. All available information on the *in vitro*, *in vivo* and clinical applications of these existing and new drugs in treating *M. ulcerans* infection are reviewed in detail in the following section.

Figure 4.1 Phylogenetic tree of slow growers members of the genus *Mycobacterium*. Strict consensus of the 230 most parsimonious trees using Paup 4.0b10 (heuristic search, gaps = fifth state) from the 1286 aligned nucleotides of the 16S rRNA DNA sequence. Sequenced genomes are highlighted in yellow. Underlined species are considered pathogens. The members of the *M. tuberculosis* complex and the *M. avium* complex are indicated. The divisions between the normal helix 18, long helix 18 and extended helix 18 of the 16S rRNA gene sequence are indicated (Figure courtesy of van Pittius, Sampson, Lee, Kim, van Helden & Warren; Van Pittius et al, BMC Evolutionary Biology 2006, 6:95)



5. PROSPECTS FOR BURULI ULCER TREATMENT

Based on the scientific work carried out so far, we have classified these antimicrobial agents into four categories:

- Antibiotics with clinical application for the treatment of Buruli ulcer
- Antibiotics suitable for or that could be suitable for the treatment of Buruli ulcer but require further investigation
- Antibiotics of unclear value due to lack of sufficient data
- Antibiotics that are not suitable for the treatment of Buruli ulcer

5.1 Antibiotics with clinical application for treatment of Buruli ulcer

5.1.1 Clinical application of combination antibiotic regimens

Data on the clinical activity of available drugs against *M. ulcerans* are presented in Table A.3 of the Appendix. Since the WHO formally published its recommendations for combination antibiotic therapy with rifampicin and streptomycin in managing Buruli ulcer disease, several studies have demonstrated the effectiveness of this regimen. A pilot study in Ghana sponsored by the WHO established that rifampicin-streptomycin was active against Buruli ulcer disease in patients [39]. This clinical trial demonstrated that, after daily treatment with rifampicin-streptomycin for at least 4 weeks, *M. ulcerans* could no longer be cultured from the lesions. Antibiotic treatment also reduced the surface area of most lesions by greater than 50%, thus allowing for less extensive surgical excision. Chauty and colleagues presented data on a cohort of patients in Benin who were treated

for Buruli ulcer disease [41]. Of the 215 patients with healed lesions after 1 year of follow-up, nearly half (47%) had received rifampicin-streptomycin alone for 8 weeks without the need for surgery. Additional studies have confirmed the clinical effectiveness of rifampicin-streptomycin alone and in combination with surgery for Buruli ulcer treatment [43-48].

Alternative antibiotic regimens also have been used clinically with reported success in treating Buruli ulcer disease. However, further systematic investigation is needed to establish these regimens as equivalent or possibly superior to the current standard of care. Several case reports have documented the successful replacement of the aminoglycoside with a macrolide clarithromycin in combination with rifampicin for Buruli ulcer treatment. Dossou *et al* [49] reported the successful treatment of Buruli ulcer in a pregnant woman with rifampicin and clarithromycin for 8 weeks, and Gordon *et al* [50] treated two pediatric patients with individualized durations of rifampicin and clarithromycin syrup. Subsequent clinical studies have been undertaken to systematically demonstrate the efficacy of clarithromycin in treating Buruli ulcer disease. In the BURULICO trial conducted by Nienhuis and colleagues [40], patients were randomized to receive either rifampicin-streptomycin for 8 weeks or rifampicin-streptomycin for 4 weeks followed by rifampicin-clarithromycin for the remaining 4 weeks. Participants were followed for one year, during which 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, a non-statistically significant difference. No participants with healed lesions had disease recurrence during the study period. Based on these data, the authors proposed that 4 weeks of streptomycin and rifampicin followed by 4 weeks of rifampicin and clarithromycin had similar efficacy to 8 weeks of streptomycin and rifampicin.

Chauty *et al* conducted a pilot study in which 30 patients in Benin were treated with rifampicin-clarithromycin for 8 weeks, all of whom had healing within 12 months of treatment initiation without any observed relapses in that 1 year of follow-up [51]. While 15 patients in that study required surgery, 8 of 10 patients with category I ulcerative lesions and 5 of 11 patients with larger lesions were cured with chemotherapy alone. Currently, a WHO-sponsored, randomized multi-center trial is being rolled out to directly compare 8 weeks of rifampicin and clarithromycin to 8 weeks of rifampicin and streptomycin in Buruli ulcer treatment [52].

Recently, O'Brien *et al* presented data on 133 patients with Buruli ulcer disease treated between 1998 and 2010 and found no difference in treatment success rates for antibiotic combinations containing fluoroquinolones compared with those not containing a fluoroquinolone[53]. The most common initial antibiotic regimens were rifampicin and ciprofloxacin (61%) and rifampicin and clarithromycin (23%). Despite several published reports of the use of fluoroquinolones in the treatment of Buruli ulcer disease, only a small pilot study of 30 patients has been conducted to study the use of fluoroquinolones [54]. In this study, patients were treated with rifampicin-streptomycin daily, rifampicin-streptomycin on alternate days, or rifampicin-moxifloxacin daily. This study showed encouraging results for the use of fluoroquinolones. Daily rifampicin-streptomycin treatment was more rapidly effective, with 9 out of 17 patients treated with this combination healed at 4 weeks compared to 1 out of 6 patients treated with rifampicin-moxifloxacin. However, after 8 weeks of treatment, comparable percentages of patients had healed lesions in the groups treated with rifampicin-streptomycin (20 of 24 patients, 2 lost to follow-up) and rifampicin-moxifloxacin (5 of 6 patients). Furthermore, a retrospective review of Buruli ulcer disease management in southeastern Australia between 1998 and 2004 demonstrated that the addition of antibiotic therapy to surgical management significantly improved treatment success rates [55]. Of the various antibiotic regimens

used, the combination rifampicin-ciprofloxacin had 100% treatment success and tolerability, with no patients prematurely stopping treatment.

Despite the documented *in vitro* efficacy of amikacin against *M. ulcerans*, clinical studies have offered mixed results regarding its effectiveness. A randomized controlled trial of 50 patients in Côte d'Ivoire with Buruli ulcer disease was performed to evaluate the efficacy of combination rifampicin-amikacin along with enoxaparin (a low molecular weight heparin product) for 3 months versus wound care alone [56]. In this study, 20 (80%) patients treated with antibiotics did not require surgery, compared to 0 of 25 patients treated with local wound care. However, clinical data published by O'Brien *et al* [55] demonstrated that the use of amikacin was associated with reduced treatment effectiveness and significant toxicity, particularly for elderly patients.

5.1.2 Laboratory-based data on individual antibiotics

The dual antibiotic regimens pursued in the clinical studies described above have been motivated by *in vitro* and *in vivo* data establishing the anti-mycobacterial activity of the individual agents as well as in combination.

Rifampicin

The *in vitro* and *in vivo* activity of rifampicin against *M. ulcerans* has been extensively documented, and several independent publications from different laboratories consistently report a high bactericidal activity of rifampicin in animal models of Buruli ulcer disease [24,25,33,34,36,57]. When administered as monotherapy for 8 weeks, rifampicin is able to clear the footpads of *M. ulcerans*-infected mice, indicating strong bactericidal activity *in vivo* [33,34]. Common adverse effects of rifampicin, such as nausea, seldom result in its discontinuation. However, there are treatment-limiting adverse effects including drug-induced hepatitis or a "flu-like" syndrome [58]. Rifamycins are also associated with significant drug-drug interactions as a result of their ability to induce metabolic enzymes, particularly cytochrome P450 (CYP) 3A, in the gut and liver. Drug-drug interactions between rifampicin and antiretroviral drugs [59], however, significantly complicates the treatment of Buruli ulcer disease in patients co-infected with HIV.

Fluoroquinolones

M. ulcerans has been shown to be susceptible *in vitro* to fluoroquinolones [27,28,34,57]. Several fluoroquinolones, including levofloxacin, moxifloxacin, ofloxacin and sitafloxacin, have been tested in the animal footpad model for activity against Buruli ulcer disease. Levofloxacin and moxifloxacin demonstrated bacteriostatic activity in the mouse footpad preventive model [23]. When moxifloxacin was tested in the mouse footpad curative model, the proportion of mice with culture-positive footpads among those that received moxifloxacin as monotherapy was significantly greater than among mice administered rifampicin, streptomycin or amikacin as monotherapy [33,34]. These data indicate that moxifloxacin is less bactericidal than rifampicin or the aminoglycosides.

The activity of ofloxacin and sitafloxacin, either as monotherapy or in combination with rifampicin, in the mouse footpad model has also been investigated [29]. Despite the small number of mice in the studies limiting the statistical interpretation, the results suggested that sitafloxacin has strong bactericidal activity even at low doses. When used as monotherapy, a low dose of sitafloxacin (with administration starting 4 weeks after infection) was sufficient to obtain culture-negative footpads in

all treated mice after 4 weeks of treatment. Footpads of mice remained culture-negative at 2 weeks of post-treatment follow-up. In order to obtain comparable results with ofloxacin treatment, a four-fold higher dose of ofloxacin had to be administered to mice. Thus, these preliminary results indicated that sitafloxacin may have great potential as an effective drug for Buruli ulcer disease. However, an important caveat is that sitafloxacin has been shown to have significant phototoxicity in Caucasian populations, which may limit its clinical applicability [60].

Macrolides

Clarithromycin and azithromycin are macrolides commonly used in the treatment of *Mycobacterium avium* complex infections [61]. *M. ulcerans* is susceptible to clarithromycin *in vitro*, with MICs on isolates and strains ranging from 0.125 to 2 mg/ml at pH 6.6 and from <0.125 to 0.5 mg/ml at pH 7.4 [25,35,62]. When tested in the mouse footpad preventive model, clarithromycin showed weak bactericidal activity. In mice infected on day 1 and started on clarithromycin treatment on day 7, footpad swelling was observed on average at week 10. In contrast, footpad swelling started at week 12 in mice treated with rifampicin and week 15 in mice treated with an aminoglycoside [23,25]. Bentoucha and collaborators[23] compared the activities of clarithromycin, azithromycin, moxifloxacin and levofloxacin using the mouse footpad preventive model. In the control group of untreated mice, swelling of 50% of footpads occurred by 6 weeks, while swelling of 50% of the infected mice footpads occurred by 7 weeks in mice treated with daily levofloxacin (200mg/kg), by 8 weeks in mice treated with daily azithromycin (100 mg/kg) and those treated with daily moxifloxacin (100mg/kg), and by 11 weeks in mice treated with daily clarithromycin (100 mg/kg). In comparison, mice treated with rifampicin, amikacin and streptomycin developed swelling of 50% of mice footpads by 13, 17 and 25 weeks, respectively. These data suggest that: i) clarithromycin has stronger anti-mycobacterial activity than the fluoroquinolones; and ii) levofloxacin activity is slightly weaker than that of moxifloxacin. Among the macrolides, azithromycin exhibited weaker, bacteriostatic activity against *M. ulcerans* compared to clarithromycin but its activity was comparable to that of moxifloxacin. In a study published by Ji and colleagues [33], clarithromycin activity was tested in the mouse footpad curative model and compared with the activity of rifampicin, streptomycin and moxifloxacin. While data generated in the mouse preventive model suggest that clarithromycin has stronger activity compared to moxifloxacin, data in the mouse curative model suggest that these two drugs have comparable activity. Indeed, after 4 weeks of treatment the mean colony forming unit (CFU) counts of mice treated with clarithromycin alone was similar to that of mice treated with moxifloxacin monotherapy. In addition, the reduction in CFU counts in these two treatment groups was significantly smaller than that seen in mice treated with rifampicin alone.

