



Antimicrobial Treatment of *Mycobacterium ulcerans* Infection

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1 Historical Aspects

MacCallum and coworkers described Buruli ulcer (BU) as an infectious disease caused by *Mycobacterium ulcerans* in Victoria, Australia. They first considered the skin lesions in their patients to be caused by tuberculosis or leprosy, when they observed numerous acid-fast bacilli in the biopsy specimens [1]. The typical duration of illness was between 1 and 2 years; treatment was essentially surgical. With the advent of chemotherapy for tuberculosis [2–4], and later for leprosy, doctors made individual attempts to treat the lesions with anti-tuberculosis and anti-leprosy drugs. The anecdotal evidence suggested poor or no response to chemotherapy with rifampicin monotherapy [5], despite the fact that in vitro susceptibility of 33 strains of *M. ulcerans* was as good as for *M. tuberculosis* [6]. A randomized clinical trial by the British Medical Research Council in Buruli county (now called Nakasongola; Uganda) failed to show any benefit from clofazimine, a drug then first marketed for leprosy [7]. A small-sized trial with cotrimoxazole (18 participants; 12 evaluable) was inconclusive [8]. A small-sized randomized study in Côte d'Ivoire compared a

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combination of dapsone and rifampicin with placebo; the follow-up was limited; the ulcer size decreased slightly faster in the intervention group but the baseline characteristics of both groups differed, and the study did not allow to draw any firm conclusions about the effectiveness of these drugs [9]. By the turn of the millennium, the discrepancy between in vitro efficacy of rifampicin [6] or clarithromycin [10] and lack of clinical response prompted to stressing the need for well-designed and well-powered drug trials, but in the meantime, to also improve early detection and surgical treatment [11].

One important question that comes to mind when looking back at earlier studies that failed to show effectiveness of antimicrobial treatment for BU is how the clinical response was assessed. Even long before the chemical structure of the toxin mycolactone was discovered [12], it was realized that a toxin secreted by *M. ulcerans* was responsible for the extensive tissue necrosis [13, 14] as well as for the immune suppression observed and replicated in experimental animals, using a culture filtrate of *M. ulcerans* [15]. Radical surgical excision was to some extent effective but at the cost of tissue loss, and the need for plastic surgery, e.g., split-skin or full-thickness skin grafting. Reported recurrence rates following surgical treatment were variable; one follow-up study from Benin reported 6.1% recurrence in a subset of patients (66/150; 44%) followed up to 7 years after surgery [16]. In a case series of 346 patients operated in three centers in Côte d'Ivoire, the recurrence rate was 17.1% [17]. A large difference in recurrence rates was reported from two centers in Ghana; 21/45 (47%) of patients had a recurrence in one hospital, as compared to 6/33 (18%) in the other hospital [18]. In a case series from the Bas Congo, DR Congo, of 51 patients seen over 2 consecutive years, 14 (39%) had recurrent disease after previous surgery [19]. Lesions at a pre-ulcerative stage can be excised successfully with primary closure, however a 1-year recurrence rate of 16% was observed in a cohort of 50 patients in Ghana [20].

With increased understanding of the dominant role of the secreted toxin mycolactone in the pathogenesis of Buruli ulcer, and realizing that perhaps the best measure of efficacy of antimicrobial treatment might be complete healing without recurrence, the effectiveness of antimicrobial treatment might have been underestimated in the past. Indeed with killing of *M. ulcerans* in lesions, as exemplified by the beneficial effects of different classes of antimicrobials in vitro [6, 10, 21], mycolactone secretion might stop, but the impact on tissue damage including necrosis would conceivably take more time than in any other infectious disease. Likewise, there was increased understanding of the profound effects of mycolactone on the immune system, with little local [22] and systemic inflammation [15]. Yet another factor perhaps blurring the observation of response to antimicrobial therapy is a common paradoxical increase in lesion size, development of new lesions, and increased inflammation following antimicrobial therapy [23–25]. Apart from the in vitro studies [6, 10, 21], several studies in animal models had shown the efficacy of anti-mycobacterial drug combinations [26–28].

