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Abstract: ABSTRACT
Background Buruli ulcer (Mycobacterium ulcerans infection) is a Neglected Tropical Disease characterised by severe subcutaneous necrosis, with occasional bone involvement. Being reported from 33 countries, it is most prevalent in West and Central Africa, and Australia. In Africa, the major burden is borne by poor rural children. If left untreated, Buruli ulcer may progress to cause severe suffering and ultimately stigmatising disability resulting in school drop-out and loss of income. Standard antimicrobial treatment with oral rifampicin 10 mg/kg and intramuscular streptomycin 15 mg/kg for eight weeks (RS8) is highly effective but streptomycin injections are painful and may cause hearing loss.
Methods Between January 2013 and December 2017, we conducted an open label randomised multicentre phase III clinical trial with non-inferiority design comparing fully oral treatment with rifampicin and clarithromycin 15 mg/kg extended release (RC8) with RS8. A sample size of 332 participants was calculated to detect inferiority of CR8 by a margin of 12%.
Findings We stopped recruitment after 310 participants, 297 of whom had PCR-confirmed Buruli ulcer; 151 were assigned to SR8 treatment, while 146 received oral CR8 treatment. In the RS8 arm, 144/151 patients had healed lesions without relapse at the pre-defined time point 52 weeks after start of treatment - 95.4 (90.7-98.1)%, while 140/146 - 95.9 (91.3-98.5)% in the CR8 arm were healed. The difference in proportion, - 0.5 (-5.2 - 4.2), was not significantly greater than zero (p= 0.5873) demonstrating non-inferiority. Median time to healing was 24 (IQR, 8-28) weeks in the RS8 arm, and 16 (IQR, 8-25) weeks in the CR8 arm. Adverse drug effects were more common with streptomycin-based therapy; no patients needed

surgical resection; four had skin grafts. Interpretation For early, limited Buruli ulcer, oral clarithromycin-rifampicin combination treatment was non-inferior to intramuscular streptomycin-oral rifampicin treatment, and was associated with less ototoxicity.
Funding the World Health Organisation, sponsor; additional support in cash or kind was provided by MAP International, American Leprosy Missions, Raoul Follereau France, Buruli ulcer Groningen Foundation, Sanofi-Pasteur France; BuruliVac (EU FP7-241500). Trial registration - clinicaltrials.gov: NCT01659437.

Randomised trial to compare clarithromycin (extended release)-rifampicin and streptomycin-rifampicin for early, limited lesions of *M. ulcerans* infection

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ABSTRACT

Background Buruli ulcer (*Mycobacterium ulcerans* infection) is a Neglected Tropical Disease characterised by severe subcutaneous necrosis, with occasional bone involvement. Being reported from 33 countries, it is most prevalent in West and Central Africa, and Australia. In Africa, the major burden is borne by poor rural children. If left untreated, Buruli ulcer may progress to cause severe suffering and ultimately stigmatising disability resulting in school drop-out and loss of income. Standard antimicrobial treatment with oral rifampicin 10 mg/kg and intramuscular streptomycin 15 mg/kg for eight weeks (RS8) is highly effective but streptomycin injections are painful and may cause hearing loss.

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Interpretation For early, limited Buruli ulcer, oral clarithromycin-rifampicin combination treatment was non-inferior to intramuscular streptomycin-oral rifampicin treatment, and was associated with less ototoxicity.

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RESEARCH IN CONTEXT

Evidence before this study: The standard treatment for Buruli ulcer is combination antibiotic therapy comprising of intramuscular streptomycin and oral rifampicin (RS8) daily for 8 weeks. Streptomycin injections are painful and can cause ototoxicity. No previous trials in humans have tested the efficacy of a fully oral antibiotic regimen. A systematic review found case reports and observational cohort studies of demonstrating potential effect of fully oral antibiotic combinations for the treatment of Buruli ulcer.

Added value of this study: This phase III open label randomised controlled study evaluated the efficacy of a fully oral treatment with rifampicin and clarithromycin 15 mg/kg extended release (RC8) compared with standard of care using a non-inferiority design. Rates of healing of Buruli lesions were comparable in both arms being 95.4 (90.7-98.1)% in RS8 vs 95.9 (91.3-98.5)%, difference of -0.5% (-5.2 - 4.2). Notably, adverse events from treatment, in particular ototoxicity rates, were higher in the RS8 arm (9 out of 151) vs the RC8 arm (1 out of 146; X^2=6.35; p= 0.011745).

Implication of all the evidence available: A fully oral treatment comprising of rifampicin and clarithromycin is non-inferior to streptomycin-rifampicin in healing of early, limited Buruli ulcer lesions and is associated with a better safety profile.

Introduction

Buruli ulcer is a necrotizing skin disease caused by *Mycobacterium ulcerans (M. ulcerans),* and one of the 20 neglected tropical diseases. [1] In Africa, the disease was first identified and described near the Nile River in the former Buruli County in Uganda. Buruli ulcer has been reported from at least 33 countries [2], with the majority of cases occurring in West Africa; and sporadic cases occur in many locales in Central America and the Western Pacific.[3] Prevalence of the disease is highly variable, ranging from 3.1 to 30.7 cases per 100,000 population.[4] Even in endemic areas, prevalence is highly focal and varies considerably in space and time.[5] In Sub-Saharan Africa, the median age of new cases is around 20 years [6,7], whereas in the temperate climate of South-East Australia, the median age is around 60 years. [8]

Before 2005, surgery was the mainstay of treatment, and often required wide excision and skin grafting. A proof-of-principle study conducted in Ghana demonstrated the efficacy of the combination of streptomycin 15mg/kg (IM) and rifampicin 10mg/kg orally for 8 weeks to kill *M. ulcerans* in early limited Buruli ulcer disease [9] and the WHO subsequently issued provisional guidelines for this antimicrobial regimen (RS8) as the standard treatment. Early antimicrobial treatment gives excellent outcomes, but when treatment is delayed or disease is diagnosed late, severe morbidity, permanent disability and social stigma with loss of productivity and school drop-out may ensue.[10-12] The antimicrobial combination RS8 has proven effective in healing all forms of Buruli ulcer disease and has led to a reduction of the recurrence rate from 6 – 47% after surgery alone, to almost 0% after antimicrobial treatment.[13,14] However, daily streptomycin injections are painful and potentially toxic[15] and this in turn jeopardizes early reporting as well as compliance with therapy. Patients have to travel long distances to health

facilities for daily treatment; hence, there is a dire need for fully oral treatment. Mouse footpad studies comparing the standard RS8 therapy with rifamycins and other antimicrobials showed that replacing streptomycin by the macrolide drug clarithromycin (RC8) was as effective as RS8.[16,17] Studies in humans have shown that switching from streptomycin to clarithromycin after 4 weeks [14] or after 2 weeks [18] yielded comparable efficacy to RS8 therapy. In addition, a pilot study conducted in Benin provided the initial data suggesting the efficacy of fully oral RC8 treatment [19] but no direct comparison was made between treatment groups, and some patients with larger lesions still needed surgical treatment. Here, we report results from an open label randomised clinical study designed to determine the efficacy and tolerability of RC8 as compared to RS8 for treatment of early Buruli ulcer lesions.

Methods

The study adhered to the CONSORT (Consolidated Standards for the Reporting of Randomized Controlled Trials) guidelines.[20]

Study Population

The study was conducted in Benin and Ghana. [21] In the south of Ghana, four study sites participated; Agogo Presbyterian Hospital, Tepa Government Hospital, Nkawie Government Hospital, and Dunkwa Government Hospital. In the south of Benin, one study site, Pobè Health Centre, participated. Trained community health workers referred patients clinically diagnosed with *M. ulcerans* disease for possible enrolment, combining active and passive case finding

approaches.[22,23] Patients were included if they were clinically identified with typical Buruli ulcer disease, if aged ≥ 5 years, and with one single lesion ≤10 cm maximum cross-sectional diameter.

Exclusion criteria were: children < 5 years, lesions > 10 cm in maximum diameter, pregnancy, drug intolerance, renal or hepatic impairment, HIV infection or previous treatment with trial medication.

Ethics

The protocol and consent forms were approved by the ethics review Committee of the Ghana Health service (GHS-ERC01/03/11) and Benin (N°108/MS/DC/SGM/DFRS/CNPERS/SA). Ethics approval was also granted by the WHO ethics Review Committee (RPC443) and the University Medical Centre Groningen Ethics Review Board reviewed the protocol (M11.097746). Written and verbal informed consent was obtained from all participants, aged ≥12 years, and from parents, caretakers or legal representatives of participants aged <18 years. All staff involved in the study received formal training in Good Clinical Practice.

Study Design

This was an open label, Phase III, randomized controlled trial with non-inferiority design. Eligible subjects were randomly assigned to receive a combination of oral rifampicin (10mg/kg) and intramuscular streptomycin (15mg/kg) daily for 8 weeks (RS8) or a combination of oral rifampicin (10mg/kg) and oral clarithromycin (extended release formulation; 15mg/kg) daily for 8 weeks (RC8). Randomization was done with minimization for study site and type of lesion (ulceration or non-ulceration). The randomization sequence was computer-generated with blocks of 6 for each centre, and kept concealed in opaque envelopes.

Procedures

After participants had given informed consent, demographic, clinical information and blood samples were obtained. Pregnancy tests were conducted in female participants aged ≥10 years and hearing tests performed for all participants (AS208 portable equipment; Interacoustics, Assens, Denmark) to obtain baseline audiometry data. HIV antibody tests (Alere Determine HIV 1/2 Kit) were carried out after pre-test counselling.

The dimensions and aspect of lesions were documented by taking digital photographs.

Confirmation of diagnosis

Two fine needle aspirates (FNA) or swab samples were collected from each lesion to confirm the diagnosis of Buruli ulcer by quantitative PCR targeting IS2404.[24] Samples from the study sites in Ghana were transported to the Kumasi Centre for Collaborative Research in Kumasi, Ghana for analysis. The study centre in Benin used IS2404 qPCR conducted locally in Cotonou and confirmed in Angers, France. These laboratories participated in an external quality assessment program conducted by the WHO reference laboratory of the Institute of Tropical Medicine, Antwerp, Belgium.