In considering clarithromycin as a possible option for Buruli ulcer chemotherapy, it is important to investigate possible drug-drug interactions that can occur when clarithromycin is used in combination with rifamycins. Indeed, clarithromycin is metabolized by the CYP3A enzyme pathway, and rifampicin (and to a lesser extent rifabutin) has been showed to induce clarithromycin metabolism, leading to reduced serum levels [63,64]. Alffenaar *et al* [65] studied the pharmacokinetics of rifampicin and clarithromycin in patients enrolled in the BURULICO trial and found that co-administration of these two drugs resulted in higher levels of rifampicin exposure compared to the rifampicin-streptomycin group and lower levels of clarithromycin exposure. Of note, low-dose clarithromycin was administered in the BURULICO trial (7.5 mg/kg/day), suggesting that higher doses of clarithromycin (7.5 mg/kg twice daily) could be more efficacious.

5.1.3 Laboratory-based data on combination antibiotic regimens

The combination of rifampicin with either streptomycin or amikacin demonstrated strong bactericidal activity in animal models, with 100% cure rates after 8 weeks of treatment. In addition, the rifampicin-moxifloxacin regimen had comparable results to the rifampicin-aminoglycoside combinations [33,34]. No relapses were observed during 28 weeks of post-treatment follow-up in mice that received 8 weeks of rifampicin-streptomycin in two studies conducted in 2007 and 2008. In comparison, mice treated with 8 weeks of rifampicin-moxifloxacin had no relapses or 1 relapse in 20 mice (5% relapse rate) during the 28-week follow-up period [32,33].

Clarithromycin has also been tested in conjunction with rifampicin with conflicting results regarding its comparable activity to a rifampicin-aminoglycoside regimen. One found that the combination rifampicin-clarithromycin-sparfloxacin was less bactericidal than rifampicin-amikacin; but of note, the triple combination regimen had greater bactericidal activity than rifampicin monotherapy [24]. Subsequently two studies in mouse footpad curative models assessed the bactericidal activity and the relapse rate after treatment completion of the rifampicin-clarithromycin and rifampicin-moxifloxacin regimens compared to the rifampicin-streptomycin regimen [32,33]. Both studies demonstrated that the macrolide-containing regimen had similar activity to that of rifampicin-streptomycin. In addition these studies showed that, although the rifampicin-clarithromycin regimen was slightly more active than the rifampicin-moxifloxacin regimen after 4 weeks of treatment, the two oral regimens had equivalent activity at treatment completion (after 8 weeks). More recently, a study in the mouse curative model compared rifampicin-clarithromycin and rifapentine-clarithromycin regimens and demonstrated that the rifapentine-containing regimen is more active [22]. Taken together, these data support a role for clarithromycin in Buruli ulcer treatment and also indicate that moxifloxacin may be a valuable alternative to clarithromycin for an all-oral regimen.

Moxifloxacin-clarithromycin, a non-rifampicin based regimen, has also been studied in the laboratory and shown to have limited activity. While 8 weeks of rifampicin-streptomycin successfully sterilized all mice, the combination moxifloxacin-clarithromycin, despite demonstrating bactericidal activity, failed to render all inoculated footpads culture-negative after 8 weeks of treatment. Furthermore, 59% of the mice treated with the oral regimen relapsed during the 28 weeks of post-treatment follow-up [32].

5.2 Antibiotics that are suitable for or could be suitable for treatment of Buruli ulcer

There are several other classes of antimicrobials that are attractive candidates for treatment of Buruli ulcer disease. Other drugs with features – such as oral administration and good safety profile – that make them attractive for Buruli ulcer treatment include other rifamycins, dapsone, epiroprim and bedaquiline, one of the newest anti-tuberculosis drugs in development.

5.2.1 Other rifamycins (rifapentine, rifabutin)

Rifapentine

Rifapentine is an analogue of rifampicin that is characterized by a longer half-life (Table 5.2) and lower MIC for *M. tuberculosis* than rifampicin [58]. The increased bactericidal activity of a rifapentine-based regimen for TB can be explained largely by increased rifamycin exposure due to the long half-life of rifapentine [66]. In clinical trials testing once weekly rifapentine, the drug has

been well tolerated and the rate of adverse reactions has been reported to be similar or lower than with twice weekly rifampicin [67,68].

A possible role for rifapentine in the treatment of Buruli ulcer disease has been explored. In mouse curative footpad models, treatment with rifapentine (administered either 5 days a week or twice a week) and rifampicin had similar levels of bactericidal activity [32]. In addition, Almeida and colleagues [22] demonstrated that rifapentine monotherapy had similar bactericidal activity to rifampicin-streptomycin.

Rifapentine has also been studied in combination treatment regimens in animal models. In mouse curative footpad models, the levels of bactericidal activity for the groups receiving twice weekly rifapentine in combination with either streptomycin or moxifloxacin did not differ significantly from the comparison groups receiving combination therapy with rifampicin 5 days a week [32]. Based on these data, the authors suggest that Buruli ulcer might be treated with twice weekly rifapentine in place of daily rifampicin, thereby simplifying the treatment regimen. In addition, moxifloxacin may be preferred over streptomycin in combination with rifapentine because the longer half-life of the fluoroquinolone may make it more effective in preventing the selection of rifamycin-resistant strains.

Of note, in the mouse model, combining clarithromycin with either rifampicin or rifapentine was associated with shorter median time to footpad swelling than monotherapy with both rifamycins. This was thought to be the result of reduced rifamycin absorption due to the higher doses of clarithromycin required in mice; separating the administration of the two antibiotics restored rifamycin absorption [22]. This is not anticipated to be of consequence clinically.

Rifabutin

Studies in the mouse footpad preventive model showed that the bactericidal activity of rifabutin against *M. ulcerans* is comparable to that of rifampicin [25]. As shown in Table 4.2, among the rifamycins, rifabutin has the lowest potential to induce CYP3A enzymes. Rifabutin is considered as a possible alternative to rifampicin in the treatment of mycobacterial infections in patients co-infected with HIV who receive boosted protease inhibitors [69].

Table 5.2 –Comparison of the interactions of rifamycins with cytochrome P450 (CYP) 3A. Figure courtesy of Burman, Gallicano, & Pelloquin, 2001 [58]

Interaction	Rifampicin	Rifapentine	Rifabutin
Major metabolic pathway	Deacetylation; hydrolysis to formyl derivatives	Deacetylation; hydrolysis to formyl derivatives	CYP3A-mediated hydroxylation; deacetylation
Serum half-life (h)	2-5	14-18	32-67
Effect on CYP3A	Pronounced	Moderate	Weak
Auto-induction of metabolism	Yes	No	Yes
Example of CYP3A induction: effect on indinavir AUC	92% decrease	70% decrease	34% decrease
Change in AUC when given with a CYP3A inhibitor [a]	No effect	No effect	293% increase

[a]From interaction studies with ritonavir (rifampicin and rifabutin) and indinavir (rifapentine).
Abbreviations: AUC, area under the concentration-time curve

5.2.2 Dapsone

Dapsone has been shown to have *in vitro* MIC values on clinical isolates and strains between 2.0 and 4.0 mg/ml against *M. ulcerans*. The activity of dapsone in a mouse footpad preventive model was first studied in 1965 by Pattyn and Royackers [70]. Mice footpads were inoculated with *M. ulcerans* (day 0) and treated for 48 days starting from day 1. Both dapsone and streptomycin were able to prevent footpad lesions. However, while all mice (n=8) treated with streptomycin were still disease-free after 36 days of post-treatment follow-up, all those treated with dapsone (n=8) developed lesions in the follow-up period.

In 2002, a randomized, placebo-controlled pilot study of 30 patients was conducted to assess the efficacy of 8 weeks of rifampicin-dapsone in the treatment of Buruli ulcer disease [18]. Although the experimental group had a median decrease in ulcer size of 14 cm^2 compared to 2.5cm^2 in the control group, there were no significant differences in treatment outcomes as measured by clinician evaluation of the lesions by photographs. It should be noted that this study had a very short follow-up period of 2 months, significantly limiting evaluation and interpretation of the efficacy of the antimicrobial regimen.

5.2.3 Epiroprim (Hoffmann-La Roche Ltd)

M. ulcerans is susceptible to epiroprim *in vitro*, with MIC values ranging between 0.5 and 1.0 mg/ml. Epiroprim was shown to be the most potent among the dihydrofolate reductase inhibitors studied. Of note, synergistic activity was seen when epiroprim was used in combination with dapsone [26]. Epiroprim has been shown to have *in vivo* activity against *M. leprae* [71], but there are no data publicly available examining the drug's *in vivo* activity against *M. ulcerans*. This drug was originally developed by Hoffmann-La Roche Ltd (Basel, Switzerland) but it is not clear whether its development has been discontinued [72]. However, there has recently been renewed interest in the anti-mycobacterial

activity of dihydrofolate reductase inhibitors [73], and compounds belonging to this class may have a role in Buruli ulcer treatment.

5.2.4 Diarylquinoline TMC207 (Johnson & Johnson)

TMC207 (bedaquiline) is a member of a new class of anti-mycobacterial agents called diarylquinolines that act by inhibiting mycobacterial ATP synthase and leading to ATP depletion and pH imbalance [74]. This drug has a broad spectrum of activity against several mycobacteria, including *M. tuberculosis*, *M. bovis*, *M. avium*, *M. kansasii*, *M. smegmatis* and *M. ulcerans* [75].

TMC207 has potent bactericidal activity in the established murine TB infection model. Pharmacokinetic and pharmacodynamic studies in mice demonstrated a long half-life in plasma and tissue and high tissue penetration. These are all attributes that are valuable for the treatment of chronic infections and also may be important for the development of simplified dosing regimens [75]. In phase II trials, TMC207 was shown to be safe and well-tolerated [76]. Of note, TMC207 was recently approved by the United States' Food and Drug Administration (FDA) for the treatment of multi-drug resistant TB (MDR-TB) and is undergoing review by the European Medicines Agency (EMA) [77].

Furthermore, *in vitro* studies have showed that TMC207 has remarkable activity against *M. ulcerans* (MIC_{50}=0.03 mg/ml) [34]. In experiments using the mouse footpad curative model, TMC207 monotherapy demonstrated bactericidal activity comparable to that of moxifloxacin but lower than the bactericidal activity of rifampicin or the aminoglycosides [34]. When combination regimens were tested, treatment with rifampicin-TMC207 had comparable results to rifampicin-streptomycin with respect to the mean number of CFU per footpad and the proportions of mice with culture-positive footpads at 4 and 8 weeks of treatment. However, because the mean CFU and the proportion of culture-positive footpads among the mice administered rifampicin-TMC207 did not differ significantly from the results among mice treated with rifampicin monotherapy, Ji and collaborators concluded that the bactericidal activity of the rifampicin-TMC207 combination was mainly attributable to rifampicin. Nevertheless the use of drugs in combination would likely be essential in order to prevent selection of rifamycin resistance. The *in vitro* activity of TMC207 suggests that it should be further explored in combination possibly with other antibiotics in the treatment of Buruli ulcer disease. Indeed, drug interaction studies in patients showed that administration of rifampicin in combination with TMC207 causes a 50% decrease in TMC207 serum level, which could easily interfere with the efficacy of the rifampicin-TMC207 combination [78]. Thus, there may be other antibiotics that could demonstrate potentially greater anti-mycobacterial activity when co-administered with TMC207 than the rifampicin-aminoglycoside regimen currently recommended.

5.3 Antibiotics of unclear value due to lack of sufficient amount of data

Of the remaining antibiotics with anti-mycobacterial activity, two oral drugs – amoxicillin-clavulanate and co-trimoxazole – are potentially viable options for treatment of Buruli ulcer disease but data are lacking regarding their effectiveness against *M. ulcerans*. In addition, many of the new anti-tuberculosis drugs coming down the drug development pipeline have limited or no data available regarding their complete spectrums of anti-mycobacterial activity.