A formal proof-of-concept study to evaluate the potential of antimicrobial treatment without additional surgery was the landmark study conducted in Ghana,

under the umbrella of the WHO Global Buruli Ulcer Initiative [29]. Patients having non-ulcerated lesions suspected to be *M. ulcerans* infection were randomly allocated to 0, 4, 8 or 12 weeks of antimicrobial treatment with a combination of streptomycin and rifampicin; later a 5th group receiving 2 weeks of treatment was added; the total number of study participants was 21. Lesions were subsequently surgically removed and submitted for culture, PCR and histopathology. As no bacteriological confirmation tests were carried out prior to the start of therapy, some of the lesions remained bacteriologically unconfirmed to be Buruli ulcer disease; none of the patients receiving at least 4 weeks of treatment had viable bacilli by culture of their excised lesion; none had recurrent disease 12 months after start of treatment. Although some questions remained unanswered, this study provided the first robust evidence that antimicrobial treatment alone was able to sterilize non-ulcerated lesions of *M. ulcerans* infection, with no recurrence or positive culture in patients treated for at least 4 weeks with the antimicrobial combination [29].

With the introduction of antimicrobial treatment, a more conservative, less aggressive approach of surgery in addition to antimicrobial treatment was subsequently advocated [30–33].

For over 10 years now, the WHO has recommended antimicrobial treatment as the primary treatment modality. Below, we systematically discuss the literature on antimicrobial susceptibility of *M. ulcerans* in vitro and in animal models; and the accumulated evidence for effectiveness as well as safety (i.e., adverse reactions) of antimicrobial treatment regimens emerging from clinical studies.

2 Antimicrobial Susceptibility of *M. ulcerans*

For the review of in vitro activity of antimicrobial agents against *M. ulcerans*, we used a systematic approach searching the literature in PubMed. Using ‘(Buruli OR ulcerans) AND (antibiotic OR antimicrobial OR in-vitro OR susceptible)’, we retrieved 520 unique results that we further analyzed for duplicates and consistent reporting. We cross-searched references obtained from articles we analyzed.

The most widely used antimicrobials for *M. ulcerans* infection have been rifampin, streptomycin and clarithromycin. *M. ulcerans* is a phylogenetically close relative of *M. tuberculosis* and *M. leprae* and has evolved from a common *M. marinum*-like ancestor [34]. Subsequently, many attempts to treat *M. ulcerans* infection have been undertaken seeking to use and repurpose antibiotics active against *M. tuberculosis* and *M. leprae*. To estimate efficacy in vitro, the usual way to screen these agents is by determining the minimal inhibitory concentration (MIC) of inoculates of *M. ulcerans* in mycobacterial culture media. Next, inoculation of viable *M. ulcerans* in the mouse footpad is a well-established in vivo model to assess efficacy. The final test is eventually to test these agents in patients affected by Buruli ulcer disease. Such studies are extremely challenging, as the majority of potential study

participants are patients (many being children) that live in underprivileged circumstances with limited access to health care, and generally low educational background [35–37].

2.1 Ansamycins/Rifamycins

Ansamycins or rifamycins act on mycobacteria by inhibiting RNA synthesis through interfering with bacterial RNA-polymerase. Rifampin is the rifamycin most widely used in the treatment of Buruli ulcer. The drug has a strong bactericidal effect and an MIC of approximately 0.5 µg/ml [38–40]. Rifampin is administered orally at a dose of 10 mg/kg to humans to treat Buruli ulcer and is the backbone-drug of most regimens evaluated to date. Other rifamycins that have been shown to kill *M. ulcerans* are the rifampin-analogues rifabutin [26] and rifapentine [41]. Rifapentine is highly active against *M. ulcerans* with an MIC of 0.125–0.5 µg/ml [41, 42]. Because of its longer half-life time compared to rifampin, an intermitted rifapentine-based regimen with only two or three times weekly administration of antibiotics has been tested in mice [41]. Such an intermittent regimen is suggested to allow outpatient management and to simplify clinical management. However intermittent regimens also bear the risk of confusion and low compliance and patients being subsequently lost during treatment. Even though antibiotic resistance is not a major concern in the slowly replicating organism *M. ulcerans*, rifampicin resistance has been detected in rare instances [43, 44]. In-vivo, resistant mutants were isolated after monotherapy with rifampin in mice, which should be avoided in the clinical setting. The resistance was conferred by mutations in the *rpoB* gene of *M. ulcerans* [45]. Albeit uncommon and of unknown clinical significance, researchers in Ghana found 5.7–11.4% of isolates from their study districts to be resistant to 40 µg/ml rifampin [40]. Yet another rifamycin tested in *M. ulcerans* is rifabutin. Rifabutin has an MIC of 0.1–0.4 µg/ml against *M. ulcerans* [39].

