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45.1 Introduction

Since the cause of Buruli ulcer (BU)—infection with *Mycobacterium ulcerans*—was first discovered and reported in 1948 [1], surgical resection has been the mainstay of treatment for more than half a century, when clinical studies were designed and conducted, based on in vitro and in vivo animal experiments. Unlike leprosy, where progress to develop evidence-based pharmacological treatment has been hampered by the fact the *M. leprae* cannot be cultured and tested in vitro for drug susceptibility, *M. ulcerans* has been tested in vitro [2, 3] and in vivo [4, 5]—in animal models [6], for a variety of antimicrobial drug classes, and subsequently also in patients [7–9]. In contrast, in leprosy, the widely accepted multi-drug treatment has been largely based on expert opinion; observational cohort studies but no well-designed, well-powered clinical trials with clearly pre-defined clinical end points

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have been conducted to provide the evidence base for current treatment recommendations [10].

Relapse and failure after surgical resection treatment alone for BU—though to some degree effective—have been well established [11, 12]. Systemic pharmacological treatment for BU, now considered standard of care, has been tested in several clinical trials involving patients with lesions limited to 10 cm cross-sectional diameter [8, 9], and prospective cohort studies have enrolled patients with even larger lesions [13], with marked reduction in treatment failure and relapse, compared to surgery alone. All these studies followed after publication of a small-scale proof-of-principle study in pre-ulcerative *M. ulcerans* infection. This study showed that lesions in patients treated for at least 4 weeks with a combination of streptomycin and rifampin had their lesions sterilized, as evidenced by the fact that these lesions, when surgically excised, were all sterile [7]. Clearly all patients with ulcers need dressings and dressing changes as topical treatment; some topical treatment modalities have been designed and propagated for cure of BU disease. Apart from surgical resection [14], topical therapeutic approaches to seek cure for BU have included heat treatment [15], phenytoin powder [16], and oxidative nitrogen creme [17], and clay [18].

Leprosy should not be understood as merely an infectious disease, but just as well as immune pathology. Patients affected by leprosy suffer from lack of social inclusion and stigma. Apart from anti-infective agents, they need measures to prevent and treat physical disabilities, while also reduction of stigma and social inclusion should be addressed. Likewise, patients affected by BU have sequelae, with contractures, disabilities, and problems with stigma and social inclusion. Apart from the important target to attain relapse-free cure, an important aspect of treatment is therefore also to prevent and manage sequelae. Indeed, many patients have ended up with disfiguring scars and contractures [12], with ensuing functional limitations and stigma [19], causing restriction in social participation. For details regarding the prevention of disabilities, we refer to Chap. 46.

Finally, treatment for BU is much more effective when patients seek medical attention early on in the course of disease. We therefore briefly discuss perceptions, beliefs, and attitudes as well as socioeconomic restrictions that may all cause patient delay. Indeed, unraveling health-seeking behavior and targeting determinants of patient delay are equally important to improve overall treatment outcome.

45.2 Systemic Therapy: Antimicrobial Therapy

Of all treatment modalities, clearly systemic antimicrobial treatment has been most studied, and taken together, antimicrobial treatment recommendations have a strong evidence base [20–22]. The Cochrane library published a systematic review of the entire preclinical evidence on antimicrobial drug treatment [20]. Here, we highlight and summarize the most important evidence of the activity of the different classes of antimicrobials tested, with some new information on novel drugs—and new evidence from a large clinical trial [9]. It is important to realize that antimicrobials

(bactericidal or bacteriostatic agents) either kill or arrest the growth of *M. ulcerans* and its metabolic activities, including the production of the major virulence factor, mycolactone [23]. As mycolactone production ceases, concentrations in affected tissues (ulcers, nodules, plaques, or edematous lesions), as well as in the system, start to decline [24], and its effects gradually wean. Obviously, the observation of ongoing tissue damage during early stages of antimicrobial therapy is confusing for clinicians, and a directly acting therapy against mycolactone molecules still present in tissues and in the system would be a tremendous asset. At this point in time, only in vitro work has shown that directly acting antibodies against mycolactone might one day be added to our therapeutic arsenal [25]. With resolution of immune depression [24], induced through various different mechanisms, Sec61 blockade being dominant [26], tissue repair and wound healing often only start after completion of the 8-week course of antimicrobial therapy. Immune reconstitution may be accompanied by an exacerbated immune response: between 2 and 26% of cases show a transient clinical worsening. This phenomenon is characterized by reduction of bacterial load but increased inflammatory response. Sometimes, inflammatory reactions emerge in the presence of dead bacilli at sites where, initially, no disease activity was noted. Most reports focus on clinically transient worsening with increase in size of lesions compared to baseline; all the above is referred to as "paradoxical reaction" [27]. These reactions that may be to some degree inherited [28] may mistakenly be interpreted as treatment failure. It is therefore important that in designing a study protocol for the evaluation of (combinations of) antimicrobial agents, the dynamics of slow wound healing and possibly transient worsening of lesions following antimicrobial treatment are taken into account. In retrospect, earlier published studies were flawed by design, with inappropriately short follow-up, at a time when the effects of mycolactone on immunity, tissue repair, and healing were incompletely appreciated. The presence of a secreted toxin in culture filtrate of *M. ulcerans* had been reported decades before the chemistry and biological activities of mycolactones were elucidated [29]. Although some patients may develop large lesions that continue to ulcerate for many years, most patients eventually heal [30]. This tendency of spontaneous healing is important to realize; it makes the interpretation of cohort studies without control groups notoriously difficult.

45.2.1 Aminoglycosides

This class of antimicrobial drugs have very low bioavailability and need to be administered parenterally. Aminoglycosides are hydrophilic compounds, and their volume of distribution reflects the lean body mass; excretion is by renal clearance. Their mode of action is by inhibiting bacterial protein synthesis, by binding tightly to the conserved A site of 16S rRNA in the 30S ribosomal subunit, and drug resistance results from genetic changes in *rrs* coding 16SrRNA, *rpsL* coding the S12 ribosomal protein, or *gidB* [31].

Streptomycin, an anti-tuberculosis drug, was repurposed for *M. ulcerans* treatment after *M. ulcerans* isolates from patients in Buruli county in Uganda (now

known as Nakasongola) tested susceptible to this drug in vitro [32]. In vitro and in vivo studies confirmed the bactericidal activity of streptomycin on *M. ulcerans* [4, 5, 33]. Streptomycin-resistant clinical isolates of *M. ulcerans* are uncommon [34]. The clinical impact of streptomycin alone has been difficult to evaluate; streptomycin has predominantly been used in combination with rifampin [7–9, 35–37]. Streptomycin has usually been administered at 15 mg/kg bodyweight; no pharmacokinetic data in patients with *M. ulcerans* infection have been published, and pharmacokinetic/pharmacodynamic modeling has not been performed, but it is likely that dosing should be based on lean body mass; in obese individuals, toxicity can be expected with dosing based on total body weight. Toxicity is a major concern anyway, especially in elderly people who risk vestibular and ototoxic effects [38]. This oto-vestibular toxicity and also renal toxicity are class effects. Amikacin, an aminoglycoside with activity similar to streptomycin [5], has therefore no clear advantage over streptomycin. Several oral treatment schedules have been successfully tested in animal experiments [39], and in Australia, where patients are typically at a more advanced age, streptomycin and amikacin have largely been avoided [40]. Clinical studies have been designed to compare streptomycin-based treatment schedules with oral schedules. Oral agents replace streptomycin, especially clarithromycin [8, 9, 37], and these studies show that streptomycin is no longer needed, as all-oral drug combinations are safer and equally effective [9]; see Fig. 45.2.