5.3.1 Amoxicillin-clavulanate and Co-trimoxazole

Amoxicillin-clavulanate is well tolerated, safe for pediatric patients and pregnant women, and inexpensive. Furthermore, it has been shown to have *in vitro* activity against various mycobacterial species including *M. tuberculosis, M. kansasii and M. marinum* [79,80]. However, there is no data on whether amoxicillin-clavulanate has *in vitro* activity against *M. ulcerans*. A potential caveat is that a case report has been published previously on the lack of improvement in Buruli ulcer disease in one patient treated with amoxicillin-clavulanate [45]. Co-trimoxazole, on the other hand, has been shown to have *in vitro* activity against *M. ulcerans*, but did not appear to have any benefit in treating Buruli ulcer disease in a small clinical study [17]. Further investigation into the potential clinical utility of co-trimoxazole is warranted given that this previous study was small, with only 12 patients who had significant variation in disease presentation. Co-trimoxazole is attractive as an oral agent that is well tolerated and can be used safely in pediatric patients.

5.3.2 Nitro-dihydro-imidazooxazole OPC-67683 (Otsuka Pharmaceuticals, Japan)

OPC-67683 (delamanid) is a new nitroimidazole derivative currently in development. Proposed mechanisms of action for the nitroimidazoles include the inhibition of essential mycobacterial cell wall components as well as intracellular nitric oxide release. Phase I and Phase IIa clinical trials demonstrated that this compound has a good safety and tolerability profile. Possible drug interactions with rifampicin and pyrazinamide have emerged and need to be further clarified. OPC-67683 was found to be active also against *M. kansasii* [81], suggesting that it can be active against other members of the genus Mycobacterium, including *M. ulcerans*. Although OPC-67683 is closely related to PA-824 – another nitroimidazole that does not have *in vitro* or *in vivo* activity against *M. ulcerans* [34] – the two chemical entities do not belong to the same subclass of molecules, suggesting that it might be worth independently investigating whether OPC-67682 is active against *M. ulcerans*.

5.3.3 Oxazolidinones: PNU-100480 (Pfizer) and AZD-5847 (AstraZeneca)

New drugs have been developed in the class of oxazolidinones, which act as protein synthesis inhibitors and boasts linezolid as a member. Linezolid has demonstrated activity against *M. ulcerans in vitro* and *in vivo*; in addition, the bactericidal activity of the rifampicin-linezolid regimen was shown to be similar to that of the rifampicin-moxifloxacin and rifampicin-TMC207 regimens in the mouse curative model [34]. Laboratory-based studies suggest that the new oxazolidinones may be as active as linezolid against *M. tuberculosis* but with fewer associated toxicities [82-84]. An initial clinical study aimed at evaluating the safety, tolerability and pharmacokinetic profile of PNU-10048 administered at different dosages (100, 300, 600 mg twice a day or 1200 mg once a day) found that all doses were safe and well-tolerated [85]. Data is pending from a recently completed, phase II study testing the early bactericidal activity (EBA) of 600 mg and 1200 mg daily doses for 14 days [86].

The AZD-5847 compound developed by AstraZeneca has a MIC similar to that of linezolid and PNU-10048 and has been showed to be active against *M. tuberculosis* both *in vitro* and in animal models [84]. AZD-5847 is currently undergoing phase II studies [87]. It is postulated that antimicrobial resistance to the oxazolidinones may be less frequent due to the need for mutations in the 23S ribosomal RNA subunit [84].

5.3.4 Benzothiazinone BTZ043 (NM4TB/AstraZeneca)

BTZ043 belongs to a new class of antimycobacterial agents called benzothiazinones whose major target is an enzyme (decaprenylphosphoryl-b-D-ribose 2' epimarase) involved in the synthesis of the cell wall component arabinans [88]. BTZ043 has been tested and shown to have *in vitro* activity against *M. tuberculosis* (including MDR- and extremely drug resistant-TB clinical isolates), *M. smegmatis* and *M. bovis* [88,89]. The drug's bactericidal activity is comparable to isoniazid when tested *in vitro* against exponentially growing *M.tuberculosis* culture, but less effective when tested in model systems (auxotrophy and starvation) involving metabolically inert *M. tuberculosis* [88]. It is worth noting that the enzyme targeted by BTZ043 (DprE1) is highly conserved in orthologous genes from various actinobacteria. The *M. ulcerans* protein YP908322.1 (NCBI reference sequence) shares 87% sequence identity with the *M. tuberculosis* Rv3790 protein, and the Cys amino-acid residue conferring sensitivity to BTZ043 is conserved. Based on these data, BTZ043 could have significant activity against *M. ulcerans* and should be further studied.

5.3.5 Diamine SQ-109 (Sequella Inc.)

SQ-109 is a novel 1,2-ethylenediamine-based ethambutol analog. Although it targets cell wall formation like ethambutol, SQ-109 acts on a different target – the transmembrane transporter encoded by *mmpL3 gene* – and thus has no cross-resistance with ethambutol [90]. A phase 2a early bactericidal activity study is underway to determine safety, sputum clearance, and pharmacokinetics of SQ-109 alone or with rifampicin in patients with smear-positive pulmonary tuberculosis [91]. The gene encoding the *M. tuberculosis* membrane transport protein MmpL3 (Rv0206c) is conserved across all mycobacteria for which genome sequences are available and the *M. ulcerans* protein YP_905156.1 (NCBI reference sequence) shares 74% identity with the *M. tuberculosis* MmpL3 protein. This suggests that SQ-109 might be active against *M. ulcerans* and further testing should be pursued.

5.4 Antibiotics that are not suitable for the treatment of Buruli ulcer

Several drugs with activity against other mycobacterial species have been determined to be unsuitable for the treatment of Buruli ulcer disease (see Appendix Table A.4), primarily taking into account the challenges associated with their use in resource-limited settings. The main criteria applied for discarding the drugs listed in the table were the following:

- lack of evidence of drug activity against *M. ulcerans*
- administration route or storage conditions not ideal (i.e. injectable or refrigeration required)
- contraindicated for use in pediatric patients
- major side effects or toxicities
- expensive

It is also worth noting that one of the new anti-tuberculosis drugs being developed, PA-824, has been shown to lack *in vitro* activity against *M. ulcerans* [34]. Developed by PathoGenesis-Chiron and currently being promoted by the TB Alliance, PA-824 is a nitroimidazole derivative like OPC-67683 that has bactericidal activity *in vitro* against both replicating and non-replicating *M. tuberculosis* [92].

When tested specifically against *M. ulcerans*, PA-824 had very high MIC values (16 mg/ml) *in vitro*, and studies in the mouse footpad curative model confirmed its lack of activity against *M. ulcerans* [34].

Post-treatment mean CFU values in mice administered PA-824 did not differ significantly from pre-treatment values. Thus, PA-824 does not appear to have application for Buruli ulcer treatment; however, given the potent bactericidal activity of this drug against tuberculosis, researchers are identifying novel nitroimidazole derivatives that could potentially have activity against *M. ulcerans* in addition to *M. tuberculosis*.

6. TOWARD IDENTIFYING NEW BURULI ULCER CHEMOTHERAPEUTICS

Although promising alternatives have been identified to the currently recommended rifampicin and streptomycin regimen for Buruli ulcer treatment, major knowledge gaps remain that prevent rigorous conclusions from being drawn with respect to all of the existing and upcoming drugs with anti-mycobacterial activity. In addition, while the rifampicin and clarithromycin combination may prove to be an effective all-oral regimen that is not inferior to the currently recommended treatment approach, it does not fully meet the criteria for end-user requirements outlined in the introduction (see Table 2.1). Notably, this treatment regimen can pose challenges for the treatment of Buruli ulcer disease in patients co-infected with TB and HIV due to drug-drug interactions with antiretrovirals. Thus, there is a critical need to strengthen and expand research efforts in the development of effective chemotherapeutic approaches for Buruli ulcer disease that can be successfully implemented in the resource-poor settings where this disease is endemic.

With the goal of resolving deficiencies in our understanding of the antimicrobial susceptibility profile of *M. ulcerans* as well as promoting the potential use of existing or newly developed drugs for Buruli ulcer disease, we herein propose a research agenda outlining short-term and long-term objectives that should be addressed.

6.1 Short-term objective: Systematically identify all existing, orally administered drugs with activity against *M. ulcerans* and their relative potencies

Chemotherapy treatment for Buruli ulcer with rifampicin-streptomycin has already had clinical success, but this regimen presents some major disadvantages, especially when considered for use in Buruli ulcer-endemic regions. Indeed, administration through intramuscular injection and the long-term toxicities associated with aminoglycosides complicate the delivery of Buruli ulcer care in less-developed countries. With the aim of identifying alternative, orally administered regimens, the mycobacterial activity of several existing and newly developed drugs as monotherapy or in combination has been investigated in animal models [15,23,25,29,32-34]. These studies have offered promising results about possible alternative regimens, some of which have been studied in clinical trials or empirically tried in patients with success. However, the data are fragmented without a clearly defined research agenda, which has made it difficult to draw robust conclusions. Systematically studying drugs, drug candidates and combination regimens in murine models under the same experimental conditions with appropriate follow-up evaluation to exclude disease relapse would enable us to thoroughly assess the sterilizing activity of different regimens and more accurately determine their potential efficacy as Buruli ulcer chemotherapeutics. Based on these rigorous experimental models, future clinical trials could be undertaken to establish clinical effectiveness.

Studies comparing the anti-mycobacterial activities of rifampicin, aminoglycosides and other orally administered drugs in animal curative models have demonstrated that: i) rifampicin and aminoglycosides have clear bactericidal activity; and ii) clarithromycin, moxifloxacin, linezolid and TMC207 also have bactericidal activity but are less potent than rifampicin and the aminoglycosides [33,34]. In combination regimens with these drugs, most of the bactericidal activity was attributable to rifampicin; however, combination therapy remains preferred over rifampicin monotherapy to prevent the development of rifampicin resistance to *M. ulcerans*. Comparable cure rates to that of the rifampicin-aminoglycoside combination were seen after 8 weeks of treatment with these alternative regimens. Whether the sterilizing activity of these alternative regimens in clinical studies is equivalent or potentially superior to the currently recommended antibiotic regimen remains to be fully answered.

As an oral antibiotic, clarithromycin is an attractive alternative to the aminoglycosides currently recommended for Buruli ulcer treatment. Clinical studies have already indicated that a fully oral regimen can successfully treat early stage, limited disease. Recent clinical studies have shown that partial or complete treatment with rifampicin-clarithromcyin may be equivalent to standard chemotherapy with rifampicin-streptomycin [40,51]. In addition, this oral regimen has been shown to be used successfully in more vulnerable populations such as pregnant patients [49]. Taken together, these data expand on earlier pilot studies to demonstrate that Buruli ulcer disease can be effectively treated with antibiotics; and importantly, early-stage, limited lesions may not even require additional surgery. Building on this work, the WHO is sponsoring a randomized multicenter trial aimed at establishing that 8 weeks of rifampicin-clarithromycin will be equivalent to the current standard of care.

While establishing the clinical efficacy of rifampicin-clarithromycin would represent a valuable step forward, there remains an urgent need to identify additional alternative chemotherapeutics. This oral combination, like the current standard of care, has significant limitations that threaten its wide-scale implementation. Drug-drug interactions between antiretrovirals and both rifampicin and clarithromycin will complicate the use of this regimen in people co-infected with HIV. Both drugs interfere with the CYP3A enzymatic pathway, thereby affecting serum levels of antiretrovirals with unclear clinical impact [93,94]. In addition, only a liquid-based formulation of clarithromycin is available for pediatric patients, which would be challenging to administer and store in rural community settings. Clarithromycin's twice a day dosing schedule would likely lower patient adherence to treatment.