2.2 Aminoglycosides: Streptomycin

Streptomycin is an injectable aminoglycoside with a strong bactericidal effect on *M. ulcerans* and one of the first agents ever shown to be effective against this organism [46, 47]. It is a protein synthesis inhibitor that hinders binding of formyl-methionyl-tRNA to the bacterial ribosomal 30S subunit. In the mouse model, streptomycin remains one of the most active agents against *M. ulcerans* and is used as intramuscular injection of 15 mg/kg in humans [48]. In single drug in vitro and in vivo studies, streptomycin was more active than amikacin and linezolid, but less active than rifampicin and moxifloxacin [49]. In preclinical studies, streptomycin-containing regimens outperform all other commonly used drug combinations both in terms of colony-forming units remaining in infected mouse footpads and recurrence rate. However, the need to inject streptomycin is very impracticable in the rural African setting where Buruli ulcer is most common. It bears the risk of parenteral

(needle-associated) infection and also threatens patient compliance due to fear of painful, daily injections, especially in children. Besides, streptomycin causes ototoxicity as well as nephrotoxicity as observed in patients followed after having been enrolled in the BURULICO trial [50].

2.3 Amikacin

Amikacin is another aminoglycoside that has been suggested for use in the treatment of patients with Buruli ulcer. It is highly active against *M. ulcerans* with an MIC of 0.5–1 µg/ml [21] and was shown to be equally efficient in the mouse model [26]. In a study testing a series of isolates, the MIC ranged between 0.25–1 (mean, 0.65) µg/ml [49]. Amikacin was as effective as streptomycin in the *M. ulcerans* mouse model [26].

2.4 Macrolides: Clarithromycin

Clarithromycin is a protein synthesis inhibitor of the macrolide family. It reversibly binds to the 23S rRNA on the 50s ribosomal subunit and subsequently prevents polypeptide synthesis. It acts mainly as a bacteriostatic drug in *M. ulcerans* therapy. The MIC of clarithromycin against *M. ulcerans* is approximately 0.12 µg/ml [10, 26, 51, 52]. Clarithromycin is well tolerated, it can be taken orally and it is widely available. It has been shown to be non-inferior when replacing streptomycin after 4 weeks in the 8 week rifampin-streptomycin regimen while treating small lesions [36]. Following preliminary results of a randomized controlled trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01659437) identifier: NCT01659437) comparing 8 weeks rifampicin plus streptomycin with 8 weeks rifampicin plus clarithromycin, the latter regimen is now recommended by WHO.¹

2.5 Azithromycin

Azithromycin is a macrolide antimicrobial agent active against a variety of organisms. The mean MIC against a collection of different strains was 0.39 µg/ml [40]. However when 100 mg of azithromycin was administered to mice infected with *M. ulcerans*, only a modest bacteriostatic activity was observed [53].

2.6 Fluoroquinolones

Various fluoroquinolones such as ciprofloxacin, ofloxacin and moxifloxacin have been shown to be active against *M. ulcerans* with MICs ranging from 0.25 to 1 µg/ml

¹http://www.who.int/neglected_diseases/events/WHO_BU_TAG_2017_report.pdf?ua=1.