45.2.2 Rifamycins

Rifampin was reported to be effective against *M. ulcerans* in vitro as early as in 1972 [41], when surgery was still deemed the only viable option for treatment. Later reports confirmed that susceptibility is excellent in vitro [3, 34] and in animal models [39, 41]. The drug is readily absorbed with excellent bioavailability; distribution is similar to total body weight; drug elimination is by enzymatic metabolism that is saturated at higher dosing, with important auto-induction of drug elimination in the liver; renal clearance is negligible [42]. Its mechanism of action is by interfering with bacterial polymerase, necessary for bacterial cell replication; resistance depends entirely on genetic changes in the *rpoB* gene encoding the polymerase [43]. As *M. ulcerans* is an environmentally acquired microorganism with low or absent antimicrobial pressure, all infections caused by this organism are typically wild-type, and resistance is generally low and effectiveness high [9]. Enhanced drug elimination by induction of hepatic (CYP3A4) enzymes is a concern. Pharmacokinetic studies in BU have been limited to only one report [40]. Clarithromycin exposure is limited by rifampin co-medication; clarithromycin increases rifampin drug exposure slightly.

In clinical trials and observational cohort studies, drug treatment including rifampin at a dose of 10 mg/kg body weight was associated with excellent tolerability, high cure rates, and negligible relapse rates [7–9, 13, 35–37, 44], although in some of the cohort studies, resection surgery was combined with drug treatment [13, 35, 36], making it difficult to evaluate drug efficacy in those studies. In

tuberculosis susceptible to rifampin, dosing up to 35 mg/kg body weight [45] has no important toxicity. Animal experiments suggest that perhaps with increased dosing, shorter duration of therapy for BU might be possible [46].

Other rifamycins include rifapentine [39, 47] that has a considerably longer half-life than rifampin and rifabutin [5] that has higher lipophilicity, longer half-life, and less drug-drug interactions than rifampin [48].

45.2.3 Macrolides

Clarithromycin has been studied most—it is a protein synthesis inhibitor that reversibly binds to the 23S rRNA on the 50S ribosomal subunit. It has been considered a companion (bacteriostatic) drug in combination with more powerful bactericidal agents like rifampin [9]. The dosing has been slightly variable, ranging from 7.5 mg/kg body weight once daily in most studies, to 12 mg/kg body weight [36], to 15 mg/kg body weight once daily in extended release (ER) formulation [9]. Although less of a concern compared to aminoglycosides like streptomycin that is contraindicated during pregnancy, there are still concerns with its use during pregnancy [49]. It is readily absorbed from the intestine and eliminated by cytochrome 450 enzymes, especially CYP3A4 into 14-hydroxy-clarithromycin that appears to have no antimicrobial effect on *M. ulcerans* [40]. Clarithromycin decreases drug elimination of certain drugs—notably also rifampin—by inhibiting P-glycoprotein [50]. Its bacteriostatic effect on *M. ulcerans* has been shown in vitro [2] and in vivo [39]. The clinical experience has been limited to combinations with rifampin [8, 9, 35–37]. An 8-week course of oral rifampin and intramuscular streptomycin appeared highly effective [13, 36], but switching from streptomycin to oral clarithromycin after 4 weeks [8] or even 2 weeks [37] did not affect treatment outcome, compared to patients that followed a full course with eight weeks of streptomycin, combined with rifampin. In a head-to-head comparison, fully oral treatment with the combination of clarithromycin in ER form in the largest clinical trial on drug treatment in *M. ulcerans* infection to date showed similar clinical effectiveness of 8-week fully oral rifampin-clarithromycin ER compared to the rifampin-streptomycin combination [9]. Among secondary end points in this study, median time to healing was better in the clarithromycin ER group (median 16 weeks) than in the streptomycin group (median 24 weeks); paradoxical reactions were similar, but toxicity, especially, oto-vestibular side effects, was significantly more common in the streptomycin-treated group.

Azithromycin has good bacteriostatic activity in vitro [34] and in vivo, summarized in [22], but clinical studies are lacking.

45.2.4 Fluoroquinolones

Ciprofloxacin, ofloxacin, and moxifloxacin have good antibacterial activity in vitro [3]. These drugs act by interfering with DNA replication by inhibiting gyrase that

catalyzes super-coiling of bacterial double-stranded DNA. Resistance is coded by mutations in the genes gyrA and gyrB that encode bacterial gyrase [51]. In Australia, oral treatment has been the preferred approach for antimicrobial treatment, and fluoroquinolones, notably, ciprofloxacin and moxifloxacin, have been recommended and incorporated in treatment schedules, usually in combination with rifampin [44]. Fluoroquinolones have excellent bioavailability and penetrate well into various different tissues including bone. Ciprofloxacin and levofloxacin are predominantly eliminated by renal clearance; dosing interval needs to be extended in patients with impaired renal function. Elimination of moxifloxacin is by metabolic inactivation into inactive metabolites that are excreted into feces and partly by renal clearance. No dose adaptation for moxifloxacin is required for patients with renal failure; comedication with rifampin is a concern as drug elimination of moxifloxacin is enhanced, with predicted reduction of drug exposure by a third [52]. Fluoroquinolones interfere with collagen formation and carry a potential adverse effect on bone formation, tendons, and vascular structures; children may suffer from arthropathy; in the elderly, vascular dissection [53] and rupture of tendons such as the Achilles tendon might occur [54]. For moxifloxacin, which is also a core drug in the treatment of drug-resistant tuberculosis, QTc time may increase with higher drug exposure, with the potential risk of fatal arrhythmia by a mechanism referred to as "Torsade de Pointes." In Africa, where patients are typically younger than in Australia, potential side effects as well as costs of fluoroquinolones have discouraged the use of these drugs. In clinical studies, especially in children with cystic fibrosis, adverse effects appear to be mild [55]. In general, fluoroquinolones are contra-indicated in pregnancy. Fluoroquinolones have not been studied in head-to-head comparisons with other agents, and many patients in Australia receive antimicrobial treatment combined with surgery, which makes it difficult to tease out the effect of these drugs in their own right. Based on in vitro studies [3, 56], moxifloxacin should be considered an effective drug for *M. ulcerans* infection.

45.2.5 Miscellaneous Antimicrobial Drugs

Cotrimoxazole has not been tested in vitro for *M. ulcerans*, although it has a potential role in drug-resistant tuberculosis, with only borderline susceptibility [57]; a small study did not show an appreciable clinical effect [58].

Clofazimine, an anti-leprosy drug, has attracted attention because of its activity against *M. tuberculosis*; it plays an important role in the management of drug-resistant tuberculosis [59]. It has reasonable activity on *M. ulcerans* in vitro [34], and in an animal model, the 6-week combination of clofazimine and rifampin gave relapse-free cure [60]; increasing the dosage of rifampin provided cure with an even shorter duration of treatment [46]. The drug has a long half-life and causes a yellow-orange discoloration of skin, especially in individuals with fair skin color. An early clinical trial with clofazimine monotherapy failed to show benefit [61], although a small observational study later suggested improved outcome [62].

Bedaquiline, a core drug in the management of drug-resistant tuberculosis, appears highly active on *M. ulcerans* in vitro and in vivo [22], but there are no data from clinical interventional or observational studies.

Linezolid, yet another core anti-tuberculosis drug for drug-resistant tuberculosis, with important toxicity (especially, polyneuropathy) with increasing drug exposure [63] has moderate activity on *M. ulcerans* in vitro [22], but there are no clinical studies to support its use in patients with *M. ulcerans* infection.

Telacebec is a compound that has recently drawn much attention due to its extremely high efficacy on *M. ulcerans* in vitro [64] potentially holding a promise for an extremely short treatment duration [22, 65], but further clinical studies are required to assess its safety and efficacy. In pregnancy, only rifampin should be considered fully safe; animal experiments suggest that beta-lactams might be an option [66] for these special patient groups, but to date, no clinical reports have confirmed that this approach might be effective.

45.2.6 Drug Treatment With Combinations of Antimicrobial Agents and Treatment Duration

After a systematic review was published reviewing the evidence emerging from clinical studies [20], the earlier mentioned large clinical trial was published [9]. It showed that fully oral treatment with rifampin and clarithromycin ER resulted in a high cure rate, non-inferior to the combination of rifampin and streptomycin injections. Based on an earlier pharmacokinetic study [40] in the framework of the BURULICO trial [8], at the time of the study design, the choice was made to increase the total dose of clarithromycin by providing 15 mg/kg in an ER formulation. Unpublished data based on a pharmacokinetic analysis of dried blood spots in a limited group of study participants showed that drug exposure was still limited with even slightly lower peak serum concentrations reached (Klis et al, unpublished data); we therefore believe that the double dosing in ER form has no clear advantage over immediate release formulations and that perhaps 10 mg/kg clarithromycin might be an appropriate dose. Importantly, with the treatment schedule used in the trial, there was no evidence of bacteriological failure among the few study participants that failed to have their lesions healed at the pre-defined time point 52 weeks after start of treatment; see Fig. 45.4.