The use of azithromycin, a once-daily macrolide closely related to clarithromycin, could alleviate some of the aforementioned concerns. In addition, it could provide an alternative oral option for patients who do not tolerate clarithromycin. However, there is very little data on the use of azithromycin against *M. ulcerans*. Azithromycin does appear to have weaker bacteriostatic activity *in vivo* than clarithromycin, but comparable to that of fluoroquinolones. The published success with clinical use of fluoroquinolones and clarithromycin against Buruli ulcer, along with the routine use of azithromycin for *M. avium* complex infections, strongly supports the potential application of azithromycin in treating *M. ulcerans*. Further investigation is needed to better define the possible role of azithromycin – first pursuing *in vivo* studies using the mouse footpad model and subsequently conducting clinical trials.

Fluoroquinolones represent another class of drugs with significant potential as an oral chemotherapeutic option for Buruli ulcer disease. Several generations of fluoroquinolones have

been developed and are currently in clinical use, including the second-generation ciprofloxacin and third-generation moxifloxacin and levofloxacin. Most of the available laboratory and clinical data have focused on ciprofloxacin and moxifloxacin, but it is expected that levofloxacin would have comparable activity against *M. ulcerans*. The later generation fluoroquinolones are preferred over ciprofloxacin because of better safety, tolerability and convenience of use with once daily dosing. It should be noted that sitafloxacin, a fourth-generation drug, was shown to have more potent *in vitro* activity against *M. ulcerans* when compared to the others. However, given the drug's increased likelihood of phototoxicity in Caucasian populations and limited market availability, it would not be pragmatic to focus on this agent over the third-generation drugs.

Fluoroquinoles boast several features that make them attractive for Buruli ulcer treatment: excellent spectrum of activity, good tissue penetration and oral route of administration. In addition, there are no major drug-drug interactions between fluoroquinolones and antiretroviral drugs, suggesting a potential role for fluoroquinolone use in patients co-infected with HIV and mycobacterial infections. The major disadvantage of this class is the concern for fluoroquinolone-induced joint or cartilage toxicity in pediatric patients based on juvenile animal studies [95,96]. However, data on the safety of fluoroquinolones in children is certainly limited. The exact frequency of side effects related to fluoroquinolone use in children is difficult to estimate because of the paucity of prospective studies. Furthermore, fluoroquinolones – specifically ciprofloxacin – are often used in children in off-label circumstances and associated adverse events are rarely published [97,98]. Available data about pediatric tolerance of fluoroquinolones come principally from two retrospective studies and one prospective observational study [99-101]. Each of these studies had a large sample size and found a low rate of articular adverse events (0-3.8%); in addition, all adverse events were minor without significant sequelae. Thus, much of the data has failed to detect an increased rate of articular side effects in children treated with fluoroquinolones. Despite these encouraging results, it is still recommended that fluoroquinolones be used with reservation in children and only as second-line agents, primarily in cases where all other previous treatments have failed or for the treatment of life-threatening infections where the use of fluoroquinolones has been showed to be of advantage (i.e. cystic fibrosis, typhoid fever, severe shigella dysenteries, enterobacterial meningitis).

Other antibiotics, specifically dapsone, amoxicillin-clavulanate and co-trimoxazole, should also receive consideration for their possible role in Buruli ulcer treatment. These drugs meet many of the end-user requirements outlined early on, including oral administration, safe for pediatric and possibly pregnant patients, and minimal drug-drug interactions with ARVs. However, their weaker activity *in vitro* against mycobacterial species including *M. ulcerans* leads us to prioritize them lower than the macrolides and fluoroquinolones

In order to draw rigorous conclusions regarding the utility of existing oral drugs for Buruli ulcer chemotherapy and ensure that their potential value is thoroughly assessed, the anti-mycobacterial activity of the drugs described in Table 6.1 should be established under standardized experimental conditions in both *in vitro* and *in vivo* models.

Table 6.1 – Antibiotics that deserves further systematic investigation

Drug	Rationale for Strong Consideration
Azithromycin	▪ In vivo studies of azithromycin indicate it has bacteriostatic activity against *M. ulcerans*, comparable to that of moxifloxacin. ▪ Successful use of clarithromycin and moxifloxacin clinically supports a potential role for azithromycin ▪ Possible use in paediatric and pregnant patients (pregnancy category B) ▪ Limited drug-drug interactions with ARVs and no dose adjustment required ▪ Cheaper than clarithromycin and more convenient administration with once daily dosing
Fluoroquinolones	▪ Reported drug-drug interactions with ARVs limited to didanosine ▪ Good safety and tolerability profile ▪ Convenient administration with once daily dosing schedule
	Rationale for Consideration
Dapsone	▪ Safe for pediatric patients ▪ No significant interactions with ARVs
Amoxicillin-Clavulanate	▪ Pregnancy category B ▪ Safe for pediatric patients ▪ No interaction with ARVs
Co-trimoxazole	▪ Drug-drug interaction with ARVs have been reported (primarily with lamivudine, ritonavir, indinavir and delavirdine) but no dose adjustment is recommended ▪ Safe for paediatric patients > 2 years old

By systematically testing these drugs under the same laboratory conditions, the relative anti-mycobacterial activity of each drug can be more clearly delineated and can inform which drugs and in what order they should be pursued in clinical trials. To some extent, this information is available with respect to the various generations of fluoroquinolones, with sitafloxacin having greater potency over moxifloxacin or levofloxacin. However, in addition to the relative anti-mycobacterial potency, the safety profile and dosing characteristics should be taken into consideration when deciding which drugs to study first. The phototoxicity associated with sitafloxacin and the drug's limited market availability make this drug a less pragmatic consideration at this time. With this in mind, we propose that top priority be assigned to investigating azithromycin and the fluoroquinolones moxifloxacin and levofloxacin for Buruli ulcer treatment.

6.2 Short-term objective: Identify an orally administered combination regimen for Buruli ulcer disease using existing drugs

Once a pool of orally administered drugs with activity against *M. ulcerans* has been identified, an important next step will be to identify the combination regimen with the greatest bactericidal potency in animal models. For example, several observational studies and case series have demonstrated the clinical efficacy of fluoroquinolones, primarily ciprofloxacin and moxifloxacin, in conjunction with rifampicin in treating Buruli ulcers [50,53,102,103]. Due to the varying durations

of treatment, measures of disease response, and methods of follow-up presented in these studies, it is difficult to directly compare the efficacy of fluoroquinolone-containing regimens to the current standard of care. Thus, the relative efficacy of the following regimens under the same laboratory conditions and clinical settings should be studied: rifampicin-streptomycin, rifampicin-clarithromycin, rifampicin-azithromycin and rifampicin-fluoroquinolone. In comparing these regimens, the levels of bactericidal activity after 8 weeks of treatment, time to culture-sterilization, and relapse/recurrence rates during post-treatment follow-up should be assessed. Subsequently, clinical trials similar to the one being conducted by the WHO studying rifampicin-clarithromycin should be pursued using other combination regimens.

If *in vitro* and *in vivo* studies show a significant MIC for amoxicillin-clavulanate and co-trimoxazole, further studies examining the activity of these drugs in combination with rifampicin also should be pursued. Rifampicin-dapsone has been studied previously in a clinical trial but the small sample size and short duration of follow-up prevent any solid conclusions being drawn. Further investigation of this dual regimen with better-designed study protocols is likely warranted given dapsone's safety and tolerability profile.

Another unanswered question is whether combination regimens with other rifamycins, such as rifapentine and rifabutin, should be explored more systematically. Mouse model studies have shown that rifapentine may have superior anti-mycobacterial activity compared to rifampicin, and rifapentine's longer half-life may allow for intermittent dosing. However, a phase II clinical trial comparing the 2-month culture conversation rate of the standard, first-line tuberculosis regimen (RIF-INH-EMB-PZA) to the same regimen with rifapentine instead of rifampicin failed to show superior activity for the rifapentine-containing regimen [104]. Several studies are in progress to determine whether rifapentine can be used to optimize and shorten the duration of TB treatment. The results of these studies may offer important lessons to guide future research on the use of rifapentine in the treatment of Buruli ulcer disease.

6.3 Short-term objective: Explore the use of multi-drug combination therapy to shorten treatment duration

Given the challenges of successfully completing 8 weeks of antibiotics in many of the rural community settings where Buruli ulcer is endemic, there is a need for identifying regimens that could shorten the duration of treatment. Combining multiple drugs with different mechanisms of action may be one approach toward this aim. Despite the successful use of quadruple drug regimens for tuberculosis, there is little data on the use of multi-drugs regimens for Buruli ulcer beyond a few published case reports. Thus, further research is needed to assess whether multi-drug regimens could result in significantly increased levels of bactericidal activity so as to shorten time to culture sterilization and cure.

Very little is known about whether *M. ulcerans* develops antimicrobial resistance similar to what is seen with *M. tuberculosis*. The development of drug resistance has been documented *in vitro*, suggesting that this is likely to become an important concern with increased antibiotic use [16]. The potential for antimicrobial drug resistance would be another reason for considering multi-drug combinations for Buruli ulcer treatment. Of note, the triple combination of rifampicin-clarithromycin-sparfloxacin was tested in animal models and found to have lower anti-mycobacterial activity when compared to the combination rifampicin-aminoglycoside [24]. It is well known that

fluoroquinolones and clarithromycin have weaker activity against *M. ulcerans* when compared to aminoglycosides, which may account for the results seen. However, no direct comparison was made between the activity of the triple regimen and the activities of rifampicin-clarithromycin or rifampicin-fluoroquinolone. Thus, we have no data available assessing whether multi-drug combinations have added value compared to dual drug regimens, either in improving clinical efficacy or preventing drug resistance.

Inclusion criteria for selecting drugs to test in multi-drug regimens should focus on targeting different biological pathways of the bacteria and capitalizing on synergistic drug-drug interactions. Animal studies could help define the most active combinations that should then be considered for further clinical studies. Based on existing data, the regimen of rifampicin, a macrolide and fluoroquinolone may be worth pursuing further in clinical studies. The other drugs described in Section 6.2 are unlikely to have sufficient individual bactericidal activity to warrant their investigation in multi-drug regimens.

There are disadvantages to multi-drug regimens that should be mentioned. Increasing the number of drugs in a regimen raises the risk of additional toxicities and complicates drug administration with more pills to be taken. Substantially shortening the duration of antibiotic therapy would be a necessary condition to warrant the increased risks and hassles associated with a multi-drug regimen. Moreover, very recent data suggests that stronger bactericidal activity does not necessarily translate into more effective sterilizing activity. Almeida and colleagues found that although treatment with daily rifapentine was more bactericidal than daily rifampicin (both in conjunction with streptomycin), the relapse rate and time to relapse after stopping treatment were not different between the two groups [105]. Interestingly, the authors suggest that treatment of Buruli ulcer is more dependent on reversal or inhibition of mycolactone-induced local immunodeficiency by the treatment, as opposed to the bactericidal activity of the drugs. Thus, specifically targeting the inactivation of mycolactone or inhibition of its immunosuppressive activity may represent a complementary or more efficacious approach to improve and shorten the duration of Buruli ulcer chemotherapy.