[21]. Fluoroquinolones are bacterial DNA gyrase inhibitors. They impede DNA replication and transcription by hindering the DNA gyrase-catalyzed super-coiling of double-stranded DNA. In Australia, Buruli ulcer patients are frequently treated with rifampicin in combination with a fluoroquinolone, such as ciprofloxacin [54]. Such fluoroquinolones are administered orally and have favorable pharmacokinetic properties such as good tissue penetration. However, there is some concern about the use of fluoroquinolones in children; in Australia most Buruli ulcer patients are adults, most of them elderly, whereas in Africa where most of the cases are reported, the median age of patients is around 15 years. Fluoroquinolones may cause serious arthropathy in children and minors. However the evidence about increased toxicity in the young is incomplete. Although caution remains warranted, fluoroquinolones have increasingly been used in children without significantly increased adverse events or toxicity [55, 56]. The most potent fluoroquinolone against *M. ulcerans* is sparfloxacin, with an MIC at 0.25 $\mu\text{g/ml}$ in most strains tested [21]. The MIC of ofloxacin in tested strains of *M. ulcerans* was 1.26 $\mu\text{g/ml}$ [40]; in another study, ofloxacin was least active of all fluoroquinolones tested with an MIC of 2 $\mu\text{g/ml}$ [21]. Moxifloxacin (MIC 0.14 $\mu\text{g/ml}$), sitafloxacin (MIC 0.125–0.5 $\mu\text{g/ml}$) and prulifloxacin (MIC 1–3 μM) are also effective [49, 57, 58]. Of note, fluoroquinolones have increasingly been used in Ghana, one of the countries highly burdened with Buruli ulcer; an alarming rate of fluoroquinolone-resistant *E. coli* was noticed [59].

2.7 Clofazimine

Clofazimine is an anti-leprosy drug that has emerged as a critically important sterilizing drug in the treatment of multi-drug resistant tuberculosis. Its effect on mycobacteria is delayed but strongly bactericidal [60]. The mechanism of action is multifactorial, complex and not fully understood. As a prodrug, it is reduced by type 2 NADH-quinone oxidoreductase resulting in the release of bactericidal quantities of reactive oxygen species. It is then believed to competitively inhibit menaquinone, a crucial electron acceptor in the mycobacterial respiration chain [61]. Clofazimine possesses also some poorly understood anti-inflammatory activity for which it is used in conditions like severe acne or discoid lupus erythematosus [62]. These anti-inflammatory properties might also be part of its mode of action in mycobacterial infections where tissue damage by inflammatory processes is a hallmark. The MIC for various strains of *M. ulcerans* was 2.19 $\mu\text{g/ml}$ on average [40]. As mentioned above, in an early trial clofazimine monotherapy was inefficacious in human Buruli ulcer patients [7]. In mice, clofazimine in combination with rifampicin was very effective with relapse-free cure following a 6-weeks oral regimen [63]. Clofazimine causes yellow-orange skin-discoloration as well as gastrointestinal disturbances as adverse effects [64]. Newer clofazimine analogs with reduced accumulation and thus less risk for skin discoloration are under development [58].

2.8 Dapsone

Dapsone is an anti-leprosy drug that has widely been used. Activity against *M. ulcerans* is moderate—the MIC ranged between 2.0 and 4.0 µg/ml in one study [39] and the mean MIC was 0.94 µg/ml in another study [40].

2.9 Doxycycline

Doxycycline, a tetracycline, was inactive against *M. ulcerans* in one study [58].

2.10 Oxazolidinones

Linezolid showed only intermediate activity with an MIC ranging from 3 to 10 µM; as posizolid and sutezolid. This oxazolidinone is currently considered as a major component of second-line tuberculosis treatment [58, 65]. In another study with 29 isolates of *M. ulcerans* tested, the mean MIC was 0.73 µg/ml [49].

2.11 Avermectins

Avermectins are a class of anti-helminth and anti-parasite drugs that are thought to have intermediate efficacy on *M. tuberculosis* [66]. Subsequent testing showed the MIC for ivermectin and moxidectin ranging from 4 to 8 µg/ml [67]. In another study, the MIC for selamectin was 2–4 µg/ml and ivermectin and moxidectin showed no activity >32 µg/ml [68].

2.12 Trimethoprim and Epiroprim

Trimethoprim was not effective against *M. ulcerans*. However, epiroprim, another dihydrofolate reductase inhibitor, showed an MIC of 0.5–1.0 µg/ml [69].