As mentioned earlier, the dosage of rifampin might also be worthwhile to be increased to e.g., 15 mg/kg, as this is clearly to be considered safe and perhaps slightly more effective. A fixed drug combination of rifampin and clarithromycin with slightly higher dosing might be the way forward, also to prevent monotherapy under service conditions. Even though drug resistance is still extremely rare, also considering the fact that there is hardly any antimicrobial pressure on the reservoir of the organism that is clearly environmental, drug combinations are the preferred approach to combat *M. ulcerans* infection; clarithromycin is to be considered as a companion drug to prevent treatment failure during monotherapy. Treatment duration has been chosen based on one single experiment in which pharmacotherapy

with at least 4-week duration resulted in sterilizing effects on lesions that were subsequently excised and cultured [7]. In lesions <10 cm cross-sectional diameter, treatment failure was rare [8, 9], and generally, drug treatment outcome has been beneficial [13, 35–37]. Whether larger lesions require longer treatment duration has remained an unresolved question, and the question whether shorter treatment duration in small lesions is acceptable or even preferable has not been addressed in clinical studies to date. Clinical observations suggest that at least some patients with small lesions do well with less than 8 weeks of treatment [67]. Whether high-dose rifampin or combinations with telacebec facilitate much shorter treatment duration should be explored in future studies.

Fluoroquinolones have been used in observational studies in Australia, with generally good outcome, though in elderly patients, drug intolerance has been a concern [68]. The updated Australian guideline mentions fluoroquinolones as a treatment option [69], but the current WHO guideline does not encourage their use [70]. Treatment during pregnancy remains difficult; in the earlier mentioned trial, one female study participant appeared to be pregnant at week 6 of the study medication which was rifampin-clarithromycin ER in her case; in consultation with the study team, she decided to continue the treatment, with no adverse effects on maternal and fetal outcome, but clearly there is a small but significant risk for fetal damage of macrolides like clarithromycin during pregnancy [49]. Alternative treatment options like surgical resection or topical heat treatment might be an option (see below).

45.3 Topical Treatments

45.3.1 Surgical Resection

Surgical resection has been reported from the 1950s in the then Belgian Congo, now DR Congo [71], and Uganda [72]. As mentioned earlier, surgery alone, even with resection of margins of apparently healthy tissue, still results in residual bacterial load in the resection margins [73], while surgery as monotherapy has been associated with variable but overall, appreciable recurrence and failure rates [11, 12]. Earlier published guidelines have therefore recommended adding antimicrobial therapy to surgery [56], while there has been a shift to recommend all-oral treatment as the first treatment option [69, 70].

The results of the largest ever trial evaluating fully oral antimicrobial treatment compared to the rifampin/streptomycin combination revealed that in neither of the treatment arms, resection surgery was necessary to obtain cure without relapse, assessed 12 months after start of therapy [9]; indeed none of 297 with *2404* PCR-confirmed *M. ulcerans* infection needed resection, and only 4 (2 in each arm) needed skin grafting. This finding questions the ongoing practice in many locales where surgery is still common practice, not only in Africa [74] but also in Victoria,

Australia [75]. Only one study addressed the question of timing of surgery; in the treatment guideline published by the WHO in 2012 [70], surgery timing was suggested to be performed after at least 4 weeks of antimicrobial therapy, but in most centers in Africa, surgery is usually planned around the end of 8 weeks of antimicrobial therapy. In a study conducted in Benin—the only study to date, to evaluate the role of surgery, study participants were randomized for the timing between this time point—at week 8 after start of therapy or, a delay of that decision, until week 14 after start of therapy. Fifty-five (96%) of 57 participants in the delayed-decision group and 52 (90%) of 58 participants in the standard-care group had healed lesions 1 year after start of antimicrobial treatment; 37 (67%) of 55 patients in the delayed-decision group had their lesions healed without surgical intervention, as did 25 (48%) of 52 in the standard-care group (RR 1·40, 95% CI 1·00–1·96). The time to heal and residual functional limitations did not differ between the two groups. Postponing the decision to operate resulted in a marked and significant reduction in the duration of hospitalization and wound care; indeed, delaying decisions to operate was highly beneficial [76]. In summary, the role of surgery has been overestimated in the past [14], and despite advocates to point at potential benefits—albeit for Victoria, Australia that has witnessed an unprecedented outbreak of BU [77], we believe the case in favor of antimicrobial treatment and against surgery to achieve cure for BU disease is really strong now. It goes without saying that limited debridement, skin grafting, and plastic and reconstructive surgery may be beneficial for selected patients, especially those with advanced tissue destruction and contractures; whether osteomyelitis complicated by sequesters needs surgery has not been addressed in the literature.

45.3.2 Heat Treatment

Mycobacterium ulcerans typically grows at temperatures below the core temperature of humans; temperatures above 37°C harm the bacilli, and heat treatment by topical application has been pilot-tested for lesions on limbs [15, 78]. Based on these pilot studies, a larger study enrolled 65 participants with category I-III lesions (for definitions of categories, see Chap. 42). The heating device used contains sodium acetate trihydrate, a phase-change material that can quickly be reheated by boiling water, and cannot exceed temperatures above 56°C. The device is easily rechargeable; it provides a skin temperature at around 40°C, for around 10 h/day. In all, 63 patients were started on topical heat treatment; 52 individuals had PCR-confirmed BU disease; treatment duration varied from 42 to 56 days. Limited debridement surgery was allowed; to the 12 patients that eventually failed, standard antimicrobial therapy was provided [79]. The treatment was well tolerated and accepted by patients and their guardians. The authors argue that their treatment has efficacy that is comparable to reported results from clinical trials with antimicrobial therapy, but their phase II study was not a randomized comparison with antimicrobial treatment.

45.3.3 Dressings; Topical Substances; Traditional Treatments

Wound care is essential for patients with BU; in Africa and in Australia, the vast majority of patients have ulcerated lesions at any point in time; see, e.g., Fig. 45.3. Wound treatment guidelines that apply to a wide range of skin lesions have been provided by the WHO [80]. Sufficient general care for patients, addressing nutritional status, and preventing anemia and uncontrolled hyperglycemia in patients with diabetes are all essential; see Fig. 45.1. Next, rinsing and cleaning of wound service using saline, if necessary, adding careful limited wound debridement using analgesics, and regular dressing changes (Figs. 45.2, 45.3, 45.4, 45.5); prevention and treatment of lymphoedema by appropriate compression therapy (Fig. 45.6); using absorptive dressing materials in discharging wounds; and applying non-adhesive wound cover especially when the wound service is not discharging are all considered part of standard wound care (see Fig. 45.6). In rural Africa, knowledge and practice around wound care differ widely in clinical practice across treatment centers for BU [81]. Although BU typically presents as a painless ulcer in early stages, many patients experience considerable pain and anxiety related to wound care, especially dressing changes [82]. Knowledge and practice to relieve and prevent procedural pain need improvement [83], and former patients indicated that this aspect needs more attention [84].