Administration of antibiotics and other supportive care

After assessment and initiation of treatment at the hospital, most patients were treated as outpatients at the Ghanaian centres. In line with routine practice all patients recruited at the Pobè Health Centre in Benin, were treated as inpatients. Patients treated as outpatients were given medication in weekly batches to take to the nearest health facility where they received

directly observed therapy for the subsequent days, with daily wound care. Participant travel costs were reimbursed and small (nutritional) incentives were provided to compensate for their time in the study. For ulcers with active discharge, absorptive dressing material (Beier Drawtex Healthcare, South Africa) was applied, in order to maintain a moist environment for wound healing. When lesions were non-exuding, vaseline gauze was applied. Short stretch bandages were used to prevent lymphedema. Only participants who had lesions that involved joints, or who resided in distant villages were admitted to hospital in Ghana. The health care worker who observed the treatment recorded drug intake on the study form. Each study site had dedicated trained staff for wound care and for prevention of disability.

Trial medication

Rifampicin tablets and extended release clarithromycin were shipped to the trial sites; the pharmacists at Agogo Presbyterian Hospital and Pobè Health Centre each were responsible for storage of the trial medication.

Follow up and data collection

Participants were followed up at two weekly intervals during the first 8 weeks and then monthly for 12 months. At review visits, clinical assessments were performed including obtaining photographs of lesions. In addition, safety outcomes were assessed every 4 weeks by measuring liver, kidney and auditory tests in all participants. Female participants ≥ 10 years received pregnancy tests.

After 8 weeks of antimicrobial treatment (RS8 or RC8), patients were followed up monthly. Lesions not healed were measured and photographed. Treatment failure was recorded if the lesion had not healed by week 52 or if the lesion had recurred within this year. Removal of necrosis and slough, along with skin grafting were part of normal care. Requirements for these procedures were not considered evidence of treatment failure. Daily dressing changes were provided if wounds were discharging excessively.

Neither the investigators who took measurements of the lesions nor the attending doctors were masked to treatment assignment. An independent technical expert panel blinded to treatment assignment reviewed a sample of individual image sets. A study monitor assessed data entries; diagnostic uncertainties and adverse events were discussed with the (co-)principal investigators and the sponsor, using an electronic web-based platform; annual auditing trips to the study sites were made. All data were entered on paper and uploaded into OpenClinica. The electronic data files were collected and managed by the Drugs for Neglected Diseases initiative, Africa Regional Office, located in Nairobi, Kenya.

Sample size

Assuming an efficacy of 96% for standard SR8 treatment [14], the sample size needed to detect inferiority of the experimental RC8 treatment by a margin of 12% for oral treatment, was calculated to be 332 study participants with PCR-confirmed Buruli ulcer.

Outcome measures

The primary clinical endpoint was lesion healing (i.e., stable scar) without recurrence at time point 52 weeks after start of antimicrobial therapy. Secondary outcome measure was time to complete healing. The safety outcome was occurrence of adverse events (AE); any suspected or detected AE were shared with all investigators and members of the Data Safety Monitoring Board, using the web-based platform. Safety outcomes were separately analysed and compared between study arms; for hearing loss, we used a threshold increase of >25 dB.

Statistical analysis

Baseline comparisons of clinical and demographic characteristics according to study arm allocation were performed using Students T-test for parametric continuous data or Mann-Whitney's U-test for non-parametric data. Categorical data were compared using Chi-squared tests. The analysis was by intention to treat using a one-sided t-test for the primary outcome. Time to healing was analysed by a Cox proportional hazards model to assess the cumulative probability of healing. Hazards ratios were reported with 95% CI.

Role of sponsor and financial donors

WHO sponsored the trial, and additional support (including trial medication, dressing materials and financial support) was received from several donors. Neither the sponsor nor the donors had a decisive role in the study design or the analysis, reporting or decisions on publication.

Registration

The trial was registered with clinicaltrials.gov: NCT01659437.

Results

Figure 1 shows the flow chart of study participants; 443 patients were assessed for eligibility; with decreasing inclusions of study participants over time, enrolment was stopped after 310 (297 PCR-confirmed) study subjects were included; this decision was based on an ad hoc interim-analysis, in deviation of the protocol.

Table 1 shows that baseline characteristics of study participants were balanced; gender distribution was almost equal between groups, and median age was around 15 years. Most lesions were on the limbs, with slight preponderance for the lower limb; around half of the lesions were ulcerated at the time of entry.

The primary end point was healing without relapse at week 52 after start of treatment; in the RS8 arm, 144/151 patients were healed - 95.4 (90.7-98.1)%, while 140/146 - 95.9 (91.3-98.5)% in the RC8 arm were healed. The difference, - 0.5 (-5.2 - 4.2), was not significantly greater than zero (p= 0.5873). The upper limit of 95% confidence interval of the difference of 4.2% is less than the non-inferiority margin of 12% demonstrating non-inferiority. Time to healing was a secondary end point; Figure 2 shows the plot of cumulative probability of healing with 95% CI and the results of Cox proportional hazard for time to healing. The median time to healing was 24 (IQR, 8-28) weeks in the RS8 arm, and 16 (IQR, 8-25) weeks in the RC8 arm.

Table 2 summarizes the data of the 13 study participants - seven in the RC8 arm, and six in the RS8 arm - who failed on treatment.

Table 3 summarizes the safety data; adverse effects were generally limited and mild, except for

hearing loss in nine participants in the SR8 study arm. One of these patients had severe persisting dizziness, with hearing impairment confirmed by audiometry. In contrast only one patient in the RC8 group had hearing loss. Blood chemistry test results did not reveal renal damage or grade 3-4 liver test abnormalities; ECG abnormalities were equally distributed and did not reach grades 3-4. Total number of adverse events was 12 in RS8 vs 1 in RC8; p= 0.0022.

Drop-out was low (1), adherence to drug treatment was excellent, and recurrences were not noted during follow-up. No patient had resection surgery, and four patients had skin graft.

Discussion

In this study, we confirm that a fully oral antimicrobial treatment for early, limited Buruli ulcer with rifampicin and an extended release formulation of clarithromycin is non-inferior to the treatment with rifampicin and injected streptomycin. Although both treatments were generally well tolerated, safety outcomes were in favour of oral treatment; ten individuals, receiving streptomycin, experienced side effects attributed to that drug. Our results support the use of fully oral treatment as first-line treatment for early, limited Buruli ulcer lesions. Oral treatment is far more convenient for patients and also prevents the toxicity (predominantly acoustic) that can occur with the use of the injectable aminoglycoside streptomycin[15]. This study addressed a question that was also deemed important by former patients with Buruli ulcer, who prioritised research into treatment avoiding painful injections and that had few side effects [25]. Age and gender distribution, and socio-economic status and educational level were all typical for the

population affected by Buruli ulcer in West Africa.

Our study had limitations, the most important being the open label design. Concealment of active study medication was not considered appropriate with placebo injections for study participants that were predominantly children. We were unable to fully rule out bias in the read-out of the primary end point, i.e. time of healing, and healing at week 52. Assessments were however made by attending team members, and all staff involved in the study had formal training in Good Clinical Practice; monitoring and digital photographic documentation at follow-up helped reaching consensus in determining the primary end point of healing. Besides, we explored any potential bias, by assessing healing by a panel of wound experts blinded for treatment allocation, and were unable to detect bias, by reading the photographic imaging.

Though this study is the largest treatment trial for Buruli ulcer disease to date, our study failed to reach the initially intended sample size of 332 participants with PCR-confirmed Buruli ulcer. The duration of the study had already been extended several times, and finally it was decided to stop accrual based on an ad-hoc interim analysis suggesting that extending the study any further would not result in different study outcomes. Since the start of the study, the number of patients has steadily declined at the study sites, a phenomenon that has been observed in most West African endemic regions.[6,26,27] The incidence of Buruli ulcer has fluctuated, and has recently emerged unprecedentedly in Victoria, Australia.[3,6]

Several aspects in the experimental design aided in producing a high quality study. These included a focus on wound care, rehabilitation, and pain management during dressing changes. However, despite these measures, the median time to wound healing was similar to that

reported in previous studies.[13,14,18]

The strengths of the study were the large sample size; the diagnostic confirmation by validated PCR technology; extended monitoring and auditing, the high healing rate at week 52, the extremely low drop-out rate, and minimal loss to follow-up. We used internet-based data storage and data collection, and had intensive interactions and consultations among our study team, using a web-based platform. Very few study participants needed any form of surgery; no debridement surgery was done in any of the study subjects, and only four patients had skin grafts. This is consistent with findings from a study conducted in Benin addressing the role of surgery in Buruli ulcer disease.[28] One limitation here is that we are unable to comment on the efficacy of RC8 on larger Buruli ulcers that were excluded.

The RC8 treatment had excellent tolerability and efficacy.

In real life, daily drug treatment for 8 weeks, in combination with regular wound dressing changes, remains challenging in the rural endemic setting in Africa. Not all patients necessarily need a full course of 8 weeks of antimicrobial treatment [29], but it is currently unknown how much treatment would suffice, and individualised treatment duration for Buruli ulcer has not been evaluated in a randomised trial. Drug-drug interactions between rifampicin and clarithromycin result in reduced exposure to clarithromycin in the course of weeks, as a result of hepatic enzyme induction by rifampicin [30], and perhaps the most important action of clarithromycin at onset of treatment is the prevention of clonal expansion of *M. ulcerans* with reduced susceptibility to rifampicin.[31] This phenomenon however appears uncommon.[32] Considering that the role of clarithromycin is primarily a companion drug, we suspect that

providing this drug as once daily extended release preparation had no marked impact on overall efficacy; at the time of designing the study, and based on the limited exposure of the immediate release preparation of clarithromycin, 7.5 mg/kg[30], we decided to double the dosage in this extended release form.

Experimental treatments with shorter duration are currently under study [33-35] but novel drugs usually take considerable time to reach the clinical arena. Our study provides evidence that this combination of oral generic rifampicin and extended release clarithromycin provides excellent tolerability and efficacy.

Conclusion

Based on its non-inferiority compared to streptomycin combined with rifampicin, we recommend the combination of oral rifampicin and clarithromycin for patients with Buruli ulcer lesions. In our study we only provided evidence for this recommendation for lesions of limited size; this oral antimicrobial treatment might also be beneficial for larger lesions.