6.4 Short-term objective: Establish the role of and timing for surgical intervention in Buruli ulcer treatment

Finally, the increasing role of antimicrobials in Buruli ulcer treatment does not exclude the potential value of surgical interventions in specific cases. While antimicrobial therapy has been shown to effectively heal small, early-stage lesions and reduce the size of larger lesions so as to allow for less extensive surgery, the exact role and timing for surgical interventions in conjunction with Buruli ulcer chemotherapy are not well defined. To that end, a randomized, parallel-assignment clinical trial is underway exploring the impact of delayed surgery on treatment outcomes in Buruli ulcer patients receiving rifampicin-streptomycin [106]. Defining the appropriate criteria for surgical intervention to optimize treatment outcomes will help ensure that limited resources in endemic regions are maximally used.

A summary of recommended short-term research aims is presented in Table 6.4.

Table 6.4 – Short-term research aims proposed to guide the identification of new Buruli ulcer chemotherapeutics.

***IN VITRO* STUDIES**	***IN VIVO* STUDIES**	**CLINICAL STUDIES**
TOP PRIORITY: ▪ Compare the anti-mycobacterial activity of single drugs and the additive or synergistic effect of drug combinations under standardized experimental conditions. Drugs and drug combinations to consider: o Clarithromycin o Azithromycin o Fluoroquinolones (moxifloxacin, levofloxacin) o Rifampicin-Clarithromycin o Rifampicin-Azithromycin o Rifampicin-Fluoroquinolone	▪ Assess and compare anti-mycobacterial activity under standardized experimental conditions of combination regimens: o Rifampicin-Clarithromycin o Rifampicin-Azithromycin o Rifampicin-Fluoroquinolone	▪ Clinical trials to assess all-oral regimens that can offer an alternative to rifampicin-clarithromycin, such as rifampicin-moxifloxacin or rifampicin-azithromycin (if animal studies are promising). ▪ Clinical trial to study early versus delayed surgical intervention in conjunction with antibiotic therapy
MEDIUM PRIORITY: ▪ Compare the anti-mycobacterial activity of single drugs and the additive or synergistic effect of drug combinations under standardized experimental conditions of the following compounds: o Dapsone o Amoxicillin-clavulanate o Co-trimoxazole o Rifampicin-Dapsone o Rifampicin-Amoxicillin-clavulanate o Rifampicin-Co-trimoxazole	▪ Consider pursuing *in vivo* studies of dapsone, amoxicillin-clavulanate or co-trimoxazole if *in vitro* studies are promising for these drugs ▪ Consider assessing the activity of rifapentine-based regimens *in vivo* based on results of ongoing studies of tuberculosis treatment	
LOW PRIORITY	▪ Consider pursuing *in vivo* studies of multi-drug regimens (i.e. rifampicin-macrolide-fluoroquinolone) and assess their potential to shorten treatment duration	

6.5 Long-term objective: Explore the use of new drugs for Buruli ulcer treatment

Importantly, there have been significant advances in the development of novel anti-tuberculosis drug candidates in recent years. Given the highly conserved sequences between mycobacterial species, some of these drug candidates may have clinically important activity against *M. ulcerans* and

should be investigated further. In addition, new drug candidates against TB are being pursued that have fewer drug-drug interactions with antiretrovirals, are orally administered and can be produced in fixed-dose combinations, all of which are end-user requirements for Buruli ulcer chemotherapeutics (see Table 2.2). Drug candidates coming down the pipeline for anti-tuberculosis treatment thus should be evaluated for potential *in vitro* and *in vivo* activity against *M. ulcerans*. For example, the diarylquinolone TMC207 was shown to have *in vitro* activity against *M. ulcerans* while the nitroimidazole PA-824 did not have any such activity [34]. Furthermore, Ji and colleagues found that treatment with the combination regimens rifampicin-TMC207, as well as rifampicin-moxifloxacin and rifampicin-linezolid, had comparable bactericidal effects to rifampicin-aminoglycoside in animal models, suggesting these may be viable orally administered options that should be further investigated. Other attractive options include the use of PNU10048 and AZD5847 – new oxazolidinones that may have similar spectrums of activity to linezolid, which has demonstrated activity against *M. ulcerans*. However, the growing multi-drug resistant tuberculosis epidemic demands a cautious approach, prioritizing the use of these agents for MDR-TB so as to protect against the selection of additional drug resistance.

Antimicrobial resistance is of particular concern because Buruli ulcer disease is endemic to areas where tuberculosis is highly prevalent. Although screening for active tuberculosis is recommended prior to initiating Buruli ulcer disease, current limitations to accurately diagnosing active disease prevent reliance on this screening strategy. While new drugs with proven efficacy against TB should be restricted in a responsible public health approach, the research and development process offer other chemical entities and classes of compounds with possible anti-mycobacterial activity. Drugs that are discarded in the process for lack of anti-tuberculosis activity, for example, could be further investigated for activity against *M. ulcerans*. In addition, classes of anti-microbials or anti-infectives with broad spectrum of activity could represent additional sources of compounds active against *M. ulcerans* with no or limited cross-activity against *M. tuberculosis*

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In considering the application of anti-tuberculosis drugs for Buruli ulcer treatment, it will be critical to better understand the epidemiology of Buruli ulcer disease since it is endemic to regions where tuberculosis is highly prevalent. Specifically, studies must be conducted to address whether, during Buruli ulcer treatment, there could be the potential to inadvertently treat active tuberculosis with only one or two drugs and the implications of this on the development of resistant *M. tuberculosis* strains. Further guidelines are needed to determine how to optimally treat both diseases while reserving the most potent anti-tuberculosis drugs for the management of tuberculosis, which has much more profound morbidity and mortality associated with it.

In the absence of epidemiological data on co-existing mycobacterial infections, there is an important caveat that arises with respect to the clinical application of rifampicin-clarithromycin. Clarithromycin and its counterpart azithromycin lack anti-tuberculosis activity. The use of a rifampicin-macrolide regimen in a patient with undiagnosed active tuberculosis could lead to rifampicin monotherapy and raise the risk of developing rifampicin resistance. While aggressive screening for tuberculosis at the initiation of and during treatment for Buruli ulcer disease would mitigate the potential risk, this is a major hurdle for implementation of rifampicin-clarithromycin beyond the closely-monitored environment of clinical studies. Ideal solutions to address this include the identification of better diagnostic tests to definitely exclude tuberculosis co-infection or alternatively he development of new chemotherapeutic regimens that are rifampicin-free and do not include any of the 1st or 2nd line agents recommended for the treatment of tuberculosis. Taking into consideration that rifampicin is the most potent oral drug with activity against *M. ulcerans* thus far

identified, a more pragmatic short-term solution is represented by the prioritization of combination regimens with at least two anti-tuberculosis agents.

Lastly, the dihydrofalate reductase inhibitors are another class of drugs that deserve future investigation given that *M. ulcerans* has demonstrated susceptibility *in vitro.* Of note, synergistic activity was seen when the dihydrofolate reductase inhibitor epiroprim was used in conjunction with dapsone. There have been no studies of epiroprim activity *in vivo* against *M. ulcerans*, and this should be pursued. Furthermore, dual and triple combinations of epiroprim, dapsone and rifamycins should be studied *in vitro* and *in vivo* under standardized conditions.

Identifying the full anti-mycobacterial activity profiles of newly developed drugs, establishing their safety and tolerability, and developing guidelines for the responsible use of these drugs will be critical components of the Buruli ulcer research and public health agenda.

7. CONCLUSIONS

This is an exciting time for Buruli ulcer research and public health programs. With the implementation of effective Buruli ulcer chemotherapy, there has been a transformation in how this disease can be treated. While major challenges persist in scaling up treatment programs due to the resource constraints in Buruli ulcer-endemic areas, there is considerable research exploring the potential use of alternative drugs currently in existence or being newly developed. However, these data are sporadic and incomplete. Recent clinical trials sponsored by the WHO are systematically attempting to realize a fully oral treatment regimen, which will be a valuable step forward. We have outlined further steps that can be undertaken to systematically identify and establish alternative chemotherapeutics. These new regimens may come even closer to meeting the end-user requirements that we believe are essential for devising the most effective and readily implementable treatment programs for Buruli ulcer disease. With continued research and public health commitment, this goal may be soon within our grasp.

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106. Timing of surgical intervention in Buruli ulcer patients treated with antibiotics. NCT01432925. ClinicalTrials.gov (2012).

APPENDIX

Table A.1 – Search strategy for identification of articles for inclusion

Database	Search Terms and Strategy
PubMed	((((“Therapeutics” [Mesh] OR (therapy OR treatment OR intervention)) AND (((“buruli ulcer” [MeSH Terms] OR (“buruli”[All Fields] AND “ulcer[All Fields]) OR “buruli ulcer”[All Fields})) OR (mycobacterium ulcerans))
EMBASE	((buruli AND (“ulcer”/exp OR ulcer) or (“mycobacterium”/exp OR mycobacterium AND ulcerans)) AND (therapy or treatment or intervention or therapeutics))
Scopus	((“mycobacterium ulcerans” or “Buruli ulcer”) AND (therapy OR treatment OR intervention))
WHO Global Health Library	“mycobacterium ulcerans” OR “Buruli ulcer”
CAB Abstracts	exp Buruli ulcer/ or Mycobacterium ulcerans.od.
Cochrane Library	(MeSH descriptor: [Mycobacterium ulcerans] OR “mycobacterium ulcerans”)

Table A.2 – Drugs with known anti-mycobacterial activity

Drugs to treat:	Group 1 – First-line agents	Group 2 – Second-line agents	Group 3 – Third-line agents (unclear efficacy)	Group 4 – New drugs in development
M. tuberculosis	▪ Isoniazid (INH) ▪ Rifampicin (RIF) ▪ Ethambutol (EMB) ▪ Pyrazinamide (PZA) ▪ Other rifamycins: Rifapentine (RFP), Rifabutin (RFB), Rifalazil	▪ Aminoglycosides (injectables): Streptomycin (STR), Kanamycin (KAN), Amikacin (AMK), Capreomycin (Cm), Viomycin (Vi) ▪ Fluoroquinolones (oral): Ciprofloxacin (CIP), Ofloxacin (OFX), Levofloxacin (LVX), Moxifloxacin (MXF), Gatifloxacin (GFX) ▪ Other oral drugs with bacteriostatic activity: Ethionamide (Eto), Protionamide (Pto), Cycloserine (Cs), Terizidone (Trd), P-aminosalicylic acid (PAS), Thiacetazone (Th)	▪ Clofazimine (CFZ) ▪ Amoxicillin/Clavulanate (AMC) ▪ Clarithromycin (CLR) ▪ Linezolid (LZD) ▪ Meropenem-clavulanate (activity against MDR-TB)	▪ TMC207 (bedaquiline) ▪ PA-824 ▪ OPC-67683 ▪ PNU-100480 ▪ AZD-5847 ▪ BTZ043 ▪ SQ-109
M. leprae	▪ Rifampicin ▪ Dapsone (DDS) ▪ Clofazimine		▪ Epiroprim	
M. avium complex	▪ Clarithromycin	▪ Telithromycin (TEL)		

	▪ Azithromycin (AZM) ▪ Ethambutol ▪ Rifabutin ▪ Amikacin ▪ Ciprofloxacin ▪ Levofloxacin
M. marinum	▪ Rifampicin ▪ Ethambutol ▪ Clarithromycin ▪ Cotrimoxazole (CTX) ▪ Minocycline (MIN) ▪ Doxycycline (DOX)
M. fortuitum	▪ Cefoxitin (FOX) ▪ Amikacin ▪ Ciprofloxacin ▪ Levofloxacin ▪ Cotrimoxazole ▪ Imipenem (IPM)