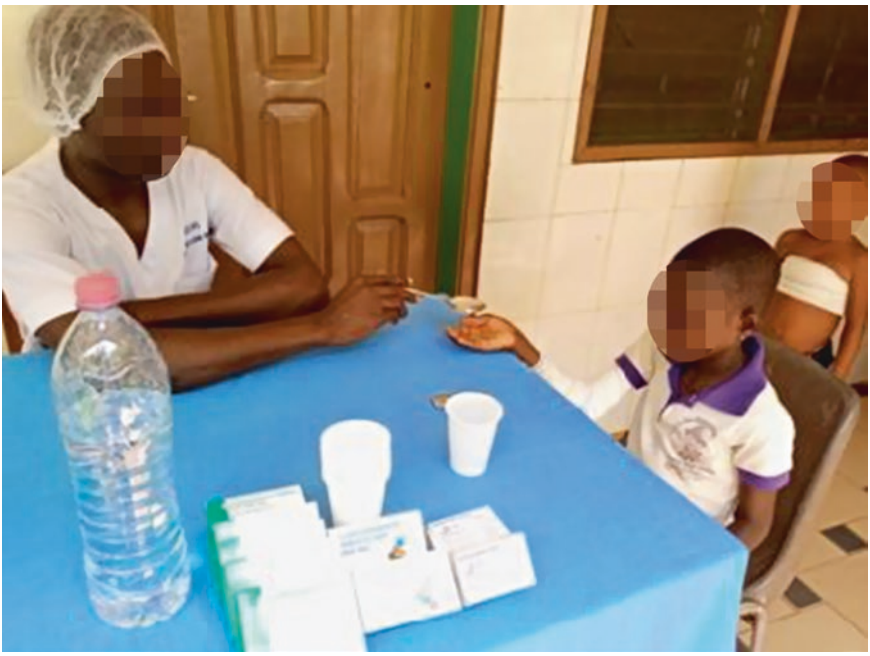

Fig. 45.1 'Look at the whole before looking at the hole': right hand side showing pallor reflecting anemia in a patient with long-standing Buruli ulcer; optimized nutritional support, deworming and malaria treatment are all essential for adequate wound healing

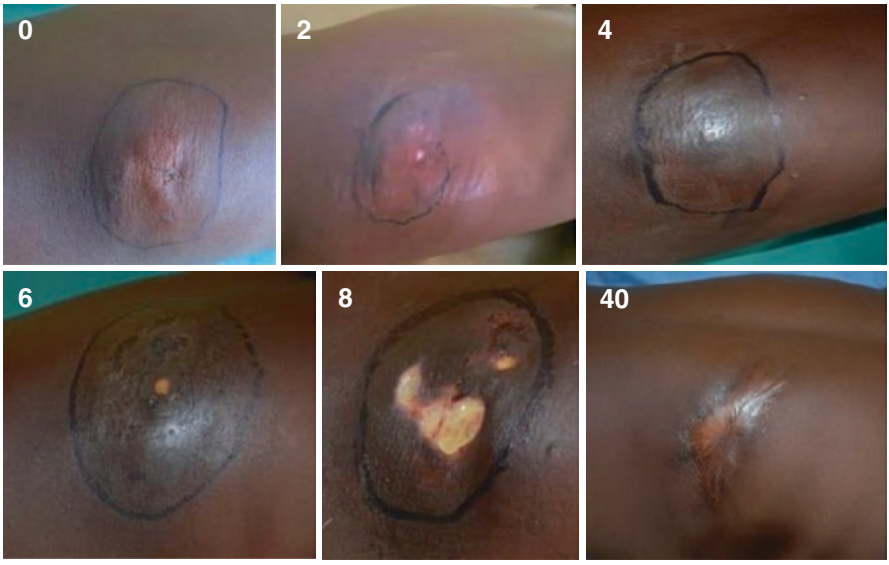

Fig. 45.2 Fully oral treatment with clarithromycin-rifampicin for 8 weeks has become the new standard treatment in Africa

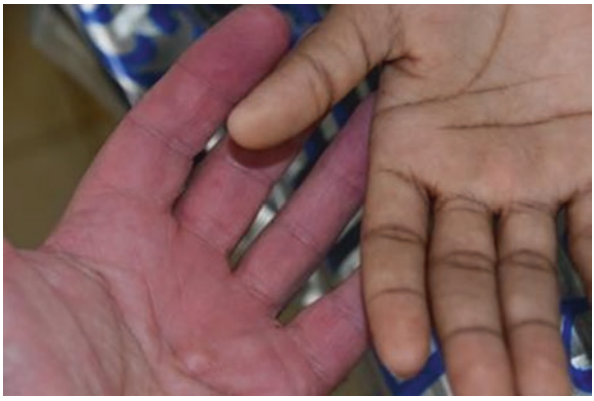

Fig. 45.3 Patient enrolled in a drug trial; plaque, PCR-confirmed as *M. ulcerans* infection located at the right lateral aspect of the trunk. Patient was followed from week 0; ulcer developing at week 6, clearly larger at week 8, but eventually healed at week 28; stable scar recorded at week 40. Without repetitive trauma, these lesions heal, even if dressing changes are irregular or sub-optimal

Many patients seek relief by traditional treatments including herbal topical dressings [85]. No randomized studies have been published to date, and most authors believe that it merely delays starting effective treatment [19].

Wound microbiota in BU are different from non-BU lesions [86]. Moreover, BU may be secondarily contaminated and/or infected by a diversity of organisms like *P. aeruginosa* and *S. aureus* [87] some of which carry resistance and virulence factors [88]. Whether these secondary infections cause delayed wound healing or not is currently uncertain; the practice around assumed secondary infection varies widely across treatment centers and is more often than not irrational and ineffective [89].

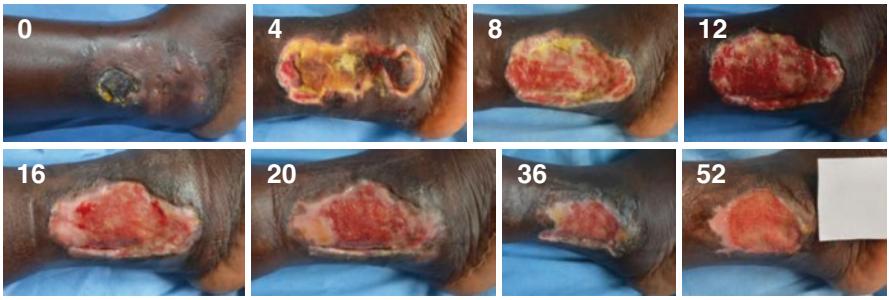

Fig. 45.4 Patient enrolled in drug trial, with PCR-confirmed *M. ulcerans* infection at the lateral malleolus of right foot; necrotic slough, purulent aspect at week 8; granulating surface at week 12. Partial healing with shallow ulceration still present at week 16; non-epithelialized shallow lesion at week 52, classified as failure, according to strict definitions used in the trial; the study team suspected that the lesion did not represent residual *M. ulcerans* infection, but rather non-healing as a result of limited compliance with topical dressings treatment and recurrent trauma

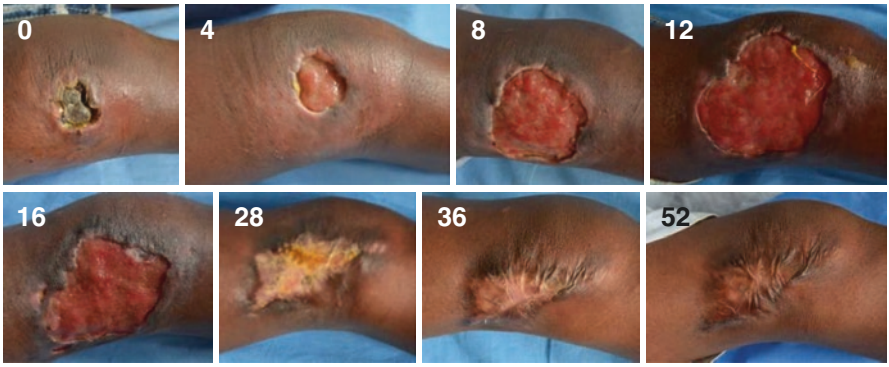

Fig. 45.5 Patient with *M. ulcerans* infection located at the lateral aspect of the right knee, enrolled in a drug trial. Although lesions located at joints run an increased risk for development of contractures, resulting in functional limitations, Prevention of Disability activities under trial conditions appeared effective, with negligible functional disabilities among study participants. At week 0, necrotic slough is clearly present; paradoxical enlargement of the lesion appears at week 4, with edema present; lesion stabilizes at around week 26, while at week 28, the lesion is almost complete epithelialized; stable scar with excellent functional results at completion of follow-up at week 52

45.4 Supportive Measures

Early detection and prompt treatment with currently available interventions, such as those described above, are critically important to achieve prompt and uncomplicated healing. Health-seeking behavior is complex and generally poorly understood; many drivers for delay in health seeking in health institutions have been identified including stigma, fear for mutilating surgery, and traditional beliefs and attitudes favoring traditional treatment [19, 90, 91].