3 Experimental Drugs

A series of experimental compounds, mainly originating from TB drug research pipelines were tested for use in *M. ulcerans* chemotherapy.

Diarylthiazoles and 1,3-diaryltriazenes are compounds with high potency against mycobacteria, including *M. ulcerans*. Different experimental 1,3-diaryltriazene analogues showed sub-micro molar inhibitory activity against *M. ulcerans* 1615 lux [70]. Similarly, diarylthiazoles, like fatostatins, were found to have a significant efficacy against *M. ulcerans* [58]. KRM-1648, a benzoxazinorifamycin with good efficacy in the TB mouse model was equally tested for use in *M. ulcerans* and showed

good killing at an MIC of 12–25 $\mu\text{g/l}$ [39]. The diarylquinoline bedaquiline, also a TB research drug had a mean MIC of 0.03 (0.015–0.12) $\mu\text{g/ml}$ when tested against a set of 29 isolates [49].

GyrB is a common drug target in TB therapy and experimental GyrB inhibitors showed good to moderate activities (MIC 0.3–10 μM) against *M. ulcerans* [58]. In the same study, pyrrolamide and aminopyrazinamide exhibited activity with an MIC of 0.3–1.0 μM [58]. These investigators found one aminopyrazole, as well as pyrazolopyrimidine and one hydroxyquinolone to kill *M. ulcerans* with MICs from 0.3 to 3.0 μM [58].

There is high genetic similarity between *M. tuberculosis* and *M. ulcerans*. Yet some of the compounds that are active against the first, failed to prove efficacy in the latter. SQ641, PA-824 a promising nitroimidazopyran for tuberculosis, semisynthetic ketolides (HMR 3647 and HMR 3004), bisbenzaldehydes, as well as quinolinyl pyrimidines and phenothiazines showed only moderate or no antimicrobial effect against *M. ulcerans* (Table 1) [21, 39, 49, 51, 52, 57, 58, 67, 70].

Table 1 Summary of compounds tested against *M. ulcerans* in vitro

Compound	Class	Mic in-vitro
Rifampin	Rifamycin	0.1–0.81 $\mu\text{g/ml}$
Rifabutin	Rifamycin	0.1–0.4 $\mu\text{g/ml}$
Rifapentine	Rifamycin	0.125–0.5 $\mu\text{g/ml}$
Dapsone	Sulfone	0.94–4.0 mg/l
Doxycycline	Tetracycline	Inactive
Streptomycin	Aminoglycoside	0.33–1.10 $\mu\text{g/ml}$
Amikacin	Aminoglycoside	0.5–0.65 $\mu\text{g/ml}$
Azithromycin	Macrolide	0.39 $\mu\text{g/ml}$
Clarithromycin	Macrolide	0.125–1.25 $\mu\text{g/ml}$
Ofloxacin	Quinolone	1.26–2.0 $\mu\text{g/ml}$
Ciprofloxacin	Quinolone	1.15 $\mu\text{g/ml}$; 1–3 μM
Sparfloxacin	Quinolone	0.25 mg/l
Moxifloxacin	Quinolone	0.14 $\mu\text{g/ml}$
Sitafoxacin	Quinolone	0.125–0.5 $\mu\text{g/ml}$
Prulifloxacin	Quinolone	1–3 μM
GyrA_NTBI-analog	Topoisomerase II inhibitor	Inactive
GYRA-NTBI PubChem_15983305	Topoisomerase II inhibitor	Inactive
GyrB_Pyrrolamide PubChem_25223515	Pyrrolamide	0.3–1.0 μM
GyrB_Aminopyrazinamide	Aminopyrazinamide	0.3–1.0 μM
Oxazolidinone PubChem_10251911	Oxazolidinone	0.3–1.0 μM
Linezolid	Oxazolidinone	3–10 μM ; 0.73 $\mu\text{g/ml}$
Posizolid	Oxazolidinone	3–10 μM
PubChem_10251911	Oxazolidinone	0.3–1.0 μM
Clofazimine	Riminophenazine	2.19 $\mu\text{g/ml}$
Selamectin	Avermectin	2–4 $\mu\text{g/ml}$
Ivermectin	Avermectin	4–32 $\mu\text{g/ml}$
Moxidectin	Avermectin	4–32 $\mu\text{g/ml}$