M. chelonae	▪ Clarithromycin ▪ Tobramycin (TOB) ▪ Imipenem ▪ Linezolid
M. kansasii	▪ Rifampicin ▪ Ethambutol ▪ Isoniazid

Table A.3– Antibiotics with clinical application for treatment of Buruli ulcer

Study Design	Population*	Intervention	Results
Randomized controlled trials:			
Double-blind, randomized controlled trial Revill WDL, Morrow RH, Pike MC, Ateng J (1973)	106 patients from Uganda with presumed Buruli ulcer disease	▪ Patients with uncomplicated non-ulcerated lesions were randomly assigned to group A with surgery withheld (2/3 of patients) or group B with immediate surgery ▪ Patients with concerning non-ulcerated lesions or who requested surgery underwent immediate surgery (group C), as did patients with ulcerated lesions (group D) ▪ Patients in all four groups were randomly assigned to either CFZ or placebo (double-blinding)	▪ CFZ alone did not heal any lesions. ▪ In patients treated with both CFZ and surgery, CFZ did not shorten disease course, reduce number of surgeries or extent of scarring. ▪ Among patients who received immediate surgery (groups B-D), time to healing was associated with initial lesion size. No differences between those treated with CFZ versus placebo. ▪ Overall recurrence rate was 17%. Group A had the highest proportion of recurrences. ▪ CFZ did not prevent recurrences, and *M. ulcerans* could be cultured from lesions after several months of treatment.
Double-blind, randomized controlled trial Fehr H, Egger M, Senn I (1994)	18 Ghanaian patients with Buruli ulcer disease diagnosed between February and June 1988	▪ Intervention: SXT (160mg/800mg) tablet twice daily, along with surgical resection of large ulcers ▪ Control: placebo tablets + surgical resection	▪ 6 patients excluded from analysis: 2 in each group were lost to follow-up; the remaining 2 patients were in the intervention group and had pneumonia and died of tetanus, respectively. ▪ All ulcers in the intervention group decreased in size and had the largest decreases in size. ▪ Lesion growth observed in 3 control patients. ▪ In both groups, all patients except for 1 needed one or more surgical interventions. ▪ During follow-up, there were 2 excisions over a 50-week period in the intervention group, compared to 4 excisions in 41 weeks with the control group. ▪ Based on these data, the authors suggest that co-trimoxazole cannot replace surgery as primary treatment of Buruli ulcer although adjuvant therapy may improve post-operative healing and reduce the number or size of further excisions needed.

Randomized controlled trial Etuaful *et al.* (2005)	33 Ghanaian patients with presumed Buruli ulcer disease enrolled between September 2001 and December 2002	▪ Intervention 1: RIF + STR X 4 wks, followed by excision ▪ Intervention 2: RIF + STR X 8 wks, followed by excision ▪ Intervention 3: RIF + STR X 12 wks, followed by excision ▪ Intervention 4: RIF + STR X 2 weeks, followed by excision. 5 patients recruited to this group after preliminary analysis of above-mentioned groups ▪ Control: immediate excision and wound closure	▪ 2 patients in intervention group 3 withdrew. ▪ 21 patients had lab-confirmed *M. ulcerans* infection. ▪ All of the patients in the control and intervention groups with laboratory-confirmation had culture-positive lesions at time of excision. In contrast, none of the lesions excised after antibiotics had been given for 4 or more weeks were culture positive. ▪ Lesions reduced in size with antibiotics, ranging from 29% reduction in surface area after 2 weeks to 52% after treatment for 4 weeks. ▪ No side effects reported during antibiotic treatment. Laboratory and hearing tests throughout treatment remained normal. ▪ During 12-month follow-up period, only 1 patient had recurrence. This patient had not received antibiotics.
BURULICO Trial: parallel, open-label randomized controlled trial Nienhuis *et al.* (2010)	151 patients in Ghana with early-stage, limited infection with *M. ulcerans* (duration < 6 months, lesions < 10cm in diameter)	▪ Intervention: 4 weeks of RIF + STR followed by 4 weeks of RIF + CLR (n=76) ▪ Control: 8 weeks of RIF + STR (n=75)	▪ Participants were followed for 1 year. ▪ 73 (96%) participants in the intervention arm had healed lesions at 1 year, compared to 68 (91) of the controls. ▪ Median time to healing for category 1 lesions was 18 weeks and 30 weeks for larger lesions. ▪ No one with healed lesions had recurrence. ▪ Vestibulotoxic events occurred in 1 and 2 patients in the control and intervention arms, respectively.
WHO-sponsored, open-label randomized controlled trial	Patients with Buruli ulcer disease from multiple centers: 1 in Benin, 4 in Ghana	▪ Intervention: 8 weeks of RIF + CLR ▪ Control: 8 weeks of RIF + STR	▪ Primary outcomes will be healing without recurrence and without surgical excision. ▪ Secondary outcomes include rates of recurrence, treatment failure, and paradoxical response within 12 months of treatment initiation.
Randomized, parallel assignment intervention study	Patients with Buruli ulcer disease	▪ Intervention A: Surgery at week 8 after antibiotic initiation ▪ Intervention B: Surgery at week 14 after antibiotic initiation	▪ Primary outcomes will be healing, extent of surgery required, and frequency of functional limitations.

Pilot studies:			
Espey *et al.* (2002)	41 patients from Côte d'Ivoire with *M. ulcerans* infection	▪ 30 patients completed the 2-month trial: ▪ Intervention: Dapsone + RIF ▪ Control: Placebo	▪ Median change in ulcer size was a decrease of 14.0 cm^2 compared to 2.5cm^2 in the treatment and control groups, respectively. ▪ 82% of ulcers in treatment group improved compared with 75% in placebo group. No significant difference between study groups in treatment effectiveness based on clinical evaluation of ulcers.
Chauty *et al.* (2011)	30 patients in Benin with Buruli ulcer disease diagnosed between December 2007 and February 2009, followed prospectively.	▪ Treatment: 8 weeks of RIF + CLR	▪ Treatment well tolerated with no adverse events reported at 18 months of follow-up. ▪ All 30 patients had treatment success within 12 months of treatment initiation. ▪ 15 (50%) healed with antibiotics alone; 11 (37%) underwent limited surgical procedures (e.g. curettage or excision). Remaining 4 required extensive surgical excision and skin grafting. ▪ 8 of 10 patients with category I lesions and 5 of 11 with category II lesions healed with antibiotics only.
Observational studies:			
Lunn HF & Rees RJW (1964)	10 patients from Uganda with *M. ulcerans* infection	▪ All patients were treated with CFZ for varying durations, along with surgical excision and skin grafting	▪ CFZ helped limit spread of disease and extent of surgery needed in 9 of 10 patients.
Oluwasanmi *et al.* (1976)	22 patients with Buruli ulcer disease in Nigeria, of which 19 were laboratory-confirmed.	▪ All patients treated with surgery, often multiple interventions ▪ Adjunctive antibiotics used: CFZ, STR, SXT ▪ 9 patients given CFZ alone or CFZ + STR ▪ 3 patients given CFZ + SXT ▪ 5 patients given SXT alone and 1 patient given SXT + STR ▪ 3 cases not treated with antibiotics	▪ 3 patients developed new lesions while on CFZ. ▪ No patient treated with SXT developed new lesions on treatment. ▪ Adverse effects of antibiotics rare: redness and hyperpigmentation seen in 3 patients with clofazimine. No other drug reactions.

Chauty *et al.* (2007)	224 patients in Benin clinically diagnosed with *M. ulcerans* infection between Jan 2003 and Dec 2004, followed prospectively.	▪ Treatment: 8 weeks of RIF + STR (n=224) ▪ Excluded: 86 patients because of pregnancy, immediate surgical excision, patient's decision to receive traditional treatment or refusal of antibiotic therapy	▪ 215 patients (96%) had treatment success; of which, 102 (47.4%) were cured with chemotherapy only. 113 cases (52.6%) cured with antibiotics and surgical excision +/- skin grafting. ▪ 22 of 27 cases (81%) with ulcers < 5 cm were cured, compared with 12 of 57 (21%) with ulcers ≥15 cm were cured with antibiotics alone. ▪ 24 of 53 (45%) with non-ulcerated lesions healed with antibiotics only. ▪ 208 patients seen in follow-up at 1 year after treatment completion, and 3 relapses identified.
O'Brien *et al.* (2007)	40 patients in southeastern Australia with *M. ulcerans* infection treated between Jan 1998 and Dec 2004. Retrospective review	▪ 59 treatment episodes: 29 with surgery alone, 26 with surgery and antibiotics, and 4 lesions with antibiotics alone ▪ Various antibiotic regimens were trialed including RIF, CLR, ETH, AMK, AZM and CIP	▪ Adjunctive antibiotic therapy significantly improved rate of treatment success: 16 (100%) of 16 lesions healed with antibiotics and surgery, compared with 16 (73%) of 23 lesions treated with surgery alone. ▪ RIF + CIP had 100% treatment success and tolerability (no patients ceased treatment), whereas amikacin was associated with reduced effectiveness and significant toxicity, particularly for elderly patients.
Schunk *et al.* (2009)	129 Ghanaian patients with *M. ulcerans* infection who received surgical treatment between Sept 2003 and Sept 2005. Retrospective review	▪ 79 (61%) patients retrieved for follow-up. The group lost to follow-up was comparable to study group ▪ 65 (82%) of 79 patients received antibiotics and surgery, but no data on antibiotics used or duration available in 11 cases. ▪ Of remaining 54, 13 received RIF only, 1 received STR only, and 40 treated with RIF +STR	▪ Mean duration of therapy was 39.2 +/- 32.4 (SD) days for STR and 26.2 +/- 21.4 days for rifampicin. ▪ Time interval between treatment and follow-up ranged from 4 to 29 months (mean 18 months; median 20 months). ▪ 7 (9%) of 79 patients had recurrences detected upon follow-up. ▪ 6 patients reported having developed recurrent lesions that were clinically diagnosed as Buruli ulcer and surgical removed in time period between excision of primary lesion and follow-up.

Kibadi *et al.* (2010)	94 patients in Democratic Republic of Congo with clinically diagnosed Buruli ulcer disease between Oct 2006 and Sept 2007, followed prospectively	▪ Treatment: RIF + STR X 4 wks, then surgery followed by additional 8 weeks of RIF + STR ▪ Only patients with category II and III lesions included ▪ 2 were excluded due to refusal to participate or loss to follow-up.	▪ 61 (66.3%) of 92 had PCR-positive lesions, confirming clinical diagnosis. ▪ All patients underwent surgical excision after week 4 except for one PCR-negative patient who refused surgery. This patient died 1 month after end of treatment due to sepsis. ▪ Of 61 patients, 60 (98.4%) had treatment success. One patient had recurrence within 2-year follow-up period.
Sarfo *et al.* (2010)	160 Ghanaian patients with *M. ulcerans* infection recruited between Sept 2005 and Dec 2007, and followed prospectively	▪ Treated with RIF + STR X 8 weeks ▪ One patient with category III ulcerated edema who responded poorly to initial regimen received additional 4 weeks of RIF + STR followed by surgery.	▪ Median diameter of lesions declined from 6.4 cm. before treatment to 2.1 cm at 4 weeks and to 1.4cm at 8 weeks. ▪ New inflammatory lesions developed in three patients; lesions aspirated but cultures were sterile. ▪ 10 patients offered surgery after antibiotics because of unsatisfactory healing, of which 8 patients accepted. ▪ All patients had complete healing except for 1 patient lost to follow. The remaining 159 patients also had no recurrences at 12-month follow-up period.
Agbenorku *et al.* (2011)	189 patients with Buruli ulcer from Bomfa sub-district of Ghana treated from Jan to Dec 2005, followed prospectively	▪ Treatment: RIF + STR X 8 wks, along with surgery	▪ 44 (23.3%) of patients had category 1 lesions and 145 (76.7%) had larger lesions. ▪ All patients had disease resolution. ▪ Recurrence identified in only 1 patient after 2 years of follow-up.
Adu EJK, Ampadu E, Acheampong D (2011)	132 Ghanaian patients with *M. ulcerans* infection treated between Sept 2005 and Sept 2009. Retrospective review	▪ Treatment: Patients with residual lesions after at least 4 weeks of RIF + STR selected for surgical treatment (wound debridement, excision, skin grafting, contracture release and flap repair)	▪ Prior to antibiotics, 7.4, 41.5 and 59.1% of lesions were category I, II and III, respectively. ▪ At time of surgery, after 4-8 weeks of antibiotics, distribution of lesions was 16.3, 34.1 and 49.6%, respectively, for category I, II and III lesions. ▪ At time of surgery, most lesions were in healing phase and required less extensive surgery.
Kotey NK & Ampadu X (2011)	68 Ghanaian patients with *M. ulcerans* infection	▪ Treated with RIF + STR X 8 weeks	▪ Average durations of primary wound healing were 29, 52, and 65 days for category I, II and III lesions. ▪ Statistically significantly differences in duration of wound healing among categories.