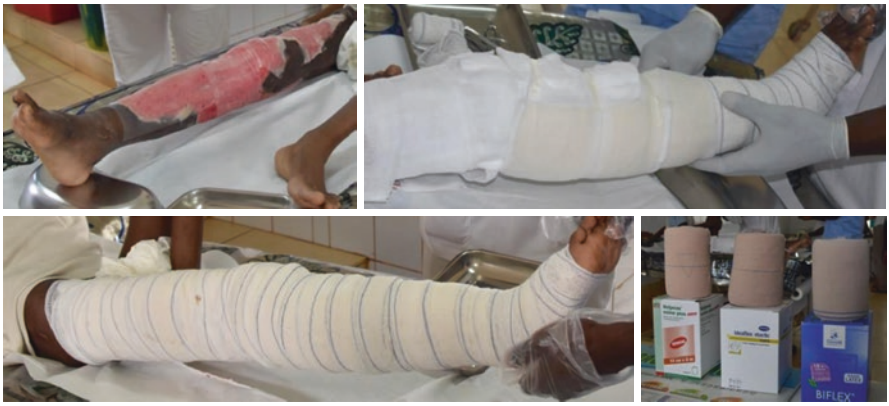

Fig. 45.6 Large Buruli ulcer lesion involving almost the entire right leg. Pain management during wound care is essential; topical treatment includes careful saline rinsing, application of non-adhesive cover with paraffine or vaseline gauze; absorptive dressing material, with gentle compression bandage, preferably using short-stretch bandage. Prevention of Disabilities that may also require careful pain management, following the WHO pain ladder, includes physiotherapy, in order to prevent contracture and functional limitation. In lesions of this size, split skin grafting helps to speed up healing

Active case finding in highly endemic regions appears to reduce the number of individuals with advanced disease [92]. Indeed, antimicrobial treatment started early on has tremendous potential to prevent disabilities, resulting in excellent quality of life [93] and societal participation and inclusion [94]. Patient delay may ultimately be the hurdle to take, to optimize outcome. Fear for disfiguring surgery kept some patients from reporting timely [19, 90], but the good news from recent work is that disfiguring resection surgery is no longer necessary to achieve relapse-free cure, without severe sequelae.

45.5 Comorbidities and Coinfections

The large trials providing the evidence for the effect of drug treatment for BU excluded potential study participants with many comorbid conditions. Drug exposure—the result of resorption, distribution, and elimination—is described by the pharmacokinetics (PK) of each individual drug. PK may change with comorbid conditions like diabetes and obesity [42]. Drug-drug interactions are common with rifampin and clarithromycin, and comedications are common with these comorbid conditions, especially with HIV [95]. Like in HIV co-infected patients with tuberculosis, protease inhibitors cannot be combined with rifampin. Otherwise, the same principles for treatment apply to patients with BU and comorbid conditions [96], although the evidence base to guide treatment for co-infected patients is small.

45.6 Conclusions

The case for early detection and prompt treatment with a combination of antimicrobial drugs, primarily, rifampin and clarithromycin, offers an excellent chance of relapse-free healing, with minimal chance of residual functional limitations; median time to healing even with small category I–II lesions remains a concern, even if supportive treatment with appropriate wound care and measures to prevent contractures are optimized. Treatment duration less than 8 weeks has been anecdotally reported, but formal studies have not addressed the question of optimal treatment duration for different categories or presentations of BU disease. In advanced lesions, there may be a place for additional surgery; the role of surgery to obtain cure has become obsolete, apart from special circumstances, e.g., in pregnant patients, who might also benefit from topical heat treatment.

References

1. Buckle G, Tolhurst JC. A new mycobacterial infection in man; cultivation of the new Mycobacterial infection in man; cultivation of the new Mycobacterium. J Pathol Bacteriol. 1948;60(1):116–22.
2. Portaels F, Traore H, De Ridder K, Meyers WM. In vitro susceptibility of *Mycobacterium ulcerans* to clarithromycin. Antimicrob Agents Chemother. 1998;42(8):2070–20703. https://doi.org/10.1128/AAC.42.8.2070.
3. Thangaraj HS, Adjei O, Allen BW, Portaels F, Evans MR, Banerjee DK, et al. In vitro activity of ciprofloxacin, sparfloxacin, ofloxacin, amikacin and rifampicin against Ghanaian isolates of *Mycobacterium ulcerans*. J Antimicrob Chemother. 2000;45(2):231–3. https://doi.org/10.1093/jac/45.2.231.
4. Feldman WH, Karlson AG. *Mycobacterium ulcerans* infections; response to chemotherapy in mice. Am Rev Tuberc. 1957;75(2):266–79. https://doi.org/10.1164/artpd.1957.75.2.266.
5. Dega H, Robert J, Bonnafous P, Jarlier V, Grosset J. Activities of several antimicrobials against *Mycobacterium ulcerans* infection in mice. Antimicrob Agents Chemother. 2000;44(9):2367–72. https://doi.org/10.1128/aac.9.2367-2372.2000.
6. Fenner F. The pathogenic behavior of *Mycobacterium ulcerans* and *Mycobacterium balnei* in the mouse and the developing chick embryo. Am Rev Tuberc. 1956;73(5):650–73. https://doi.org/10.1164/artpd.1956.73.5.650.
7. Etuaful S, Carbonnelle B, Grosset J, Lucas S, Horsfield C, Phillips R, et al. Efficacy of the combination rifampin-streptomycin in preventing growth of *Mycobacterium ulcerans* in early lesions of Buruli ulcer in humans. Antimicrob Agents Chemother. 2005;49(8):3182–6. https://doi.org/10.1128/AAC.49.8.3182-3186.2005.
8. Nienhuis WA, Stienstra Y, Thompson WA, Awuah PC, Abass KM, Tuah W, et al. Antimicrobial treatment for early, limited *Mycobacterium ulcerans* infection: a randomised controlled trial. Lancet. 2010;375(9715):664–72. https://doi.org/10.1016/S0140-6736(09)61962-0.
9. Phillips RO, Robert J, Abass KM, Thompson W, Sarfo FS, Wilson T, et al. Rifampicin and clarithromycin (extended release) versus rifampicin and streptomycin for limited Buruli ulcer lesions: a randomised, open-label, non-inferiority phase 3 trial. Lancet. 2020;395(10232):1259–67. https://doi.org/10.1016/S0140-6736(20)30047-7.
10. Lockwood DN. Leprosy. BMJ Clin Evid. 2007;2007:0915.
11. Amofah G, Asamoah S, Afram-Gyening C. Effectiveness of excision of pre-ulcerative Buruli lesions in field situations in a rural district in Ghana. Trop Doct. 1998;28(2):81–3. https://doi.org/10.1177/004947559802800208.