Compound	Class	Mic in-vitro
HMR 3647	Ketolide	5–40 µg/ml
HMR 3004	Ketolide	5–40 µg/ml
1,3-diaryltriazenes		
Diarylthiazoles		
SQ641		8 µg/ml
KRM-1648	Benzoxazinorifamycin	0.012 and 0.025 µg/ml
R207910	Diarylquinoline	0.03 µg/ml
PA-824	Nitroimidazopyran	13.1 µg/ml
Aminopyrazoles		0.3–1.0 µM
Pyrazolopyrimidines		0.3–1.0 µM
hydroxyquinolones		1–3 µM
Quinoliny pyrimidines		Inactive
Phenothiazines		Inactive
Diazene-1,2-dicarboxamides		5.67–7.25 µg/ml

4 Clinical Studies

As mentioned above, the shift to antimicrobial therapy was made following the landmark study by Etuaful et al. using 15 mg/kg body weight intramuscular streptomycin and 10 mg/kg body weight oral rifampin [29]. The earlier mentioned randomized placebo-controlled study from Côte d'Ivoire with 41 study participants testing a combination of rifampicin and dapsone lacked power and had too many dropouts with only 2 months follow-up [9].

During the past decade, case series and observational cohort studies of patients with BU were reported that we discuss here; case reports will not be discussed as BU patients may occasionally heal spontaneously [71, 72]. One observational cohort study in Benin reported on 224 patients, the majority with large (category III; >15 cm) lesions. 215 (96%) were categorized as treatment successes, and 9, including 1 death and 8 losses to follow-up, were treatment failures. Of the 215 successfully treated patients, 102 (47%) were treated exclusively with antibiotics and 113 (53%) were treated with antibiotics plus surgical excision and skin grafting; 73% of patients with lesions of >15 cm in diameter underwent surgery, whereas only 17% of patients with lesions of <5 cm had surgery [73]. Compliance with therapy was excellent; 208 of the 215 patients were actively retrieved 1 year after treatment and 3 (1.44%) of the 208 retrieved patients had recurrence of *M. ulcerans* disease, 2 among the 107 patients treated only with antibiotics and 1 among the 108 patients treated with antibiotics plus surgery. In a report from Australia, 40 patients received combined antimicrobial and surgical treatment. Failures occurred often but twice as often in the group that received surgery as the only treatment modality [74]. The Australian guidelines of 2006 (published in 2007) still proposed radical surgery including the removal of a rim of apparently healthy tissue for treatment [75]. Several other groups have reported on outcome of combined surgical and antimicrobial treatment—of 79 (61%) patients retrieved, 7 (9%) had a recurrence [76]. In a series of 92 patients treated in the Bas-Congo, DR Congo, patients received surgery

and antimicrobial (streptomycin/rifampicin) treatment sometimes more than 8 weeks, with a high success rate (98.4% in PCR-confirmed patients) and low recurrence rate (1.1%).

With a substantial proportion of study participants receiving both surgery and antimicrobial therapy it has been difficult to tease out the potential of antimicrobial treatment alone. In larger lesions, the general assumption is that surgery as added therapy is essential to obtain wound closure; in smaller lesions, the hypothesis that antimicrobial treatment alone without extensive surgical debridement could heal BU with no recurrence, was tested in a controlled clinical trial including 151 study participants with small (<10 cm cross-sectional diameter) lesions, almost all being confirmed by PCR to have *M. ulcerans* infection. After 4 weeks of streptomycin/rifampicin combination therapy, patients were randomized to either continue for 4 more weeks or switch to oral treatment with rifampicin/clarithromycin combination therapy. A switch from streptomycin to clarithromycin after 4 weeks was non-inferior to 8 weeks streptomycin and rifampicin in these early lesions; recurrence or failure was very low—success rates were 96% in the group receiving 8 weeks of streptomycin/rifampicin, and 91% in the group that switched to oral treatment which was statistically non-inferior [36]. In an observational cohort of 160 PCR-confirmed patients with BU, treated with streptomycin/rifampicin for 8 weeks, 152 healed without surgery; of 158 patients seen 1 year later, no recurrences were noted [77]. In three centers in Ghana, 43 patients—16 of them having category II/III lesions—received the streptomycin/rifampicin combination regimen that was switched after 2 weeks to oral rifampicin/clarithromycin. Ninety-three percent had successful outcome, only one had surgery, and none had recurrence at follow-up [37]. A recent report reflects perhaps better what happens under routine service conditions [78]. Patients (n = 50) with confirmed BU in two centers in the Brong-Ahafo Region of Ghana were followed over time; a majority had first used traditional treatment; the patient population consisted predominantly of peasant farmers with no formal education, or children. Only 40 completed treatment and of those, only 28 healed; in the others the lesions reduced in size [78].