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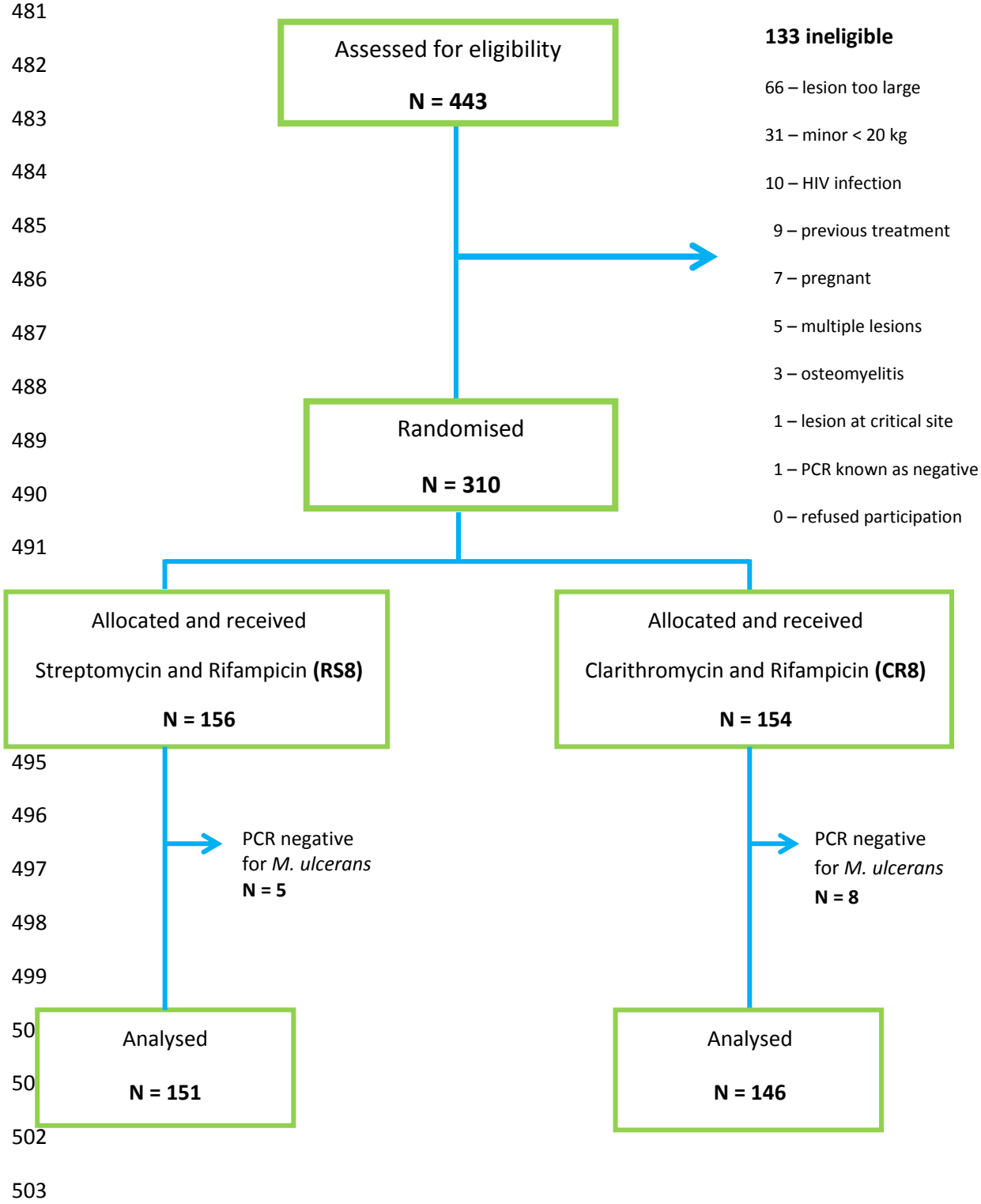
Figure 1: flow chart of study participants



504 **Table 1**: baseline characteristics of study participants

		RS8 (N=151)	**RC8 (N=146)**	**total (N=297)**
gender	Male, n (%)	74 (49.0)	70 (48.0)	144 (48.5)
	Female, n (%)	77 (51.0)	76 (52.0)	153 (51.5)
age	Mean (SD)	20.4 (16.3)	22.9 (17.7)	21.6 (17.0)
	Median (IQR)	14 (10 to 25)	15.5 (10 to 32)	14 (10 to 29)
Education level	None, n (%)	35 (23.5)	43 (30.1)	78 (26.7)
	Primary/middle school, n (%)	100 (67.1)	92 (64.3)	192 (65.8)
	Secondary/above, n (%)	14 (9.4)	8 (5.6)	22 (7.5)
Weight (Kg)	Mean (SD)	41.5 (15.9)	43 (17.5)	42.3 (16.7)
	Median (IQR)	41 (27 to 55)	44 (26 to 58)	42 (26 to 57)
Height (m)	Mean (SD)	1.5 (0.2)	1.5 (0.2)	1.5 (0.2)
	Median (IQR)	1.5 (1.3 to 1.6)	1.5 (1.3 to 1.6)	1.5 (1.3 to 1.6)
Lesion location	Upper limb, n (%)	47 (31.3)	61 (41.8)	108 (36.5)
	Lower limb, n (%)	90 (60.0)	73 (50.0)	163 (55.1)
	Buttocks and perineum, n (%)	3 (2.0)	1 (0.7)	4 (1.4)
	Head and neck, n (%)	1 (0.7)	4 (2.7)	5 (1.7)
	Thorax, n (%)	4 (2.7)	3 (2.1)	7 (2.4)
	Abdomen, n (%)	2 (1.3)	3 (2.1)	5 (1.7)
	Back, n (%)	3 (2.0)	1 (0.7)	4 (1.4)
Lesion type	Nodule, n (%)	28 (18.5)	34 (23.3)	62 (20.9)
	Plaque, n (%)	39 (25.8)	36 (24.7)	75 (25.3)
	Oedema, n (%)	1 (0.7)	3 (2.1)	4 (1.4)
	Ulcer, n (%)	83 (55.0)	73 (50.0)	156 (52.5)

505 RS=Rifampicin & Streptomycin; RC=Rifampicin & Clarithromycin; SD=standard deviation;
506 IQR=Inter-quartile range

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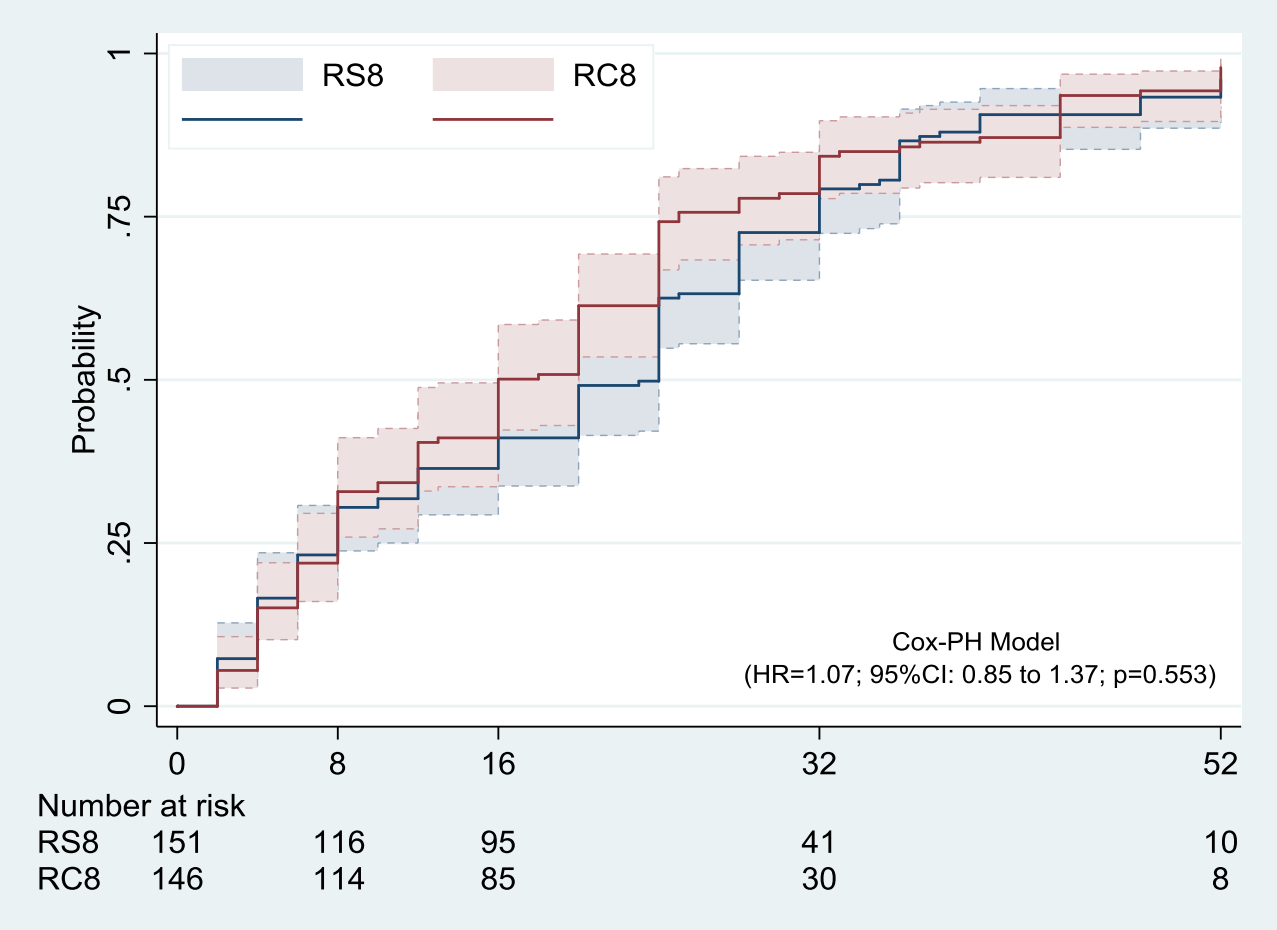
Figure 2: Cumulative probability of healing over time in weeks of Buruli Ulcer patients with 95% confidence intervals for each study arm