12. Teelken MA, Stienstra Y, Ellen DE, Quarshie E, Klutse E, van der Graaf WTA, et al. Buruli ulcer: differences in treatment outcome between two centres in Ghana. Acta Trop. 2003;88(1):51–6. https://doi.org/10.1016/s0001-706x(03)00170-0.
13. Sarfo FS, Phillips R, Asiedu K, Ampadu E, Bobi N, Adentwe E, et al. Clinical efficacy of combination of rifampin and streptomycin for treatment of *Mycobacterium ulcerans* disease. Antimicrob Agents Chemother. 2010;54(9):3678–85. https://doi.org/10.1128/AAC.00299-10.
14. Radford AJ. The surgical management of lesions of ulcerans infections due to *Mycobacterium ulcerans*, revisited. Trans R Soc Trop Med Hyg. 2009;103(10):981–4. https://doi.org/10.1016/j.trstmh.2009.04.009.
15. Meyers WM, Shelly WM, Connor DH. Heat treatment of *Mycobacterium ulcerans* infections without surgical excision. Am J Trop Med Hyg. 1974;23(5):924–9. https://doi.org/10.4269/ajtmh.1974.23.924.
16. Adjei O, Evans MR, Asiedu A. Phenytoin in the treatment of Buruli ulcer. Trans R Soc Trop Med Hyg. 1998;92(1):108–9. https://doi.org/10.1016/s0035-9203(98)90977-4.
17. Phillips R, Adjei O, Lucas S, Benjamin N, Wansbrough-Jones M. Pilot randomized double-blind trial of treatment of *Mycobacterium ulcerans* disease (Buruli ulcer) with topical nitrogen oxides. Antimicrob Agents Chemother. 2004;48(8):2866–70. https://doi.org/10.1128/AAC.48.8.2866-2870.2004.
18. Adusumilli S, Haydel SE. In vitro antibacterial activity and in vivo efficacy of hydrated clays on *Mycobacterium ulcerans* growth. BMC Complement Altern Med. 2016;16(1):40–8. https://doi.org/10.1186/s12906-016-1020-5.
19. Stienstra Y, van der Graaf WTA, Asamoa K, van der Werf TS. Beliefs and attitudes toward Buruli ulcer in Ghana. Am J Trop Med Hyg. 2002;67(2):207–13. https://doi.org/10.4269/ajtmh.2002.67.207.
20. Yotsu RR, Richardson M, Ishii N. Drugs for treating Buruli ulcer (*Mycobacterium ulcerans* disease). Cochrane Database Syst Rev. 2018;8(8):CD012118. https://doi.org/10.1002/14651858.CD012118.
21. van der Werf TS, Barogui YT, Converse PJ, Phillips RO, Stienstra Y. Pharmacologic management of *Mycobacterium ulcerans* infection. Expert Rev Clin Pharmacol. 2020;13(4):391–401. https://doi.org/10.1080/17512433.2020.1752663.
22. Omansen TF, van der Werf TS, Phillips RO. Antimicrobial treatment of *Mycobacterium ulcerans* infection. In: Pluschke G, Röltgen K, editors. Buruli Ulcer. Cham: Springer; 2019. p. 203–20. https://doi.org/10.1007/978-3-030-11114-4_11.
23. George KM, Chatterjee D, Gunawardana G, Welty D, Hayman J, Lee R, et al. Mycolactone: a polyketide toxin from *Mycobacterium ulcerans* required for virulence. Science. 1999;283(5403):854–7. https://doi.org/10.1126/science.283.5403.854.
24. Sarfo FS, Phillips RO, Ampadu E, Sarpong F, Adentwe E, Wansbrough-Jones M. Dynamics of the cytokine response to *Mycobacterium ulcerans* during antibiotic treatment for *M. ulcerans* disease (Buruli ulcer) in humans. Clin Vaccine Immunol. 2009;16(1):61–5. https://doi.org/10.1128/CVI.00235-08.
25. Dangy JP, Scherr N, Gersbach P, Hug MN, Bieri R, Bomio C, et al. Antibody-mediated neutralization of the exotoxin mycolactone, the main virulence factor produced by *Mycobacterium ulcerans*. PLoS Negl Trop Dis. 2016;10(6):e0004808. https://doi.org/10.1371/journal.pntd.0004808.
26. Demangel C, High S. Sec61 blockade by mycolactone: A central mechanism in Buruli ulcer disease. Biol Cell. 2018;110(11):237–48. https://doi.org/10.1111/boc.201800030.
27. Nienhuis WA, Stienstra Y, Abass KM, Tuah W, Thompson WA, Awuah PC, et al. Paradoxical responses after start of antimicrobial treatment in *Mycobacterium ulcerans* infection. Clin Infect Dis. 2012;54(4):519–26. https://doi.org/10.1093/cid/cir856.
28. Barogui YT, Klis S-A, Johnson RC, Phillips RO, van der Veer E, van Diemen C, et al. Genetic susceptibility and predictors of paradoxical reactions in buruli ulcer. PLoS Negl Trop Dis. 2016;10(4):e0004594. https://doi.org/10.1371/journal.pntd.0004594.
29. Hockmeyer WT, Krieg RE, Reich M, Johnson RD. Further characterization of *Mycobacterium ulcerans* toxin. Infect Immun. 1978;21(1):124–8. https://doi.org/10.1128/IAI.21.1.124-128.1978.

30. Marion E, Chauty A, Kempf M, Le Corre Y, Delneste Y, Croue A, et al. Clinical features of spontaneous partial healing during *Mycobacterium ulcerans* infection. Open Forum Infect Dis. 2016;3(1):ofw013. https://doi.org/10.1093/ofid/ofw013.
31. Okamoto S, Tamaru A, Nakajima C, Nishimura K, Tanaka Y, Tokuyama S, et al. Loss of a conserved 7-methylguanosine modification in 16S rRNA confers low-level streptomycin resistance in bacteria. Mol Microbiol. 2007;63(4):1096–106. https://doi.org/10.1111/j.1365-2958.2006.05585.x.
32. Clancey JK. Mycobacterial skin ulcers in Uganda: description of a new mycobacterium (*Mycobacterium buruli*). J Pathol Bacteriol. 1964;88(1):175–87. https://doi.org/10.1002/path.1700880123.
33. Ji B, Lefrançois S, Robert J, Chauffour A, Truffot C, Jarlier V. In vitro and in vivo activities of rifampin, streptomycin, amikacin, moxifloxacin, R207910, linezolid, and PA-824 against *Mycobacterium ulcerans*. Antimicrob Agents Chemother. 2006;50(6):1921–6. https://doi.org/10.1128/AAC.00052-06.
34. Owusu E, Newman MJ, Addo KK, Addo P. In vitro susceptibility of *Mycobacterium ulcerans* isolates to selected antimicrobials. Can J Infect Dis Med Microbiol. 2017;2017(4):5180984–6. https://doi.org/10.1155/2017/5180984.
35. Kibadi K, Boelaert M, Fraga AG, Kayinua M, Longatto-Filho A, Minuku J-B, et al. Response to treatment in a prospective cohort of patients with large ulcerated lesions suspected to be Buruli Ulcer (*Mycobacterium ulcerans* disease). PLoS Negl Trop Dis. 2010;4(7):e736. https://doi.org/10.1371/journal.pntd.0000736.
36. Chauty A, Ardant M-F, Marsollier L, Pluschke G, Landier J, Adeye A, et al. Oral treatment for *Mycobacterium ulcerans* infection: results from a pilot study in Benin. Clin Infect Dis. 2011;52(1):94–6. https://doi.org/10.1093/cid/ciq072.
37. Phillips RO, Sarfo FS, Abass MK, Abotsi J, Wilson T, Forson M, et al. Clinical and bacteriological efficacy of rifampin-streptomycin combination for two weeks followed by rifampin and clarithromycin for six weeks for treatment of *Mycobacterium ulcerans* disease. Antimicrob Agents Chemother. 2014;58(2):1161–6. https://doi.org/10.1128/AAC.02165-13.
38. Klis S, Stienstra Y, Phillips RO, Abass KM, Tuah W, van der Werf TS. Long term streptomycin toxicity in the treatment of Buruli Ulcer: follow-up of participants in the BURULICO drug trial. PLoS Negl Trop Dis. 2014;8(3):e2739. https://doi.org/10.1016/S0140-6736(20)30047-7.
39. Ji B, Chauffour A, Robert J, Jarlier V. Bactericidal and sterilizing activities of several orally administered combined regimens against *Mycobacterium ulcerans* in mice. Antimicrob Agents Chemother. 2008;52(6):1912–6. https://doi.org/10.1128/AAC.00193-08.
40. Alffenaar JWC, Nienhuis WA, de Velde F, Zuur AT, Wessels AMA, Almeida D, et al. Pharmacokinetics of rifampin and clarithromycin in patients treated for *Mycobacterium ulcerans* infection. Antimicrob Agents Chemother. 2010;54(9):3878–83. https://doi.org/10.1128/AAC.00099-10.
41. Stanford JL, Phillips I. Rifampicin in experimental *Mycobacterium ulcerans* infection. J Med Microbiol. 1972;5(1):39–45. https://doi.org/10.1099/00222615-5-1-39.
42. Svensson RJ, Aarnoutse RE, Diacon AH, Dawson R, Gillespie SH, Boeree MJ, et al. A population pharmacokinetic model incorporating saturable pharmacokinetics and autoinduction for high rifampicin doses. Clin Pharmacol Ther. 2018;103(4):674–83. https://doi.org/10.1002/cpt.778.
43. Beissner M, Awua-Boateng N-Y, Thompson W, Nienhuis WA, Klutse E, Agbenorku P, et al. A genotypic approach for detection, identification, and characterization of drug resistance in *Mycobacterium ulcerans* in clinical samples and isolates from Ghana. Am J Trop Med Hyg. 2010;83(5):1059–65. https://doi.org/10.4269/ajtmh.2010.10-0263.
44. Friedman ND, Athan E, Walton AL, O'Brien DP. Increasing experience with primary oral medical therapy for *Mycobacterium ulcerans* disease in an Australian cohort. Antimicrob Agents Chemother. 2016;60(5):2692–5. https://doi.org/10.1128/AAC.02853-15.
45. Boeree MJ, Diacon AH, Dawson R, Narunsky K, Bois Du J, Venter A, et al. A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis. Am J Respir Crit Care Med. 2015;191(9):1058–65. https://doi.org/10.1164/rccm.201407-1264OC.