O'Brien *et al.* (2012)	147 patients with *M. ulcerans* infection treated between March 1998 and May 2010 in south-eastern Australia	▪ 133 patients followed for 12 months after treatment initiation; 4 patients had 2 lesions, for total of 137 lesions ▪ 14 patients excluded because of death, loss to follow-up or ongoing follow-up ▪ 61% received RIF+CIP, 23% received RIF+CLR; of remaining, 4 received CIP + CLR, and 2 received RIF + MXF	▪ 47 (34%) lesions treated with surgical excision alone, while 90 (66%) treated with antibiotics and surgery ▪ Proportion of cases receiving antibiotics increased pre-2005 compared to post-2005, from 45 to 74%. ▪ No treatment failures among those receiving antibiotics and surgery, compared with 14 (30%) of 47 treated with surgery alone who failed treatment. ▪ Complication rates comparable for RIF (GI intolerance, hepatitis, rash, hypoglycemia), CIP (GI intolerance, joint/tendon effects, rash, hallucinations) and CLR (GI intolerance, hepatitis, palpitations).
Saka *et al.* (2012)	119 patients in Togo with Buruli ulcer disease between June 2007 and Dec 2010	▪ All patients treated with RIF + STR X 8 weeks ▪ 30 patients received surgery	▪ 7 patients had complications including superinfection, osteomyelitis and bleeding. 3 patients had amputations. ▪ 12 patients lost to follow-up after 6 months. ▪ Of the remaining patients, 10 had functional limitations, but no recurrences were observed.
Case series:			
Pettit JHS, Marchette NJ, Rees RJW (1966)	4 patients from Malaysia with clinical or proven *M. ulcerans* infection	▪ Case 1: 5-year-old girl treated with CFZ for at least 5 months ▪ Case 2: 3-year-old boy treated with CFZ for 5 weeks ▪ Case 3: 4-year-old boy treated with CFZ for 6 months ▪ Case 4: 27-year-old woman treated for 3 ½ months with CFZ	▪ Cases 1 and 4 were microbiologically proven *M. ulcerans* infection, while cases 2 and 3 were clinically presumed. ▪ Complete healing seen in all four patients. ▪ Diarrhea and skin discoloration were adverse effects associated with CFZ.

Reid IS (1967)	13 patients from Papua treated between Sept 1964 and Oct 1965, of which 6 were presumed and 7 with laboratory confirmation.	▪ Case 4: 6-week-old boy was started on pencillin and chloramphenicol, and then taken for excision and grafting. 7 days after surgery, ulcer was larger, so started dapsone + STR for 5 weeks with additional small full-thickness graft ▪ Case 5: 6-year-old girl with partial surgical excision, then chloramphenicol + dapsone until skin grafting performed. Subsequently treated with dapsone, penicillin and STR but continued spread of lesion. Then treated with heat therapy ▪ Case 8: 12-year-old boy treated with penicillin + STR and then taken for surgical excision with grafting. Also received heat therapy	▪ Case 4 used dapsone + STR to arrest ulcer spread and allow complete healing within 5 weeks of treatment initiation. ▪ Cases 5 and 8 had complete healing.
Goutzamanis JJ & Gilbert GL (1995)	8 patients with *M. ulcerans* infection in Melbourne, Australia between 1968 and 1993. 3 patients did not have microbiological confirmation. Retrospective review	▪ All patients underwent excision and either primary closure or skin grafting. ▪ 3 patients received antibiotics: o STR o STR+RIF, and o RIF+INH+ETH+PZA	▪ Susceptibility patterns obtained for 4 of 5 positive *M. ulcerans* cultures: o Susceptible to STR, RIF; Resistant to INH, ETH o Susecptible to STR, RIF, ETH; Resistant to INH, PAS o Susceptible to STR, RIF; Resistant to INH, ETH, PZA o Susceptible to RIF; Resistant to INH, ETH ▪ One patient had disease progression despite antibiotics and surgery, which responded to adjunctive heat therapy.

Ouoba K, Sano D, Traore A, Ouedraogo R, Sakande B, Sanou A (1998)	6 patients from Burkino Faso with presumed Buruli ulcer disease diagnosed between 1991 and 1996	▪ Case 1: 23-year-old woman treated with RIF X 3 months, and surgical excision and debridement ▪ Case 2: 22-year-old man with CIP X 2 wks, acetic acid treatment and skin grafting ▪ Case 3: 10-year-old boy received amoxicillin X 2 weeks, nystatin X 10 days, and metronidazole X 6 days, along with acetic acid treatment and skin grafting ▪ Case 4: 11-year-old boy treated with ampicillin + metronidazole X 1 month ▪ Case 5: 7-year-old boy treated with gentamicin + metronidazole X 3 weeks, with amputation ▪ Case 6: 8-year-old girl treated with RIF X 3 months	▪ Cases 1-3 and 5 had complete healing. ▪ Case 4 was lost to follow-up. ▪ Case 6 died of sepsis.
Journeau P, Fitoussi F, Jehanno P, Padovani J-P, Penecot G-F (2003)	3 pediatric patients diagnosed with Buruli ulcer disease in France between 1995 and 2000	▪ Case 1: 9-year-old boy originally from Côte d'Ivoire. Treated with multiple excisions, skin grafting and RIF + CLR X 6 months ▪ Case 2: 9-year-old boy originally from Côte d'Ivoire. Treated with wide excisions and skin graft. Also received RIF + CLR X 6 months ▪ Case 3: 10-year-old boy from Mali. Treated with wide excision, skin grafting and RIF + CLR X 6 months.	▪ All 3 cases had complete healing with surgery and antibiotics. No recurrence seen over 5-year, 18-month and 1-year follow-up periods for Cases 1, 2 and 3 respectively.

Coloma JN, Navarrete-Franco G, Iribe P, Lopez-Cepeda LD (2005)	2 patients from Central Mexico with *M. ulcerans* infection	▪ Case 1: 76-year-old woman treated with INH + RIF X 1 month, then RIF stopped due to hepatitis. After LFTs normalized, treatment reinitiated with ETH + STR for 60 days. ▪ Case 2: 23-year-old man treated with RHZE X 2 months, then surgical excision of nodules. Additional STR + ETH X 90 days, then RIF + ETH X 10 months	▪ Case 1 had complete healing of all 10 nodules (between 2 and 4 cm in diameter) and 7 ulcers (between 2 and 10 cm in diameter). ▪ Case 2 had healing of all cutaneous lesions (10 ulcers between 1 and 5cm in diameter).
Guerra *et al.* (2008)	8 patients with *M. ulcerans* infection in Peru. 3 patients lost to follow-up.	▪ Cases 1, 2 and 5 lost to follow-up ▪ Case 3: RIF + ETH X 5 wks; abx d/c for hepatotoxicity. Then, CIP + TMP-SMX X 15 days. ▪ Case 4: initially diagnosed with leismaniasis and treated with sodium stibogluconate, but developed new lesions and treated with herbal medicine. Then diagnosed as Buruli ulcer. Lesions improved after drainage so no other treatment initiated. ▪ Case 6: RHZE X 6 months and surgery ▪ Case 7: RIF + STR X 8 wks and surgery ▪ Case 8: RHZE and surgery. Then 4 months of MIN + CIP + SXT	▪ Case 3 had 2 lesions on both knees, and 8 months after start of treatment, only a small ulcer remained on right knee. Complete remission of lesions seen at follow-up visits 3 and 5 years after diagnosis. ▪ Case 4 was treated with herbal treatments until total cure. No recurrence at 5-year follow-up. ▪ Cases 6 – 8 had complete resolution without recurrence after 2 years of follow-up.
Gordon *et al.* (2010)	4 patients with *M. ulcerans* infection in coastal Victoria, Australia.	▪ Case 1, 2: RIF + MXF X 6 wks, prior to surgery, followed by 6 additional weeks of antibiotics ▪ Case 3: RIF + CLR X 4 wks, prior to surgery, followed by 3 additional weeks of antibiotics ▪ Case 4: RIF + CLR X 8 wks, without resection	▪ Cases 1, 2 and 3 had complete resolution of lesions with no recurrence at 36, 13 and 12 months, respectively. ▪ In case 4, the ulcer had reduced to a small palpable nodule at end of treatment course. Then, 4 weeks after stopping antibiotics the lesion became inflamed and spontaneously drained. Culture of drainage was culture-negative, suggesting this represented an immune-mediated "paradoxical reaction".

Kouame *et al.* (2010)	8 patients from Côte d'Ivoire with *M. ulcerans* infection involving the face	▪ 6 patients received RIF + OFX for 3 to 6 months, along with enoxaparin for 2 months ▪ 2 patients received KAN + RIF, as well as enoxaparin.	▪ Two patients with non-ulcerative lesions cured without any sequelae; 6 patients with ulcerated disease cured with sequelae. ▪ No disaggregation of outcomes by treatment regimen.
Nakanaga K, Hoshino Y, Yotsu RR, Makino M, Ishii N (2011)	19 Japanese patients with Buruli ulcer disease	▪ All patients treated with various antibiotic regimens, the most common shown here: o Single-drug: CLR (n=2) o Two-drugs: RIF+CLR (n=2), o 3-drugs: RIF+CLR+LVX (n=3) ▪ 13 patients underwent surgical excision and 9 needed skin grafting	▪ Most common antibiotics used were CLR and RIF, used in 12 and 9 patients, respectively. ▪ In 2 cases, initial choice of antibiotics ineffective and were changed. ▪ In 2 other patients, antibiotics discontinued due to adverse effects. ▪ All patients had healing with no relapse at at least 3-months of follow-up. ▪ *M. ulcerans* subsp. *Shinshuense* identified in all patients. *In vitro* susceptibilities of these isolates to STR, KAN, and CLR are higher than those of *M. ulcerans* strains from W. Africa.
Phanzu *et al.* (2011)	13 patients from the Democratic Republic of Congo with clinically diagnosed with *M. ulcerans* infection involving the face	▪ 10 patients with laboratory confirmation of Buruli ulcer disease ▪ 9 patients treated with RIF + STR, then surgery ▪ 3 patients treated with surgery only, and 1 with antibiotics alone	▪ Antibiotic duration between 60 and 90 days ▪ Clinical response to antibiotics seemed to be associated with disease stage: earlier-stage lesions had better responses. ▪ 1 patient of the 10 treated with antibiotics had relapse, although could not rule out possible paradoxical reaction.
Case reports:			
Delaporte E, Alfandari S, Piette F (1994)	30-year-old pregnant woman with HIV diagnosed with *M. ulcerans* infection from Democratic Republic of Congo	▪ Treated with INH + RIF + ETH X 2 months, then treated with RIF + CLR X 5 months.	▪ Patient's CD4 count > 500/mm^3 ▪ Rapid remission of lower extremity lesion with complete healing in 8 months. ▪ No relapse in 6-month period after antibiotics completed.