535 **Table 2**: details of study participants in whom treatment failed

TREATMENT ARM	AGE	SEX	LESION TYPE	LESION CATEGORY	COMMENT
RS8	72	Male	Ulcer	II	Lesion not healed due to trauma which caused enlargement, possibly due to the location of lesion (lateral malleolus)
RC8	26	Female	Ulcer	I	Patient was lost to follow up at week 12 when lesion was unhealed. Could not be traced to establish healing
RS8	9	Female	Ulcer	II	Lesion healed at week 56
RS8	9	Male	Ulcer	II	Lesion healed at week 77
RC8	11	Female	Ulcer	II	Patient was lost to follow up, reported at week 97 but not healed possibly due to improper wound management
RC8	32	Male	Plaque	II	Suspected malignancy. Patient was lost to follow up when referred for further medical checks
RS8	23	Female	Ulcer	II	Patient developed paradoxical reaction, suspected daily trauma due to location of lesion (medial malleolus) and lack of proper wound care
RS8	7	Male	Plaque	I	Patient lost to follow up after week 12. Unable to establish healing at week 52
RC8	7	Female	Oedema	II	Lesion not healed due to unresolved osteomyelitis
RS8	75	Female	Ulcer	II	Patient was lost to follow up, traced after week 52 but not healed
RS8	14	Male	Ulcer	II	Lesion not healed after 52 weeks due to superstitious beliefs of patient and hence improper wound care
RC8	84	Female		II	Patient could not be traced after baseline. No documentation to support healing although lesion said to heal at week 20
RC8	45	Female	Ulcer	I	Patient was lost to follow up after week 20. Patient reported lesion not healed when called by phone after week 52 - possibly due to improper wound care

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Table 3: summary of adverse drug reactions

		RS8	RC8	total
Number enrolled and receiving at least one dose		151	146	297
Number of patients with at least one SAE: n (%)		1 (0.7)	1 (0.7)	2 (0.7)
	Adverse drug reaction	1 (100)	0 (0.0)	1 (50.0)
	Not related to study treatment	0 (0.0)	1 (100)	1 (50.0
Patients with at least one AE (serious or not): n (%)		18 (11.9)	9 (6.2)	27 (9.1)
	Adverse drug reaction	9 (50.0)	1 (11.1)	10 (37.0)
	Not related to study treatment	10 (55.6)	8 (88.9)	18 (66.7)
Total number of Adverse drug reactions		12	1	13*
Number of Adverse drugs reaction per patient, median (range)		0.5 (0 to 2)	0 (0 to 1)	0 (0 to 2)
Patients whose treatment was stopped due to an AE, n (%)		2 (1.3)	0 (0.0)	2 (0.7)
	Adverse drug reaction	0 (0.0)	0 (0.0)	0 (0.0)
	Not related to study treatment	2 (100)	0 (0.0)	2 (100)

RS=Rifampicin & Streptomycin; RC=Rifampicin & Clarithromycin; AE=adverse event; SAE=serious adverse event

** p=0.0022

Table 4: accrual of study participants at study sites over time

Year / Site	2013	2014	2015	2016	Total	%
Agogo, Ghana	41	51	33	16	141	45.7
Tepa, Ghana	13	11	21	17	62	20.3
Dunkwa, Ghana	4	11	9	6	30	9.5
Nkawie, Ghana	5	7	5	4	21	6.8
Pobè, Benin	8	22	8	18	56	17.7
Total	**71**	**102**	**76**	**61**	**310**	100

World Health Organization

PROTOCOL

RANDOMIZED CONTROLLED TRIAL COMPARING EFFICACY OF 8 WEEKS TREATMENT WITH CLARITHROMYCIN AND RIFAMPICIN VERSUS STREPTOMYCIN AND RIFAMPICIN FOR BURULI ULCER (*M. ULCERANS* INFECTION)

Version Number: 06
25 May 2012

BENIN AND GHANA

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Sponsor: WHO

Registry File Number : T9-370-115

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1. American Leprosy Missions, USA
2. Raoul Follereau Foundation, France
3. MAP International, USA (provision of supplies)
4. Sanofi, France (donation of rifampicin)
5. 7th Framework Programme of the European Union: Burulivac project (241500)

SIGNATURE PAGE

The signatures below document the approval of this protocol and the attachments, and provide the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality and according to local legal and regulatory requirements.

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Signed: _____

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University of Groningen, Netherlands.

Sponsor:

Signed: _____ Date: Friday, 25 May 2012

(Dr. Kingsley Asiedu)
Global Buruli Ulcer Initiative,
Department of Control of Neglected Tropical Diseases,
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Contents

LIST OF ABBREVIATIONS

AE	Adverse Event
AFB	Acid-fast bacilli
AIDS	Acquired immunodeficiency syndrome
ART	anti-retroviral therapy
BUD	Buruli ulcer disease
BUFLS	Buruli ulcer functional limitation score
C	Clarithromycin
CI	Co-Investigator, Medical Doctor in charge of the study at the study site
CRF	Case Report Forms
C&T	Counseling and Testing (i.e., HIV testing)
Co-PI	Co-Principal Investigators
DOT	Directly Observed Treatment
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
FNA	Fine Needle Aspiration
GBUI	Global Buruli Ulcer Initiative
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HAART	Highly active anti-retroviral therapy
HIV	Human immunodeficiency virus
ITM	Institute of Tropical Medicine
LRM	Laboratoire de Référence des Mycobactéries
NACP	National AIDS Control Programme
NBUCP	National Buruli Ulcer Control Programme
NGOs	Non-governmental organizations
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
NTM	Nontuberculous mycobacteria
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PNLLUB	*Programme national de lutte contre la lèpre et l'ulcère de Buruli*
PNLS	*Programme national de lutte contre sida*

R	Rifampicin
RCT	Randomized Controlled Trial
S	Streptomycin
SAE	Serious Adverse Event
SC	Study Coordinators
SUSAR	Serious Unexpected Suspected Adverse Reaction
SOP	Standard Operating Procedure
TAG	WHO Technical Advisory Group on Buruli ulcer
TB	Tuberculosis
VAS	Visual Analogue Scale
WHO	World Health Organization
ZN	Ziehl–Neelsen stain

ABSTRACT

The study will be a single blinded, randomized, controlled open label non-inferiority phase II/III, multi-center trial (1 center in Benin and 4 centers in Ghana), with two parallel groups. The ultimate goal is to search for an effective alternative treatment to the current standard WHO-recommended therapy in all stages of BUD, with inherent logistical, operational and safety disadvantages. A total of 415 clinically diagnosed BUD patients will be included in the study, which will consist of 332 cases of PCR- confirmed category I & II BUD ($\leq$ 10 cm) plus other 83 non PCR-confirmed BUD. Patients will be randomized to receive treatment with the two antibiotic regimens as follow: (i) Regimen I (SR8): 15 mg/kg streptomycin per day intramuscular injection for 8 weeks plus 10 mg/kg per day oral rifampicin for 8 weeks (ii) Regimen II (CR8): 15 mg/kg per day oral clarithromycin extended-release for 8 weeks plus 10 mg/kg per day oral rifampicin for 8 weeks. The follow-up period of patients will be 12 months. Assessments before, during and after the course of antibiotic treatment will include full medical history, clinical assessments and monitoring of vital signs, assessment of the lesion, laboratory investigations, hearing test, electrocardiogram (ECG), pregnancy test, HIV counseling and testing, and functional limitation assessment. The primary efficacy parameters are healing without recurrence and without excision surgery 12 months after start of treatment.

1.0 PROTOCOL SUMMARY

Title: RANDOMIZED controlled trial comparing efficacy of 8 weeks treatment with clarithromycin and rifampicin *versus* streptomyci*n and r*ifampicin for Buruli ulcer (*M. ulcerans* infection)
Protocol Number: T9-370-115

Background:

A combination therapy with streptomycin and rifampicin (SR) has been the standard antibiotic treatment for *M. ulcerans* infection since 2004 (1). Healing was common after SR treatment in an observational study with at least 4 weeks of S injections daily (S, 15 mg/kg body weight intramuscularly) combined with R (10 mg/kg body weight daily) (2). In an observational study, SR treatment administered to 160 patients with a wide range of BUD lesions was highly successful; only 8 individuals required excision and skin grafting (3). In a randomized trial, SR treatment had a success rate of 96% at 12 months after start of treatment, and a switch to oral treatment, replacing S by clarithromycin (C), 7.5 mg/kg body weight did not significantly change the chance of healing (4). The WHO Technical Advisory Group (TAG), meeting in March, 2010, was in favor of trying fully oral treatment (CR8: CR x 8 weeks), considering that a long duration of injected therapy may lead to potential pain and infection at site of injection. Several small observational unpublished studies presented at the GBUI meeting in Geneva, March 2010, showed that fully oral treatment is promising.

Objectives:

Primary objectives:

To assess whether the complete lesion healing rate at 12 months of treatment without the need for additional excision surgery and without recurrence (relapse) in BUD patients with category I lesions and category II ≤10 cm cross-sectional diameter lesions, is in favor of CR8 or within an acceptable difference of 12% point in favor of SR8.

Secondary objectives:

- To compare the *recurrence rates* within 12 months of treatment initiation with the two antibiotic regimens (CR8 and SR8).
- To compare the *treatment failure rates* within 12 months of treatment initiation with the two antibiotic regimens (CR8 and SR8).
- To compare the *incidences of paradoxical response* within 12 months of treatment initiation with the two antibiotic regimens (CR8 and SR8).
- To assess whether *lesion healing* depends upon lesion category (I or II ≤10 cm in diameter) and lesion type (nodule; plaque; ulcer; or edema) and treatment regimen in terms of:
  - (i) lesion sizes (surface area)
  - (ii) time to complete lesion healing
  - (iii) healing without additional surgery or relapse
  - (iv) type of adjunctive surgical therapy
  - (v) time from treatment initiation to surgery
- To compare the *rates of residual functional limitations* at 12 month following treatment with the two antibiotic regimens (CR8 and SR8) and lesion category (I or II) and lesion type (nodule; plaque; ulcer; or edema).
- To compare the *incidences and relative risk* of all Adverse Events (AEs), including treatment-related AEs, Serious Adverse Events (SAEs) and grade 3-4 toxicity in patients treated with CR8 and SR8 regimens.
- To compare treatment *discontinuation rates* in patients treated with CR8 and SR8 regimens.
- To compare the *compliance rates* in patients treated with CR8 and SR8 regimens.

- To explore any emergent hypothesis or any explanation of an unexpected result in randomized patients meeting all inclusion criteria.

Study locations:

The study will be carried out at five study sites:

- Pobè Treatment Center (Benin)
- Agogo Presbyterian Hospital (Ghana)
- Tepa Government Hospital (Ghana)
- Nkawie-Toase Government Hospital (Ghana)
- Dunkwa Government Hospital (Ghana)

Study design:

Randomized, controlled open label non-inferiority phase II/III, multi-center trial, with two parallel groups.

Study population:

A total of 415 clinically diagnosed BUD patients, consisting of 332 cases of PCR- confirmed category I & II BUD (≤ 10 cm) plus approximately 83 non PCR-confirmed BUD

Inclusion criteria:
All patients (both genders) with a clinical diagnosis of BUD (categories I or II; lesion size, ≤10 cm cross-sectional diameter).