In summary, based on the above evidence from various studies and reports—notably, the potential to achieve cure without relapse, at much higher rates than previously with surgery alone, the role of antimicrobial treatment in the management of BU has become standard of care [79]. All drugs used for BU have significant side effects, especially in the elderly [80]; but aminoglycoside drugs—streptomycin and amikacin—are notoriously the most toxic compounds [81], and although most of the evidence supporting antimicrobial treatment for BU has been provided for the combination of rifampicin and streptomycin, there has been a search for an alternative, fully oral treatment for over a decade now. The first report came from Australia where the patient population is typically elderly and more vulnerable for the ototoxicity of aminoglycosides; the results of a small (n = 4) case series [82] was soon followed by larger series of 43 patients that had oral antimicrobials combined with surgery with only one patient that had a relapse [83]. Later, a series of 132 patients had oral therapy with less surgery than before and with excellent healing rates and only one relapse [84]. In Benin, all-oral treatment was given for 8 weeks to 30 patients with BU with a slightly higher daily dose of clarithromycin (12 mg/kg once daily, combined with rifampicin 10 mg/kg);

they all healed without relapse [35]. The WHO initiated a randomized clinical trial in 2012, that was recently completed (clinicaltrials.gov identifier: NCT01659437) comparing all-oral, 8-week rifampicin plus clarithromycin (in sustained release, once daily, 15 mg/kg) with standard streptomycin/rifampicin treatment; interim analysis showed non-inferiority of the all-oral group compared to the streptomycin/rifampicin group, and in the bi-annual meeting on BU in Geneva in the Spring of 2017, it was decided that sufficient evidence had been provided to change the treatment recommendation to all-oral treatment, using rifampicin and clarithromycin.² The Australian guidelines had already recommended all-oral antimicrobial treatment [85] earlier.

4.1 Secondary Infection

Wounds caused by *M. ulcerans* often harbor a multitude of secondary pathogens. Isolates of *S. aureus*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with a high degree of resistance to commonly used antibiotics were found in BU lesions in Ghana and Nigeria [86, 87]. *S. aureus* isolated from *M. ulcerans* lesions were found to harbor a large array of virulence factors. In Ghana, an association between the presence of these organisms and delayed healing was observed. The question whether these secondary bacterial invading pathogens are merely bystanders of *M. ulcerans* infection or actual contributors has not been resolved [88]. Apart from the question whether these secondary invaders inhibit and delay wound healing or not, rational use of antimicrobials other than the combination recommended by WHO, should be discouraged, in order to prevent further antimicrobial selection pressure and the further emergence of resistance among these secondary organisms [89]. The skin microbiome was significantly changed, however it is not clear if and how this contributes to pathology [90]. The option to explore topical instead of systemic antimicrobial agents, e.g., nitric oxide needs perhaps further attention [91]. For more detailed information the reader is referred to chapter “Secondary Infection of Buruli Ulcer Lesions” of this book.