46. Omansen TF, Almeida D, Converse PJ, Li S-Y, Lee J, Stienstra Y, et al. High-dose rifamycins enable shorter oral treatment in a murine model of *Mycobacterium ulcerans* disease. Antimicrob Agents Chemother 2019;63(2):e01478–18. https://doi.org/10.1128/AAC.01478-18.
47. Almeida D, Converse PJ, Ahmad Z, Dooley KE, Nuermberger EL, Grosset JH. Activities of rifampin, Rifapentine and clarithromycin alone and in combination against *Mycobacterium ulcerans* disease in mice. PLoS Negl Trop Dis. 2011;5(1):e933. https://doi.org/10.1371/journal.pntd.0000933.
48. Blaschke TF, Skinner MH. The clinical pharmacokinetics of rifabutin. Clin Infect Dis. 1996;22(Suppl 1):S15–22.
49. Fan H, Li L, Wijlaars L, Gilbert RE. Associations between use of macrolide antibiotics during pregnancy and adverse child outcomes: a systematic review and meta-analysis. PLoS One. 2019;14(2):e0212212. https://doi.org/10.1371/journal.pone.0212212.
50. Rodvold KA. Clinical pharmacokinetics of clarithromycin. Clin Pharmacokinet. 1999;37(5):385–98. https://doi.org/10.2165/00003088-199937050-00003.
51. Maruri F, Guo Y, Blackman A, van der Heijden YF, Rebeiro PF, Sterling TR. Resistance-conferring mutations on whole-genome sequencing of fluoroquinolone-resistant and -susceptible *M. tuberculosis* isolates: a proposed threshold for identifying resistance. Clin Infect Dis. 2020;5:e12245. https://doi.org/10.1093/cid/ciaa496.
52. Pranger AD, van Altena R, Aarnoutse RE, van Soolingen D, Uges DRA, Kosterink JGW, et al. Evaluation of moxifloxacin for the treatment of tuberculosis: 3 years of experience. Eur Respir J. 2011;38(4):888–94. https://doi.org/10.1183/09031936.00176610.
53. Pasternak B, Inghammar M, Svanström H. Fluoroquinolone use and risk of aortic aneurysm and dissection: nationwide cohort study. BMJ. 2018;360:k678. https://doi.org/10.1136/bmj.k678.
54. Alves C, Mendes D, Marques FB. Fluoroquinolones and the risk of tendon injury: a systematic review and meta-analysis. Eur J Clin Pharmacol. 2019;75(10):1431–43. https://doi.org/10.1007/s00228-019-02713-1.
55. Principi N, Esposito S. Appropriate use of fluoroquinolones in children. Int J Antimicrob Agents. 2015;45(4):341–6. https://doi.org/10.1016/j.ijantimicag.2015.01.004.
56. Johnson PDR, Hayman JA, Quek TY, Fyfe JAM, Jenkin GA, Buntine JA, et al. Consensus recommendations for the diagnosis, treatment and control of *Mycobacterium ulcerans* infection (Bairnsdale or Buruli ulcer) in Victoria. Australia Med J Aust. 2007;186(2):64–8. https://doi.org/10.5694/j.1326-5377.2007.tb00802.x.
57. Alsaad N, van der Laan T, van Altena R, Wilting KR, van der Werf TS, Stienstra Y, et al. Trimethoprim/sulfamethoxazole susceptibility of *Mycobacterium tuberculosis*. Int J Antimicrob Agents. 2013;42(5):472–4. https://doi.org/10.1016/j.ijantimicag.2013.07.011.
58. Fehr H, Egger M, Senn I. Cotrimoxazol in the treatment of *Mycobacterium ulcerans* infection (Buruli ulcer) in West Africa. Trop Doct. 1994;24(2):61–3. https://doi.org/10.1177/004947559402400206.
59. Collaborative Group for the Meta-Analysis of Individual Patient Data in MDR-TB treatment–2017, Ahmad N, Ahuja SD, Akkerman OW, JWC A, Anderson LF, et al. Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis: an individual patient data meta-analysis. Lancet. 2018;392(10150):821–34. https://doi.org/10.1016/S0140-6736(18)316-1.
60. Converse PJ, Tyagi S, Xing Y, Li S-Y, Kishi Y, Adamson J, et al. Efficacy of rifampin plus clofazimine in a murine model of *Mycobacterium ulcerans* disease. PLoS Negl Trop Dis. 2015;9(6):e0003823. https://doi.org/10.1371/journal.pntd.0003823.
61. Revill WD, Morrow RH, Pike MC, Ateng J. A controlled trial of the treatment of *Mycobacterium ulcerans* infection with clofazimine. Lancet. 1973;2(7834):873–7. https://doi.org/10.1016/s0140-6736(73)92005-9.
62. Lunn HF, Ree RJ. Treatment of mycobacterial skin ulcers in Uganda with a riminophenazine derivative (B.663). Lancet. 1964;1(7327):247–9. https://doi.org/10.1016/s0140-6736(64)92351-7.