Exclusion criteria:

(1) Children < 5 years, or ≤ 20 kilograms body weight

(2) Pregnancy (self-reported, clinically diagnosed, or urine test (beta-hCG) positive.

(3) Patients with previous treatment of Buruli ulcer, tuberculosis or leprosy with at least one of the study drugs (rifampicin, streptomycin, clarithromycin)

(4) Patients with history of hypersensitivity to rifampicin and/or streptomycin and/or clarithromycin

(5) Patients with previous treatment with macrolide or quinolone antibiotics, or anti-tuberculosis medication, or immuno-modulatory drugs including corticosteroids within one month.

(6) Patients with current treatment with any drugs likely to interact with the study medication, e.g, anticoagulants, cyclosporin, phenytoin, and phenobarbitone. Users of oral contraceptives should be notified that such contraceptive is less reliable if taken with rifampicin; alternative (mechanical) contraceptive methods will be discussed with the study participant (*Appendix 8*).

(7) Patients with co-infection with HIV

(8) Patients with history or having current clinical signs of ascites, jaundice, partial or complete deafness, myasthenia gravis, renal dysfunction (known or suspected), diabetes mellitus, and severe immune compromise (e.g., immunosuppressive drugs after organ transplant), or evidence of (previous) tuberculosis, Buruli ulcer or leprosy; or terminal illness (e.g., metastasized cancer).

(9) Patients who are unable to take oral medication or having gastrointestinal disease likely to interfere with drug absorption.

(10) Patients with known or suspected bowel strictures who cannot tolerate macrolide antibiotics such as clarithromycin

(11) Patients with mental condition, including addiction with substance abuse (alcohol, qat, etc.) likely to interfere with possibility to comply with the study protocol.

(12) Patients who are not willing to give informed pre-consent, and consent (patient and/or parent/legal representative), or withdrawal of consent.

Treatment allocation and study drugs:

A total of 415 BUD patients. Patients will be block-randomized to receive one of the two different antibiotic regimens as follow:

- *Regimen I (SR8):* Streptomycin (15 mg/kg per day, intramuscularly) in combination with rifampicin (10 mg/kg per day, orally) for 8 weeks
  - *166 PCR*-confirmed BUD + approximately *41* non-PCR confirmed BUD
- *Regimen II (CR8):* Clarithromycin (150 mg/kg per day. Oral extended release formulation) in combination with rifampicin (10 mg/kg per day, orally) for 8 weeks
  - *Approximately 166 PCR*-confirmed BUD + approximately *41* non-PCR confirmed BUD

Discontinuation criteria:

For individual subjects

- Rapid enlargement of the lesion despite drug treatment not attributed to a paradoxical response
- Serious adverse event (SAE) or adverse event of NIH/NCI CTC grade 3 or 4
- Withdrawal of consent or assent
- Need to use any drug with anti-mycobacterial activity or likely to interfere with interpretation of the results
- Any new condition or situation (including pregnancy, major trauma, or severe intercurrent disease) developing during drug treatment in the trial which may interfere with continued participation in the study.

For part of, or for the entire study

- If the first interim analysis shows failure >20% in the CR8 arm after the first 100 patients have been followed for six months, and at least 50% more failure than in the SR8 arm, or the second interim analysis shows failure >20% in the CR8 arm

after the first 200 patients have been followed for six months and at least > 40% more failures than in the SR8 arm.

- If the chance of success of the trial is less than 10% once 100 patients were followed for one year.
- Presence of statistically significantly more SUSAR or adverse drug reaction of NIH/NCI Common Toxicity Criteria (CTC) grade 3 or 4 in CR8 relative to CS8 or observation of a number of patients that is considered by the DSMB as too large to be acceptable for toxicity.
- Rapid enlargement of the lesion despite drug treatment (increase in size of the lesion to 150% of the original maximum diameter) in >20% of cases in any (sub-) group. The protocol may need to be amended if lack of efficacy is suspected in such sub-group.

Study duration:

• Recruitment and treatment of patients	24 months (1 July 2012–1 July 2014)
• Follow-up of patients	12 months (ending 1 July 2015)
• Laboratory studies	(continued throughout the study)
• Data collection and analysis	(continued throughout the study)

Assessment procedures:

- Pre-treatment assessments: full medical history, clinical assessments and monitoring of vital signs, assessment of the lesion (full description, size measurement, photography, fine needle aspirates and swabs for *M. ulcerans*, drug sensitivity and mycolactone), laboratory investigations (clinical chemistry, urinalysis, full blood count), hearing test, electrocardiogram (ECG), pregnancy test, HIV counseling and testing, and functional limitation assessment.

- During (8 weeks) and after treatment assessments (8 weeks to 12 months and at the time of treatment failure): full medical history, clinical assessments and monitoring of vital signs, assessment of the lesion (full description, size measurement, photography, fine needle aspirates and swabs for *M. ulcerans*, drug sensitivity and mycolactone), laboratory investigations (clinical chemistry, urinalysis, full blood count, hearing test, ECG, pregnancy test, HIV counseling and testing), and functional limitation assessment.

Efficacy and safety parameters:

Primary parameters

- Rate of complete lesion healing at 12 months in BUD patients with category I lesions and category II ≤10 cm cross-sectional diameter lesions.

Secondary parameters

- Recurrence rate within 12 months of treatment initiation.
- Rate of treatment failure within 12 months of treatment initiation.
- Rate of paradoxical response within 12 months of treatment initiation.
- Proportion of patients with reduction in lesion surface area within 12 months of treatment initiation.
- Time taken for complete lesion healing within 12 months of treatment initiation.
- Proportion of patients with complete lesion healing (Δ% points) without additional surgery or relapse within 12 months of treatment initiation.
- Iinterval of time between healing and recurrence in case of occurrence within 12 months of treatment initiation.
- Proportion of each type of surgery within 12 months of treatment initiation.
- Time from treatment initiation to surgery if any within 12 months of treatment initiation.

- Proportion of patients with residual functional limitations within 12 months of treatment initiation.
- Incidence of all adverse effects (AEs) within 12 months of treatment initiation.
- Treatment discontinuation rate
- Treatment compliance rate

Statistical analyses:

- Descriptive statistics: mean (95& CI), median (95% CI) or geometric mean, proportion and percentage, where appropriate
- Inferential statistics: Chi-square, Wilcoxon Signed Rank test, logistic model, generalized linear model, Cox model, multinomial logistic model, Hodge-Lehman estimator, Blackwelder test, where appropriate
- Level of significance: $\alpha = 0.05$

2.0 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Mycobacterium ulcerans infection causes ulcerating skin lesions known as Buruli ulcer. Its existence was first recorded by Sir Albert Cook, a British missionary doctor who worked in Uganda, in the Mengo Hospital, that he established in 1897 (5). Although he did not publish his findings, the descriptions of patients with lesions are very likely to have been Buruli ulcer. The name: Buruli ulcer is derived from the Buruli district in Uganda (now Nakasongola), close to the borders of a portion of the Nile that constitutes lake Kyoga. In the 1960's, Buruli County harboured refugees from a tribal conflict in Burundi. Many patients were identified with ulcers that were described and studied by researchers of the British Medical Research Council (6-8). Cases were however also described from Zaire / Congo (9-11).

2.1.1 Epidemiology

The disease exists or has been suspected in at least 32 countries in Africa, South East Asia, Western Pacific region and the Americas. After Cook described the disease in his Mengo Hospital Notes in 1897, cases have subsequently been reported from Central and West Africa. Sporadic cases have been reported from other areas such as Malaysia, Papua New Guinea, Mexico and French Guiana and from Victoria, Australia with its temperate climate. It was in Australia that the relationship with *M. ulcerans* infection was first recognized and proved in 1948 (12). Gradually, case series have been reported from West Africa, i.e., from Benin (13-14), Cameroon (15), Gabon (16), Nigeria (17;18), Liberia (19), and Ghana (20). The disease generally affects poor farming communities in remote rural areas with limited access to health services. In Africa, a little over 50% of those affected are children under the age of 15 years while in Australia, patients are older and attack rate appears to increase with age. The disease starts as a painless nodule, papule, plaque or oedema and without specific treatment, it frequently progresses to massive ulceration. Up to one third of patients with nodules heal spontaneously (21). The lesion may however heal at any stage, and the onset of healing is generally believed to follow the development of an immune response to the organism. Lesions can extend to cover

large areas of the body and this, together with the protracted course of the disease, results in scarring and deformity with debilitating sequelae (22).

2.1.2 WHO – GBUI

In 1998, the Director General of the World Health Organization (WHO), Dr. Hiroshi Nakajima, helped establishing the Global Buruli Ulcer Initiative (GBUI) in response to the growing spread and impact of Buruli ulcer disease (*M. ulcerans* infection). The primary objectives of the GBUI are to raise awareness of the disease, mobilise support to assist affected countries to deal with the disease, promote and co-ordinate research activities, and co-ordinate the work of non-governmental organizations (NGOs) and other partners. Since 1998, Dr Kingsley Asiedu has headed the WHO GBUI. In 2004, the 57th World Health Assembly passed a resolution on Buruli ulcer that reflected for the first time international recognition of this neglected tropical disease as a global health threat. Subsequently, WHO has listed Buruli ulcer among 17 Neglected Tropical Diseases (23).

2.1.3 *M. ulcerans* transmission

M. ulcerans is generally believed to be an environmental micro-organism, but only recently has recovery from the environment been achieved, using culture, after passage in experimental animals (24). Many live species harbour the micro-organism (25-33) but the mode of transmission remains largely unknown. Exposed body parts are most vulnerable (34) and transmission might occur by direct skin penetration either by trauma (35), or insect bites (26, 28, 30, 36-37). The role of insects is still debated (38-41). Distribution on body surface has been studied but while some studies show unequal distribution between left and right extremities (4, 42), other studies show a remarkably even distribution (34). Possibly different modes of transmission occur in different disease foci.

2.1.4 Mycolactone toxins

The micro-organism is phylogenetically closely related to *M. marinum* (43-44). It had long been recognized that *M. ulcerans* exerts its tissue damage predominantly by a secreted toxin (45-48). In 1999, A research group led by Prof. Pam Small identified the toxin as a ketolide lipid structure, mycolactone (49). A whole range of mycolactone molecules (A-F) have been

identified, with decreasing toxicity from A to F, and all are produced by a limited number of Mycolactone-Producing Mycobacteria (50). *M. ulcerans* is the only MPM affecting humans. MPM produce mycolactone via a specialized system of mycolactone synthase enzymes, coded for by genes located on a plasmid (51-52). A sub-species, *M. ulcerans* subsp. *shinshuense* causes similar lesions in Japan (53) but some researchers argue that these MPM are all one single species (54).