4.2 HIV Co-infection

HIV co-infection complicates diagnosis and treatment of BU [92]. In 2015, a consensus statement was published by WHO; HIV testing is recommended for all patients with BU. For more detailed information the reader is referred to chapter “Management of BU-HIV Co-infection” of this book. Combined antiretroviral treatment might be postponed 4 weeks after starting streptomycin/rifampicin treatment for BU, because of drug-drug interactions and adverse drug effects, and the importance to use the most effective bactericidal drug combination available for *M. ulcerans* infection.³

² http://www.who.int/neglected_diseases/events/WHO_BU_TAG_2017_report.pdf?ua=1.

³ http://apps.who.int/iris/bitstream/10665/154241/1/WHO_HTM_NTD_IDM_2015.01_eng.pdf?ua=1.

5 Conclusions; Areas of Uncertainty; and Future Directions

There is no doubt that antimicrobial therapy—preferably, all-oral rifampicin-based treatment is essential for cure without relapse. Not all questions have been resolved, however. Despite the overwhelming evidence that the combination of clarithromycin and rifampicin works well, with acceptable levels of toxicity especially in the relatively young patient population in West Africa, the dosage of these drugs has been different across different studies and regions. Drug-drug interactions of rifampicin and clarithromycin are complex—rifampicin induces the metabolism of clarithromycin into its inactive metabolite 14-OH clarithromycin; and it induces its own hepatic elimination as well. Clarithromycin inhibits the elimination of rifampicin [93]. Increased drug exposure to rifampicin is much safer than previously thought [94].

An important next step could be to improve the dosage by extensive pharmacokinetic modeling; a fixed dose combination drug might have considerable advantage for compliance, logistics and in order to prevent monotherapy; this approach has been shown to be highly successful in tuberculosis, malaria and HIV infection.

Further, duration of therapy has not been individualized; based on the initial observations in the small study by Etuaful et al. [29], where no viable *M. ulcerans* bacilli were recovered from lesions in patients treated for at least 4 weeks, an arbitrary safety margin of 4 more weeks was chosen. The question whether larger lesions need longer treatment, or smaller lesions perhaps less has not been addressed in well-designed studies. In a follow-up study among 56 patients in Ghana, who defaulted before the end of planned treatment, 92% of patients with category I lesions (<5 cm) were healed with treatment duration of 32 days or more [95], suggesting that smaller lesions indeed might do well with much less than standard 8-weeks treatment.

Next, the treatment of BU extends well beyond administration of antimicrobials alone. During the active phase of the disease adequate wound care is imperative to ensure healing and to prevent disabling scarring. Non-adhesive, absorbent dressing materials have been shown to improve the wound microenvironment and improve time to healing [96, 97], and might also play a role to prevent painful dressing changes. While *M. ulcerans* is generally thought to be a pain-free disease as the toxin mycolactone causes hypoalgesia [98–100], later on, as lesions start to heal and the mycolactone is washed out from the lesions and the system, patients report considerable pain especially during wound dressing changes and during physiotherapy needed to prevent and treat contractures and disability [101]. For patient comfort and compliance, a sound wound-care program is essential, with adequate pain management [102].

During and after the completion of antimicrobial treatment, many patients still experience restrictions through either physical disability caused by wound contractures or joint involvement of lesions; or psycho-social participation restrictions caused by social stigmatization [103, 104]. Physical therapy and societal inclusion and de-stigmatization are therefore important components of BU control programs

[81, 105]. Community-based approaches with early case finding are essential to prevent the large disease burden in individuals and communities [106, 107].

Future research thus should focus on evaluating shorter treatment for limited lesions while supplementing standard treatment with clofazimine or fluoroquinolones to provide a treatment tailored to the lesion size and estimated bacterial burden of individual patients.

Such attempts have so far been hampered by the fact that no good measurable surrogate parameter of treatment success, such as a blood marker, is available. However recent advances in the use of mass-spectrometry to carefully measure mycolactone in patient samples could aid in this regard.

Furthermore, there is dire need for the integration of control programs for tropical skin conditions such as BU, yaws, leprosy, etc. on a public health level [108]. Health officials should identify opportunities for systematic integration for the control of tropical neglected diseases depending on the prevalent diseases and available resources in any given setting.

Finally, BU treatment should be accompanied by surgery where needed, as well as good wound care, analgesia, physiotherapy and advocacy to reduce stigma.

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