Farber ER & Tsang A (1967)	20-year-old Peace Corp volunteer with probable Buruli ulcer disease after return from 20-month stay in Nigeria	▪ Treated with numerous antibiotics without improvement. Then underwent amputation and treated with CFZ	▪ Given no improvement with antibiotics, patient underwent amputation but postoperatively had wound dehiscence so was treated with CFZ. No data on response to CFZ.
Song M, Vincke G, Vanachter H, Benekens J, Achten G (1985)	22-year-old patient with *M. ulcerans* infection from Democratic Republic of Congo	▪ Treated initially with SXT, then switched to RIF based on culture results for 6 months. ▪ Fistulous tracts identified and minocycline added to rifampin for additional 6 months. ▪ Dextran polymer applied topically to help with wound debridement	▪ Lesion healed after 1-year of therapy.
Tsukamura M, & Mikoshiba H, (1982)	19-year-old Japanese woman with *M. ulcerans* infection	▪ Treated with 2 weeks of RIF	▪ Initial lesion of 5cm in diameter healed completely.
Semret M, Koromihis G, MacLean JD, Libman M, Ward BJ (1999)	36-year-old Canadian man with *M. ulcerans* infection after travel to northern, West and Central Africa.	▪ Treated with cloxacillin X 7 days, then metronidazole and doxycycline X 10 days, without benefit ▪ Once acid-fast bacilli identified on tissue biopsy, started on RIF + ETH + SXT, for 8 weeks. Then CLR + CIP added. Underwent multiple debridements ▪ 5-drug regimen continued for 10 weeks. ETH stopped due to paresthesias. ▪ Paresthesias continued for 3 more months until CIP and TMP-SMX stopped ▪ RIF + CLR continued for total of 18 months	▪ In addition to multiple debridements, patient also underwent radical excision of the lesion and split thickness skin graft about 8 months into treatment. ▪ Complete healing of lesion after 18-month treatment period. ▪ No recurrence within 4-year follow-up period.

Faber *et al.* (2000)	40-year-old Chinese woman with Buruli ulcer disease	▪ Treated with RIF + CLR X 2 months. Then *M. ulcerans* strain found to be resistant to rifampicin, so switched to CIP + RFB X 4 weeks.	▪ After 2 months with RIF + CLR, ulcer was nearly completely healed with only small defect. Lesion was culture-negative at this time. ▪ Treated with CIP + RFB for 4 weeks before abx had to be stopped due to leukopenia. ▪ Ulcer healed with scarring. Several months after treatment completed, scarring with erythematous induration was excised by patient request (tissue biopsy was culture-negative).
Johnson *et al.* (2002)	27-year-old HIV-positive woman with disseminated *M. ulcerans* infection (including osteomyelitis)	▪ Initial lesion excised with skin grafting. New lesion appeared within months and was excised. 3 months of RIF + ETH + CLR given. Continued to have disease progression requiring multiple debridements and excisions.	▪ Disseminated Buruli ulcer disease with multifocal lesions and osteomyelitis despite numerous surgical excisions and debridements and 3 months of RIF + ETH + CLR. ▪ Patient left against medical advice to pursue care by traditional healers. Passed away shortly thereafter.
Pszolla N *et al.* (2003)	4-year-old boy from Angola with disseminated *M. ulcerans* infection (including osteomyelitis)	▪ Multiple lesions treated with repeat debridements, and RFB + CLR + Pto. ▪ Some of the lesions progressed, so repeat debridements. RFB + CLR continued but Pto stopped and SXT added. ▪ After 3 months of antibiotics, CLR stopped. New metastatic lesions developed, and antibiotic regimen changed to CLR + RFB ▪ 5 months into treatment, hyperbaric oxygenation therapy (HBO) initiated. Within 6 weeks of HBO, wound care and antibiotics all lesions had closed	▪ Shortly after all lesions appeared to have healed, patient found to have osteomyelitis of left distal femur. Whole-body scan showed new focus of infection in distal part of left humerus. ▪ ETH and CFZ added to CLR + RFB. Antimicrobial therapy was ongoing until patient returned to Angola and was lost to follow-up.
Millay *et al.* (2006)	14-year-old boy from West Victoria, Australia, with *M. ulcerans* infection	▪ Treated with AMK + AZM + RIF, along with surgical debridement and skin grafting ▪ After 3 weeks, patient discharged home on RIF + AZM	▪ *M. ulcerans* was cultured from tissue biopsy after 11 weeks of antibiotics. ▪ After 3 months of treatment, wound was healing well with residual scarring.

Kibadi K. (2008)	9-year-old boy with *M. ulcerans* infection from the Democratic Republic of Congo	▪ Treated with surgical excision and grafting, and antibiotic course of RHZE for 2 months ▪ Received steroid taper	▪ Patient had healed lesion without recurrence after 6-year follow-up.
O'Brien DP, Athan E, Hughes A, Johnson PD (2008)	87-year-old woman from southeastern Australia with *M. ulcerans* infection involving osteomyelitis	▪ Treated with multiple surgical interventions and adjunctive antibiotic courses.	▪ Initial right calf ulcer surgically excised with primary closure and clean margins. ▪ 14 days later, new left heel lesion, treated with wide excision and free flap. ▪ Adjunctive RIF + ETH + AMK + CLR, but new lesion on right buttock that was also excised. ▪ Had drug reaction to CLR, so abx changed to RIF + AMK. AMK stopped due to ototoxicity. ▪ Continued on RIF X 4 months, but disease progression requiring multiple debridements. CIP added. ▪ Patient elected for no further surgery, completed 6 months of RIF + CIP, with wound healing. No disease recurrence over 36-month follow-up period.
Ezzedine *et al.* (2009)	24-year-old Caucasian man, returning from long-term travel to Senegal, with *M. ulcerans* infection	▪ RIF + MXF, plan for 12 weeks	▪ Patient lost to follow-up after 15 days of treatment initiation.
Ezzedine *et al.* (2010)	44-year-old Malian visitor to France with *M. ulcerans* infection	▪ RIF + CLR, plan for 8 weeks	▪ Patient returned to Mali and lost to follow-up.
Kibadi *et al.* (2010)	35-year-old man with HIV in Democratic Republic of Congo	▪ RIF + STR for 12 weeks	▪ After 8 weeks of antibiotics, enlargement of all lesions observed and development of new ulcer. Antibiotics continued for additional 4 weeks. ▪ Patient died 2 weeks after treatment completed, just when ARV scheduled to begin.
Minime-Lingoupou *et al.* (2010)	2 patients from the Central African Republic diagnosed with *M. ulcerans* infection	▪ Case 1: 62-year-old man with large ulceration of right lower extremity. Died of unknown case before treatment could be started ▪ Case 2: 62-year old man with left ankle ulceration treated with RIF + STR X 8 weeks.	▪ The second patient's lesion had receded in size after 8 weeks of antibiotics. ▪ First case report of Buruli ulcer disease in Central African Republic.

Watanabe *et al.* (2010)	46-year-old man Japanese man with Buruli ulcer disease	▪ Treated initially with steroids. After diagnosis as Buruli ulcer, treated with LVX + CLR for unspecified duration, followed by debridement and skin graft closure.	▪ Initial lesion misdiagnosed as insect bite and treated with steroids. Had improvement at first but then worsening of ulcer, so underwent skin biopsy. Histopathology showed dense neutrophilic infiltration so presumed neutrophilic dermatosis and steroids resumed. Lesions gradually formed painless ulceration with necrotic mass, and AFB identified using direct smear examination. PCR positive for *M. ulcerans*. ▪ Treated with LVX + CLR, disease progression halted. ▪ Given poor epithelization/healing, patient underwent debridement and skin graft closure ▪ No recurrence during 6-month follow-up period.
Onoe *et al.* (2012)	52-year-old Japanese woman with *M. ulcerans* infection	▪ Initial lesion: CLR + RIF + LVX X 6 wks, then surgery ▪ Recurrent lesion: CLR + RIF X 10 weeks	▪ No improvement in facial ulcer after 6 weeks of antibiotics so patient underwent excisional surgery. ▪ Then developed recurrent lesion, with reduction in size by half after antibiotics.
Tsukagoshi S, Dehn TCB (2012)	9-month-old boy in Uganda with presumed Buruli ulcer	▪ Right forearm edema (Ziehl-Neelson staining negative): gentamicin, metronidazole and ceftriaxone initially. Then underwent multiple surgeries, and started on RIF + INH (only preparation of RIF available; STR not available)	▪ Right forearm edema evolved into 3 ulcers with undermined edges. Multiple debridements in first 10 days after presentation, with improvement in lethargy and appearance of wounds. Lesions reduced in size by 50% with antibiotics. However, diagnosed with osteomyelitis of distal radius and transferred to another hospital for planned sequestrectomy with skin grafting.

* Buruli ulcer cases were laboratory-confirmed unless otherwise specified.
Abbreviations: amikacin, AMK; azithromycin, AZM; ARV, antiretrovirals; ciprofloxacin, CIP; clarithromycin, CLR; clofazamine, CFZ; levofloxacin, LVX; minocycline, MIN; rifabutin, RFB; rifampin + isoniazid + pyrazinamide + ethambutol, RHZE; rifampicin, RIF; rifapentine, RFP; streptomycin, STR; co-trimoxazole, SXT

Table A.4 – Drugs not suitable for application in the treatment of Buruli ulcer disease

Drug	Activity against *M. ulcerans*	Route of administration	Contraindications	Side effects	Other
Isoniazid	No			Hepatitis	
Ethambutol	No				
Pyrazinamide	No			Hepatotoxicity	
Streptomycin	Yes	Injectable	Pregnancy category D	Ototoxicity; nephrotoxicity	
Kanamycin	Yes	Injectable	Pregnancy category D	Ototoxicity; nephrotoxicity	
Amikacin	Yes	Injectable	Pregnancy category D	Ototoxicity; nephrotoxicity	
Capreomycin	No clear activity	Injectable	Pregnancy category C	Ototoxicity; nephrotoxicity	
Viomycin	No data	Injectable	Pregnancy category C	Ototoxicity; nephrotoxicity	
Sparfloxacin				Phototoxicity – Withdrawal from U.S. market	
Cycloserine	Weak activity in animal models		Pregnancy category C	CNS toxicity	Difficult procurement
Terizidone	No data available, but as a different formulation of cycloserine, anticipate would have weak activity			CNS toxicity	Difficult procurement
Ethionamide	No				
Prothionamide	No data		Pregnancy category C	Severe GI intolerance; Hepatic dysfunction,	Difficult procurement
PAS	No			GI disorders, hepatotoxicity	
Thioacetazone	No			GI disorders	
Clofazimine	*In vitro* activity, but no convincing evidence of efficacy in mouse models or patients		Pregnancy category C	Skin discoloration	
Linezolid	Weak bactericidal activity in mouse curative model		Pregnancy category C	Thrombocytopenia	High cost
Minocycline	Weak bacteriostatic activity in mouse footpad preventive model		Pregnancy category D. Not recommended in children <8 years old		
Doxycycline	No data		Pregnancy category D.		

Drug	Activity against *M. ulcerans*	Route of administration	Contraindications	Side effects	Other
			Not recommended in children <8 years old		
Imipenem	No data	Injectable			
Meropenem-clavulanate	No data	Injectable			
Cefoxitin	No data	Injectable			
Tobramycin	No data	Injectable			
Telithromycin	Moderately susceptibility *in vitro*. Weak bacteriostatic activity in mouse model				
PA-824	No				

Abbreviations: CNS, central nervous system; GI, gastrointestinal; U.S., United States.

MSF Access Campaign

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