2.1.5 Immune phenomena & pathogenesis

After passing the natural barrier of the skin, *M. ulcerans* is recognized by innate immune mechanisms and phagocytosed by specialised immune cells, i.e., Dendritic Cells and macrophages (55-58). There is abundant evidence that many healthy people in endemic areas have been exposed to *M. ulcerans* but never develop overt disease (59-62). Host genetic susceptibility as well as yet unknown other factors determine whether bacterial multiplication and mycolactone production follow (63). Many studies have addressed the role of protective immunity, generally considered to be Th1 phenotype, both locally at the site of infection as well as systemically (64-71). Mycolactone clearly impairs the host immune response early on in BUD (72-77). Typically, the immune response improves over time, usually following surgical or antimicrobial treatment (58, 66, 71, 75, 78-80). Mycobacterial infections in their own right cause down-regulation of Th1 type immuno-protection (77, 81) but probably mycolactone is the dominant factor causing local and systemic down-regulation of protective immunity (56, 72-77). In the course of time following treatment, enhancement of immune response might be mistaken for disease progression. Increase in size of lesions, with inflammatory changes, and new lesions appearing during or after effective antimicrobial treatment have been referred to as paradoxical responses (82). A paradoxical response is defined as increase in number and/or size of lesions – usually, after initial improvement - during effective reduction of bacterial load. This has been described in reversal reactions in leprosy (83-85) and tuberculosis (86-89).

2.1.6 Diagnosis

Although the classical way to confirm the presence of *M. ulcerans* – like the diagnosis of any micro-organism is culture (12), this test is rather insensitive (90-95). Before polymerase chain

reaction (PCR) targeting a repetitive insertion segment 2404 was developed, histopathology was considered the diagnostic test with the highest diagnostic yield (96-98). Although experienced pathologists may easily recognise BUD in tissue specimens by typical features of coalescent necrosis in addition with the finding of acid-fast bacilli in early lesions and granuloma formation in later lesions (90, 99-100), the inherent weakness of histopathology is the need to provide representative tissue samples. Besides, the presence of acid-fast bacilli in tissue specimens may not differentiate between BUD and other mycobacterial infections. The same is true for direct microscopy of cotton-wool swabs and fine needle aspirates (FNA) of lesions. With the development of PCR (101-102), it has gradually become evident that this test had the highest diagnostic yield. The complete sequencing of the genetic code of *M. ulcerans* (51-52) made it clear that among potential targets for PCR, IS2404 had the highest number of copies (>200/cell) thus providing an ideal target for a diagnostic assay. PCR targeting IS2606 (with some 100 copies/cell) has been tried but generally has lower diagnostic yield (103-104), probably because of the lower number of copies present in the genome of *M. ulcerans*. Several different groups have reported on the diagnostic yield of PCR targeting IS 2404 (105-110). A potentially important development is the introduction of real-time PCR technology that although more expensive may be more reliable and less vulnerable to contamination (111). Inclusion of multiple *M. ulcerans*-specific DNA sequence targets may increase the usefulness for environmental samples (112-113).

Sufficient evidence has emerged for the diagnostic yield of FNA (93-95, 114). A more invasive approach like punch biopsy is no longer justified as a routine diagnostic procedure (115).

2.1.7 Treatment

Since the days of Sir Albert Cook, surgery has been recommended as treatment of choice (12, 21, 97). Thinking along the lines of oncological surgery, wide, radical excision was recommended with the aim of removing all bacilli, followed by spilt-skin grafting. Indeed treatment centres that followed this strategy reported very few recurrences (116) while other centres with less aggressive approach had persistent or recurrent lesions in 18-47% (117). Even with excision of a fairly large rim of apparently healthy tissue however, PCR signals suggesting presence of *M. ulcerans* can still be demonstrated at the edge of resected tissue specimens (118-120).

The option to treat BUD with anti-mycobacterial agents has been subject to debate. *In vitro* evidence has invariably suggested that *M. ulcerans* is highly susceptible to rifampicin (121-125), aminoglycosides notably streptomycin and amikacin (124-126); clarithromycin (125, 127); and some of the fluoroquinolones (125-126).

In animal models, combinations with rifampicin were likewise effective (126, 128-135). There has been controversy about the need to add aminoglycosides to treatment regimens. Animal studies showed that combinations with amikacin (or streptomycin) were highly effective in killing *M. ulcerans* in lesions (129-132, 136). However some oral treatment schedules were also effective in these animal models (126, 137-138).

In humans, an earlier study evaluating clofazimine mono-therapy compared with placebo did not find any important benefit for this treatment (21). Clinical observations in patients with BUD empirically treated with several different antimycobacterial agents were disappointing. Rifampicin combined with dapsone was tried in a small study in which baseline characteristics differed and no convincing advantage for patients using this combination therapy was observed compared with placebo (139). An inconclusive small study was reported on cotrimoxazole with no apparent benefit (140). The overall impression has been that *in vitro* and *in vivo* susceptibility results would not reliably predict response in humans. A proof-of-principle study was therefore designed to test whether *M. ulcerans* could be efficiently killed in human tissue. The treatment schedule selected for this study was derived from *in vivo* animal model data, and SR combination (streptomycin plus rifampicin) was used in a study that enrolled patients with non-ulcerated lesions of BUD. Patients were selected based on clinical criteria with no confirmation testing was done prior to treatment. Patients were allocated to either 2, 4, 8, or 12 weeks of SR treatment after which lesions were surgically removed and submitted for testing by PCR targeting IS2404, histopathology, direct microscopy with acid-fast stain, and culture (141). Although in only 21 of 30 individuals included, the diagnosis could be confirmed, culture of tissue specimens was negative in all patients treated with SR for 4 weeks or longer (1). All patients either had healed or at least their lesion had decreased in size prior to surgical resection. Based on this report, as well as observational data reported on patients with ulcerated and non-ulcerated BUD lesions treated with SR, many with PCR-confirmed disease, the majority of whom had favourable response to

treatment (2), a study was designed to evaluate the response to 8 weeks SR treatment (SR8), in comparison with the same treatment for 4 weeks, with a switch to oral treatment for 4 weeks with rifampicin combined with clarithromycin (SR4CR4) (4). The primary endpoint of this study was healing without the need for debridement surgery, and without recurrence, 12 months after start of treatment. Patients allocated to SR8 treatment had 96% success, which was not significantly different from the 91% success rate for those on SR4CR4.

One observational study reporting on 160 patients with a wide range of BUD lesions treated with SR for eight weeks was highly successful; only 8 individuals required excision and skin grafting (3). In another observational study in 92 patients with large BU lesions, 62 of whom had PCR-confirmed disease, surgery was combined with SR drug treatment. Recurrence rate was only 1.1% (142).

2.1.8 The case for oral treatment

The preliminary results of the study testing a switch to CR4 after SR4 in comparison with SR8 were reported during the annual meeting of the Global Buruli ulcer Initiative, WHO, in Cotonou, March 30 – April 3, 2009. Discussion fuelled the idea to evaluate the clinical efficacy of oral therapy alone, without adding S. Recurrence of BUD after surgery occurring in 27% of cases could be eliminated entirely with combinations of oral treatment including CR (143). A case of a pregnant woman was successfully treated with CR combination was reported (144). Several groups started small pilot studies that were subsequently reported at the WHO Annual meeting on BU in Geneva, March 22-24, 2010:

(1) Chauty et al (145) showed a case series of 30 patients with PCR-confirmed BUD <10 cm cross-sectional diameter, duration < 6 months. Surgery was conducted based upon assessment at week 4; 4 patients (category II) had debridement surgery, 10 more had only minor surgery; all healed and none had recurrence in 12-18 months follow-up.

(2) Couppié et al (146) presented data from a small case series from French Guyana. Patients were increasingly treated without surgery, and with oral (CR) antimicrobial treatment.

(3) Gordon et al (147) presented 4 cases of BUD treated with oral antimicrobial treatment; two were given CR, two others had R-moxifloxacin; three were operated in addition but one had CR only. None had recurrence but one had a culture-negative inflammatory paradoxical response.

(4) Humphris (148) presented data from Cameroon. Of 19 patients on oral CR treatment, 13 were treated as out-patient. Adherence to therapy was satisfactory. During follow-up no recurrences were noted.

(5) Phillips (149) presented interim data from a pilot study in Ghana with patients with BUD randomized to SR2CR6 (n=43) or SR8 (n=40). At 20 weeks follow-up, no difference was noted between the two study arms. A later report presented at the recent GBUI meeting in March 2011 confirmed that the regimen was well tolerated with excellent efficacy.

(6) O'Brien (150) presented two cases suspected to have failed on oral antimicrobial treatment; both had evidence of a paradoxical inflammatory response, no failure with persistently negative culture results.

Based on the preliminary evidence provided, the lack of robust intermediate end-points predicting recurrence-free cure 12 months after start of therapy, and the promising responses observed in the open-label series presented, it was generally agreed that a head-to-head comparison of fully oral (CR8) treatment with current WHO-standard-recommended SR8 treatment should now follow.

2.1.9 Paradigm shift: Bacterial killing vs reducing mycolactone production

The design of the drug trial conducted in Ghana [1] was based on the assumption that a bactericidal anti-mycobacterial drug combination would be required to eliminate all bacteria present in human tissues. Essentially, the trial answered the question whether this bactericidal combination could safely be limited to four weeks only, and this question has been answered to some degree. The paper reporting the study results (4) mentions several positive culture results from lesions that

[1] http://clinicaltrials.gov/ct2/show/NCT00321178?term=buruli&rank=1

were not healed after completion of the treatment. These patients nonetheless went on to heal without recurrence. These findings challenge the hypothesis that bactericidal drugs should be used, and that killing of all bacteria is required for healing. Adding the aminoglycoside streptomycin (S) to the regimen reflects this hypothesis, and leaving this drug class out entirely, implying a deviation from that paradigm. It has generally been agreed, both for tuberculosis (151), leprosy (152), as well as for Non-Tuberculosis Mycobacterial (NTM) infections (153) that multi-drug regimens are required to prevent drug-resistant mutants to replace wild-type drug-sensitive micro-organisms. This is especially true during initial disease when a high microbial burden with inherent chance of presence of resistant mutants facilitate drug resistant clones to repopulate disease foci. Conceptually, drug-resistant clones are a major concern if humans are the reservoir of offending pathogens like *M. tuberculosis* and *M. leprae* due to the inherent danger of transmission of drug-resistant organisms. In NTM and *M. ulcerans* infection, the reservoir is not in humans but nonetheless, drug resistance has important consequences for infected individuals themselves. Once they harbour drug-resistant organisms, their chances for successful treatment outcome diminish. Monotherapy with R has been shown to have potential of development of drug resistance in experimental animals (154). For selection of the necessary companion drug of R, which is considered the more powerful component of oral regimens, it is important to consider toxicity issues, as well as drug-drug interactions, and pharmacokinetics. Although moxifloxacin is a powerful drug (126), the class of fluoroquinolone drugs is relatively contraindicated in pregnant women and young children (144). Based on safety and efficacy, clarithromycin (C) is probably the best choice for an oral companion drug for R.