63. Bolhuis MS, van der Werf TS, Kerstjens HAM, de Lange WCM, Alffenaar J-WC, Akkerman OW. Treatment of MDR-TB using therapeutic drug monitoring: first experiences with sub-300 mg linezolid dosages using in-house made capsules. Eur Respir J. 2019;54(6):1900580. https://doi.org/10.1183/13993003.00580-2019.
64. Scherr N, Bieri R, Thomas SS, Chauffour A, Kalia NP, Schneide P, et al. Targeting the *Mycobacterium ulcerans* cytochrome bc1:aa3 for the treatment of Buruli ulcer. Nat Commun. 2018;9(1):5370–9. https://doi.org/10.1038/s41467-018-07804-8.
65. Thomas SS, Kalia NP, Ruf MT, Pluschke G, Pethe K. Toward a single-dose cure for buruli ulcer. Antimicrob Agents Chemother 2020;64(9):e00727–20. https://doi.org/10.1128/AAC.00727-20.
66. Arenaz-Callao MP, González Del Río R, Lucía Quintana A, Thompson CJ, Mendoza-Losana A, Ramón-García S. Triple oral beta-lactam containing therapy for Buruli ulcer treatment shortening. PLoS Negl Trop Dis. 2019;13(1):e0007126. https://doi.org/10.1371/journal.pntd.0007126.
67. Klis S, Kingma RA, Tuah W, van der Werf TS, Stienstra Y. Clinical outcomes of Ghanaian Buruli ulcer patients who defaulted from antimicrobial therapy. Trop Med Int Health. 2016;21(9):1191–6. https://doi.org/10.1111/tmi.12745.
68. O'Brien DP, Friedman D, Hughes A, Walton A, Athan E. Antibiotic complications during the treatment of *Mycobacterium ulcerans* disease in Australian patients. Intern Med J. 2017;47(9):1011–9. https://doi.org/10.1111/imj.13511.
69. O'Brien DP, Jenkin G, Buntine J, Steffen CM, McDonald A, Horne S, et al. Treatment and prevention of *Mycobacterium ulcerans* infection (Buruli ulcer) in Australia: guideline update. Med J Aust. 2014;200(5):267–70. https://doi.org/10.5694/mja13.11331.
70. WHO. Treatment of *Mycobacterium ulcerans* disease (Buruli Ulcer) [Internet]. Geneva, WHO; 2012. 66 p. Available from: https://www.afro.who.int/publications/treatment-mycobacterium-ulcerans-disease-buruli-ulcer
71. Janssens PG, Pattyn SR, Boveroulle MT, Quertinmont MJ, Demuynk A. A tropical necrotic ulcer originating in Bas-Katanga. Ann Soc Belg Med Trop 1920. 1963;43:729–37.
72. Clancey JK, Dodge OG, Lunn HF, Oduori ML. Mycobacterial skin ulcers in Uganda. Lancet. 1961;2(7209):951–4. https://doi.org/10.1016/s0140-6736(61)90793-0.
73. Rondini S, Horsfield C, Mensah-Quainoo E, Junghanss T, Lucas S, Pluschke G. Contiguous spread of *Mycobacterium ulcerans* in Buruli ulcer lesions analysed by histopathology and real-time PCR quantification of mycobacterial DNA. J Pathol. 2006;208(1):119–28. https://doi.org/10.1002/path.1864.
74. Wadagni AC, Steinhorst J, Barogui YT, Catraye PM, Gnimavo R, Abass KM, et al. Buruli ulcer treatment: rate of surgical intervention differs highly between treatment centers in West Africa. PLoS Negl Trop Dis. 2019;13(10):e0007866. https://doi.org/10.1371/journal.pntd.0007866.
75. O'Brien DP, Hughes AJ, Cheng AC, Henry MJ, Callan P, McDonald A, et al. Outcomes for *Mycobacterium ulcerans* infection with combined surgery and antibiotic therapy: findings from a south-eastern Australian case series. Med J Aust. 2007;186(2):58–61. https://doi.org/10.5694/j.1326-5377.2007.tb00799.x.
76. Wadagni AC, Barogui YT, Johnson RC, Sopoh GE, Affolabi D, van der Werf TS, et al. Delayed versus standard assessment for excision surgery in patients with Buruli ulcer in Benin: a randomised controlled trial. Lancet Infect Dis. 2018;18(6):650–6. https://doi.org/10.1016/S1473-3099(18)30160-9.
77. Carswell JW. Surgery for Buruli ulcer in the antibiotic era. Lancet Infect Dis. 2018;18(9):947. https://doi.org/10.1016/S1473-3099(18)30474-2.
78. Junghanss T, Um Boock A, Vogel M, Schuette D, Weinlaeder H, Pluschke G. Phase change material for thermotherapy of Buruli ulcer: a prospective observational single centre proof-of-principle trial. PLoS Negl Trop Dis. 2009;3(2):e380. https://doi.org/10.1371/journal.pntd.0000380.
79. Vogel M, Bayi PF, Ruf MT, Bratschi MW, Bolz M, Um Boock A, et al. Local heat application for the treatment of Buruli ulcer: results of a Phase II open label single center non comparative clinical trial. Clin Infect Dis. 2016;62(3):342–50. https://doi.org/10.1093/cid/civ883.

80. WHO. In: Asiedu K, editor. Wound and Lymphoedema Management. Geneva: WHO; 2010. p. 1–136. Available from: https://www.who.int/lymphatic_filariasis/resources/9789241599139/en/.
81. Velding K, Klis S-A, Abass KM, Tuah W, Stienstra Y, van der Werf TS. Wound care in Buruli ulcer disease in Ghana and Benin. Am J Trop Med Hyg. 2014;91(2):313–8. https://doi.org/10.4269/ajtmh.13-0255.
82. Woolley RJ, Velink A, Phillips RO, Thompson WA, Abass KM, van der Werf TS, et al. Experiences of pain and expectations for its treatment among former Buruli ulcer patients. Am J Trop Med Hyg. 2016;95(5):1011–5. https://doi.org/10.4269/ajtmh.16-0419.
83. Alferink M, de Zeeuw J, Sopoh G, Agossadou C, Abass KM, Phillips RO, et al. Pain associated with wound care treatment among Buruli ulcer patients from Ghana and Benin. PLoS One. 2015;10(6):e0119926. https://doi.org/10.1371/journal.pone.0119926.
84. Velink A, Woolley RJ, Phillips RO, Abass KM, van der Werf TS, Agumah E, et al. Former Buruli ulcer patients' experiences and wishes may serve as a guide to further improve buruli ulcer management. PLoS Negl Trop Dis. 2016;10(12):e0005261. https://doi.org/10.1371/journal.pntd.0005261.
85. Fokou PVT, Kissi-Twum AA, Yeboah-Manu D, Appiah-Opong R, Addo P, Yamthe LRT, et al. In vitro activity of selected West African medicinal plants against *Mycobacterium ulcerans* disease. Molecules. 2016;21(4):455. https://doi.org/10.3390/molecules21040455.
86. Van Leuvenhaege C, Vandelannoote K, Affolabi D, Portaels F, Sopoh G, de Jong BC, et al. Bacterial diversity in Buruli ulcer skin lesions: challenges in the clinical microbiome analysis of a skin disease. PLoS One. 2017;12(7):e0181994. https://doi.org/10.1371/journal.pone.0181994.
87. Yeboah-Manu D, Kpeli GS, Ruf MT, Asan-Ampah K, Quenin-Fosu K, Owusu-Mireku E, et al. Secondary bacterial infections of Buruli ulcer lesions before and after chemotherapy with streptomycin and rifampicin. PLoS Negl Trop Dis. 2013;7(5):e2191. https://doi.org/10.1371/journal.pntd.0002191.
88. Amissah NA, Chlebowicz MA, Ablordey A, Tetteh CS, Prah I, van der Werf TS, et al. Virulence potential of *Staphylococcus aureus* isolates from Buruli ulcer patients. Int J Med Microbiol. 2017;307(4–5):223–32. https://doi.org/10.1016/j.ijmm.2017.04.002.
89. Barogui YT, Klis S, Bankolé HS, Sopoh GE, Mamo S, Baba-Moussa L, et al. Towards rational use of antibiotics for suspected secondary infections in Buruli ulcer patients. PLoS Negl Trop Dis. 2013;7(1):e2010. https://doi.org/10.1371/journal.pntd.0002010.
90. Aujoulat I, Johnson C, Zinsou C, Guédénon A, Portaels F. Psychosocial aspects of health seeking behaviours of patients with Buruli ulcer in southern Benin. Trop Med Int Health. 2003;8(8):750–9. https://doi.org/10.1046/j.1365-3156.2003.01089.x.
91. Alferink M, van der Werf TS, Sopoh GE, Agossadou DC, Barogui YT, Assouto F, et al. Perceptions on the effectiveness of treatment and the timeline of Buruli ulcer influence prehospital delay reported by healthy individuals. PLoS Negl Trop Dis. 2013;7(1):e2014. https://doi.org/10.1371/journal.pntd.0002014.
92. Barogui YT, Sopoh GE, Johnson RC, de Zeeuw J, Dossou AD, Houezo JG, et al. Contribution of the community health volunteers in the control of Buruli ulcer in Bénin. PLoS Negl Trop Dis. 2014;8(10):e3200. https://doi.org/10.1371/journal.pntd.0003200.
93. Klis S, Ranchor A, Phillips RO, Abass KM, Tuah W, Loth S, et al. Good quality of life in former Buruli ulcer patients with small lesions: long-term follow-up of the BURULICO trial. PLoS Negl Trop Dis. 2014;8(7):e2964. https://doi.org/10.1371/journal.pntd.0002964.
94. de Zeeuw J, Douwstra M, Omansen TF, Sopoh GE, Johnson C, Phillips RO, et al. Psychometric properties of the participation scale among former Buruli ulcer patients in Ghana and Benin. PLoS Negl Trop Dis. 2014;8(11):e3254. https://doi.org/10.1371/journal.pntd.0003254.
95. O'Brien DP, Ford N, Vitoria M, Christinet V, Comte E, Calmy A, et al. Management of BU-HIV co-infection. Trop Med Int Health. 2014;19(9):1040–7. https://doi.org/10.1371/journal.pntd.0004075.
96. O'Brien DP, Comte E, Serafini M, Ehounou G, Antierens A, Vuagnat H, et al. The urgent need for clinical, diagnostic, and operational research for management of Buruli ulcer in Africa. Lancet Infect Dis. 2014;14(5):435–40. https://doi.org/10.1016/S1473-3099(13)70201-9.