2.1.10 Pharmacokinetics consideration

An important problem in CR combination therapy is drug-drug interaction (155). R is known to induce cytochrome P450 enzymes, e.g., CYP 3A4 involved in the elimination of C, while C is also known to inhibit enzyme activity of CYP 3A4 (156-157). During the conduct of the trial in Ghana, the pharmacokinetics of CR-treated individuals was investigated (4, 158-159). The study confirmed that the area under the plasma-concentration time curve (AUC) of C was reduced during co-medication with R. The 14-hydroxy metabolite of C was increased compared to the parent compound C. The 14–OH C metabolite appeared not to contribute significantly to the

antimicrobial effect of the parent compound C on several strains of *M. ulcerans* tested. On the other hand, AUC of R was non-significantly increased. Taking into consideration that in the trial, three individuals failed on the primary end point in the SR arm, while seven failed in the SR4CR4 arm, one should consider the possibility that the latter treatment might be slightly less effective, even though the difference did not reach statistical significance. Slightly more individuals in the SR4CR4 arm had additional (non-protocol driven) microbial testing than those in the SR8 arm. Although two of the five individuals who had positive cultures during follow-up after treatment completion still went on to heal, and were indeed completely healed at the pre-defined time point (52 weeks after start of treatment), there is concern that the CR treatment may be slightly inferior to SR treatment. This might be due to the fact that C blood concentrations were slightly lower in some patients, even though no failure to prevent R-resistant mutants to escape and multiply was demonstrated in those participants that failed in the CR arm. R is an inducer of cytochrome P450 enzymes that metabolize C into its less effective 14-OH form. Alternatively, if C is only required as a companion drug to suppress R-resistant mutants, all of these considerations might be less critical, but to suppress R-resistant mutants, inhibitory concentrations of C should also be obtained, especially if CR is considered from the start. In Pobe in Benin (145), CR treatment was given with a single dose of 12 mg/kg body weight C, which is not an EMA- or FDA approved dosage for any indication. In the current trial, due to the propensity of pharmacokinetic drug interaction which may result in inadequate therapeutic concentration of C when used in combination with R, the EMA- and FDA- registered extended release formulation (15 mg/kg, ER) will be used instead of the single dose once daily treatment (7.5 mg/kg, once daily) as used in the earlier trial in Ghana (4). Increase of C exposure without risking toxicity and without the need of twice-daily dosing would be expected following the extended release formulation. Twice daily dosing would make supervised treatment virtually impossible, and adherence to therapy significantly more difficult. As the best way to overcome these problems, the once-daily double-dose extended release formulation has been selected.

2.1.11 Co-infection with HIV

In general, the HIV prevalence in West and Central Africa remains comparatively low, with the adult HIV prevalence estimated at 2% or under in 12 countries in2009 (Benin, Burkina Faso,

Democratic Republic of the Congo, Gambia, Ghana, Guinea, Liberia, Mali, Mauritania, Niger, Senegal, and Sierra Leone)[2]. The role of co-infections may influence outcome. This is especially true for HIV co-infection (160-162), although generally, HIV co-infection has not been identified as a risk factor for BUD in highly endemic areas in West Africa, notably, Ghana (163), Benin (164) and Cameroon (165). In the randomized drug trial in Ghana, 2% of the study population under investigation were found HIV positive (4). In Benin, 6 (3.6%) out of 156 patients treated at Pobe Buruli Ulcer Treatment Center in 2006 were positive for HIV, and in 2010, 2 (1.5%) out of 135 patients were positive (personal communication, Annick Chauty). The issue of HIV testing may still be delicate among rural populations in Ghana and Benin, even though the national policies are in favor of providing counseling and testing in individuals with increased risk for HIV infection. Since HIV infection may confound efficacy and safety analyses, and because some of the drugs used in treating HIV infection may interact with some of the drugs used to treat Buruli ulcer, participants will be offered counseling and testing as inclusion criteria. Those who are HIV- positive will be offered appropriate treatment. Those who test negative and consent to participate in the randomization study will be enrolled. The national program for HIV/AIDS in Ghana provides services with 85% successful treatment for those adherent to treatment; 31% were however lost to follow-up (166). Because of greater access to treatment, HIV counseling and testing is beneficial. HIV counseling will be incorporated in the written and oral informed consent, and those testing positive will be initially treated for Buruli ulcer (8 weeks of antibiotics) and referred to HIV treatment services for care in collaboration with the National HIV Programmes in both countries. In Benin, the Pobe General Hospital will be the referral hospital for HIV treatment and in Ghana, *Agogo Presbyterian Hospital, Tepa Government Hospital and Dunkwa Government Hospitals are known ART centres. Nkawie hospital does not offer ART and patients who test positive will be referred to the Suntreso Government Hospital in Kumasi (nearest place) for confirmation and treatment. This is the normal arrangement under the National AIDs Control Programme* If testing is refused at intake, study participants will be offered counseling and testing during consecutive follow-up visits.

[2] UNAIDS Report on the Global AIDS Epidemic 2010: http://www.unaids.org/documents/20101123_GlobalReport_em.pdf

2.1.12 Co-infection with tuberculosis

Co-infection of Buruli ulcer and tuberculosis has not been reported in the literature and appears exceedingly rare. However, because limited numbers of TB drugs are used for the treatment of Buruli ulcer for a shorter period of time (2 months compared with 6 months in TB) and to avoid sub-treatment of TB patients, a careful history (cough for more than 2 weeks, sometimes productive of sputum that can be blood-stained, chest pains, weakness, weight loss, fever and night sweats) and physical examinations will be carried out for each study participants (see *Appendix 1:* Diagnosis of tuberculosis disease, CDC factsheet). Patients with suspected TB will be further examined – chest X-ray and sputum for laboratory examination for AFB will then be requested. Those confirmed to have tuberculosis will be referred for TB treatment according to national treatment policies but if streptomycin is not included in the TB treatment regimen, it will be added to adequately cover *M. ulcerans*. In Benin, the Pobe General Hospital will be the referral facility. In Ghana, TB treatment is integrated into the general health services so all the study sites also offer TB care.

2.1.13 Paradoxical response and stopping rule

Another important issue is the concept of paradoxical response, which is defined as increase in inflammatory changes with increase in lesional size, typically after initial improvement and decrease in size, and/or the appearance of new lesions following or during antimycobacterial treatment. The word ‘paradoxical’ refers to the fact that these lesions do not contain increasing numbers of viable bacilli, but instead, decreasing and/or non-viable or dead bacilli. This has been well known in tuberculosis (86-89;167) and leprosy (83;84). It has also been recognized in BUD (79, 82, 168). This should caution against very strict clinical criteria for failure early on, and soon after completion of antimycobacterial treatment.

A paradoxical response is defined as increase in number and/or size of lesions, usually after initial improvement during effective reduction of bacterial load. Failure is therefore not defined as any increase of the lesion size (surface area) compared to the initial size, but > 150% of initial surface area at any time point after start of treatment. Furthermore, new lesions appearing during or weeks after completion of treatment need not necessarily be interpreted as failure. After the first 100 patients have been followed for at least 6 months, the Data Safety Monitoring Board

(DSMB) will need to consider stopping the trial if the conditional power is less that 10%, *i.e.* if the chance to succeed is less than 10%. Likewise, stopping will be considered if the first interim analysis shows failure >20% in the CR8 arm after the first 100 patients have been followed for six months, and at least 50% more failure than in the SR8 arm, or the second interim analysis shows failure >20% in the CR8 arm after the first 200 patients have been followed for six months and at least > 40% more failures than in the SR8 arm.

Finally, at 12 months, residual functional limitations will be assessed by a formally validated questionnaire (169). At pre-defined time points, the study team will use a 3-point Likert scale for ability to walk, and for hand function.

2.2 Ethics and community involvement

This study follows the principles of ethical conduct of studies in humans, in accordance with the Helsinki Declaration and its updates; and the study will be conducted respecting Good Clinical Practice (GCP). Ethics Committees in the Netherlands (UMCG, University of Groningen), Switzerland (WHO, Geneva), Ghana (HRU, ACCRA) and Benin (Cotonou) have already approved, or will need to approve the protocol prior to commencing the study. Special attention will be given to illiterate candidate study participants, and the study team will be trained in the process of seeking informed consent in low resource settings with participants with limited educational background.

Community mobilization plans: Health seeking behavior in BUD in endemic African regions has been shown to be highly influenced by beliefs and attitudes towards chronic infectious diseases like BUD (170-173), anticipated and perceived stigma (170), fear for surgery (170, 173), and financial barriers (173). In this study, several culturally sensitive issues will be addressed. Consent and assent will be asked from participants and their parents or guardians about co-infections, notably HIV. Pregnancy testing is also required for female minors after menarche. The cultural context demands an approach in which the study team will therefore need to discuss these issues with community members. The study team will organize meetings with opinion leaders in endemic areas in each district. These opinion leaders include health care workers, i.e., village health workers, traditional birth attendants, traditional and herbal healers, as well as other important social leaders, e.g., school teachers, chiefs and elders, and the outline of the study

protocol will be discussed with them, and the culturally sensitive issues will be addressed in detail. The leading principle will be to respect privacy and autonomy of potential study participants, even if they are minors. Although the rights of vulnerable groups women and children is generally considered a basic human right, this may not necessarily be appreciated in some traditional societies. The consent and assent procedures and the language and procedures will therefore be explained to community leaders, and trained with the study team using video, slide presentations and role play. Traditional healers will be mobilized in the communities and get involved in case finding and referral to the study sites, and possibly, to help in the follow-up of patients /study participants. Communities will be sensitized to detect (early) presentations of BUD, using the graphics material (comic books) written in English and French. The general principle here is to show hope for cure without sequelae with antibiotic treatment. Identifying possible early BUD lesions are pivotal, and the recognition of such lesions is emphasized; individuals that might have such early lesions will be encouraged to present to the study team at the study site. We will use photographic images of typical early BUD lesions to be used in the field; these images will be coated with plastic to prevent decay. As there is no planned pre-consent at village level, the community leaders are not directly involved in this process. The procedures will be explained to them, for their information. Furthermore, the satellite studies will be explained in such fashion that we will emphasize that we seize the opportunity; the randomized study provides a platform that may help to address additional scientific questions to be answered without causing additional harm or disadvantages to study participants.

Community education: The community education sessions will be organized to ensure that the same level of information is provided in all locations irrespective of the language the information is in. Key communities endemic communities have been selected by the districts and these will be initially targeted for education and mobilization of study participants (see Appendix 7) The study coordinators involved in the training of study staff will attend and coordinate the community meetings. All of this will be part of the launching of the study at each study site. The various different study sites will be opened one at the time to ensure that community education sessions follow additional training of staff at each study site.

2.3 Risk-benefit consideration

2.3.1 Potential risks

(1) The patients may experience a brief moment of physical discomfort or pain during the venepuncture procedure and there is a possibility of brusing and/or infection at the venipuncture site. All procedures will be conducted by trained personnel under sterile conditions as to minimize the risk for the patients.

(2) Patients may experience adverse effects from the study drugs:

Rifampicin (R): The most serious adverse effect of R is hepatotoxicity (hepatitis, jaundice, and liver failure) and detection of liver test abnormalities helps to detect liver damage early. Other possible adverse effects include respiratory (breathlessness), cutaneous (flushing, pruritus, rash, redness and watering of eyes), abdominal effects (nausea, vomiting, abdominal cramps with or without diarrhea), flu-like symptoms (chills, fever, headache, arthralgia, and malaise), and rarely, dysphoria. Furthermore, R is an effective liver enzyme-inducer (such as CYP2C9 and CYP3A4 enzymes), and therefore, co-administration with other drugs metabolized by these enzymes may result in inadequate therapeutic drug concentrations of the co-administered drugs.

Clarithromycin (C): Most common side-effects are gastrointestinal (diarrhea, nausea, extreme irritability, abdominal pain and vomiting), facial swelling. Patients with known or suspected bowel strictures cannot tolerate this drug and they will be excluded from the study. Less common side-effects include headaches, dizziness/motion sickness, rashes, alteration in senses of smell and taste, including a metallic taste. The drug may also aggravate or cause Qtc prolongation.

Streptomycin (S): The nephrotoxic potential of S is very low when compared to other aminoglycosides. However, risk of ototoxicity is higher. Hearing loss may begin in the high-tone range and can only be detected with an audiogram.

All the adverse effects related to the study drugs including long-term disabilities will be responsible by the research team and the costs of treatment will be covered by the insurance.

(3) There is also a risk that recurrence following treatment regimens. All patients will therefore be closely monitored and treated should any signs and symptoms of BUD be detected.

2.3.2 Potential benefits

(1) Patients may be hospitalized during antibiotic therapy (8 weeks) free of charge and will be closely monitored for any signs and symptoms of BUD. They will have benefit of close medical supervision. All doses of the study drugs will be administered by medical/nursing staff trained in drug administration, and any change in the course of their infection or any adverse experiences will be recognized and treated more rapidly than would normally occur if they were treated as outpatients in a government facility. In addition, while admitted to the hospital, the patients may be examined and treated for other concurrent illnesses. Patients will receive medical attention and appropriate standard medical care or referral should they become ill during the study. Patients will also be provided with food, accommodation, and transport during the study.

(2) Patients in whom BUD could not be ascertained will receive standard care. If they have BUD but meet exclusion criteria to participate in the study, they will receive medical care in the respective hospitals as deemed best by the attending medical team. Only if treatment for BUD follows, patients will be reimbursed for treatment as in the study protocol.

(3) Patients who fail treatment therapy will be treated with a standard regimen following the national treatment guideline that is known to be effective.

(4) When a patient is withdrawn from the study medication prior to completion of trial treatment, the investigator medical team will provide the best proven treatment for the patient's condition. Additionally, the team will carry out all the safety and efficacy assessments that would have been carried out at the next scheduled visit (unless the patient is lost to follow up).

(5) Female participants with child-bearing potential will be notified that the study team provides contraceptives free of charge for the duration of drug treatment for those

that show interest in having these. Condom use is preferred as oral contraceptives are unreliable during co-medication with R. The procedure will be such that by covering the package, this will not be discernable by others attending the clinic or Out-Patient Department where potential study participants are received.

(6) The national control program of Benin and Ghana will benefit from human resource capacity building to modern laboratory methodology and GCP training for study staff.

2.4 Working hypotheses

(i) Healing rate, defined as recurrence-free healing at time point 12 months of antibiotic treatment initiation is either better in the CR8 group than, or equal to, or at worst less than 12% point inferior to (acceptable limit) the SR8 group.

(ii) In patients with BUD category I and category II with ≤10 cm cross-sectional diameter lesions, the proportion of individuals with complete lesion healing without additional excision surgery is higher in the CR8 group than, or equal to, or at worst less than 12% point inferior to the SR8 group.

(iii) In patients with BUD category I and category II with ≤10 cm cross-sectional diameter lesions, the proportion of individuals with complete lesion healing without additional excision surgery in the CR8 arm is higher than, or at worst less than 12% inferior to the SR8 group.

(iv) The difference in surgery extent (measured by ARANZ equipment or by conventional surface area measurement) immediately before and after surgery, with measurements assessed by a panel of experts unaware of treatment given in the CR8 group is lower than, or at worst 20% point in favor of the SR8 group.

(v) The proportion of grade 3-4 NIH-NCI toxicity in the CR8 group is lower than, or at worst lower than 10%-points in favor of the SR8 group.

(vi) The proportion of patients with residual functional limitations at 12 months in the CR8 group is lower than, or at worst less than 10% to that of the SR8 group.

3.0 TRIAL OBJECTIVES

The ultimate goal is to search for an effective alternative treatment (CR8) to the current standard WHO-recommended therapy with SR8 in BUD category I lesions and category II ≤10 cm cross-sectional diameter lesions, with inherent logistical, operational and safety disadvantages.

3.1 Primary objectives:

To assess whether the complete lesion healing rate at 12 months of treatment without the need for additional excision surgery and without recurrence (relapse) in BUD patients with category I lesions and category II ≤10 cm cross-sectional diameter lesions, is ≥, or equal to, or less than 12% point (acceptable limit) in favor of the group treated with CR8, compared with the group treated with SR8 regimen.

3.2 Secondary objectives:

To compare secondary parameters between groups, as listed below -

- Recurrence rate within 12 months of treatment initiation.
- Rate of treatment failure within 12 months of treatment initiation.
- Rate of paradoxical response within 12 months of treatment initiation.
- Proportion of patients with reduction in lesion surface area within 12 months of treatment initiation.
- Time taken for complete lesion healing within 12 months of treatment initiation.
- Proportion of patients with complete lesion healing (Δ% points) without additional surgery or relapse within 12 months of treatment initiation.

- Iinterval of time between healing and recurrence in case of occurrence within 12 months of treatment initiation.
- Proportion of each type of surgery within 12 months of treatment initiation.
- Time from treatment initiation to surgery if any within 12 months of treatment initiation.
- Proportion of patients with residual functional limitations within 12 months of treatment initiation.
- Incidence of all adverse effects (AEs) within 12 months of treatment initiation.
- Treatment discontinuation rate
- Treatment compliance rate

To explore any emergent hypothesis or any explanation of an unexpected result in randomized patients meeting all inclusion criteria.

4.0 TRIAL DESIGN

4.1 Study sites

The study will be conducted at the five study sites, one in Benin and four in Ghana as follow:

(i) Pobe Treatment Center (Benin);
(ii) Agogo Presbyterian Hospital (Ghana)
(iii) Tepa Government Hospital (Ghana)
(iv) Nkawie-Toase Government Hospital (Ghana)
(v) Dunkwa Government Hospital (Ghana)

Descriptions of these sites are summarized in *Appendix 2.* Depending on the recruitment rate, additional study sites in other districts may be required. Approval from the Ethics Committee will be processed before the recruitment of the new study site(s).

Table 1 – *BU data of selected drug trial sites*

District	Trial site	2008	2009	2010	2011	Total
Ahafo-Ano North	Tepa Government Hospital	76	51	57	51	235
Asante-Akim North	Agogo Presbyterian Hospital	102	110	130	112	454
Atwima-Nwabiagya	Nkawie-Toase Government Hospital	101	104	60	58	323
Upper Denkyira East	Dunkwa Government Hospital	73	56	19	13	161
Upper Denkyira West	St. John Community Clinic, Nkotumso	100	94	81	32	307
Ouémé Plateau	Centre Dépistage TB & Ulcère de Buruli, Pobe	222	156	140	150	668
Total		674	571	487	416	2148

4.2 Study design

The study will be conducted as a randomized, controlled open label non-inferiority phase II/III, multi-center trial, with two parallel groups. The rationale for selecting the non-inferiority study design is that, considering the efficacy of the standard treatment with injected streptomycin (SR8) of as high as 96% (4), the question whether the experimental treatment (CR8) could be better or worse than SR8, is not the relevant question. Rather, the main consideration is to estimate to what extent the oral treatment with CR8 could be inferior if so to the current standard of care with SR8. During the last GBUI Meeting held at Geneva, March 2011, the protocol and the comments by the WHO Ethics Committee were discussed during the main session. In addition, during a special meeting by the Drug Treatment Group of the Technical Advisory Group of GBUI, with a large group of attendants from Buruli ulcer-endemic regions in Africa including National BU Program managers from Ghana, Benin, as well as Togo, and DR Congo at the national level were consulted. Specifically, attendants from endemic African regions agreed that it would be justified to accept a margin of 12% in the currently proposed trial design, considering

the fact that the experimental treatment (CR8) would provide a very valuable alternative to standard care (SR8). The advantages are no injections, which is not only much more comfortable for the patients, but also less dangerous with respect to local (injection abscess) and general infectious complications (inadvertent needle re-use at village level, with associated risk for blood-born infections, especially, infectious hepatitis - HBV, HCV, and HIV). Moreover, the logistic challenge of proving injected therapy is enormous, with the need to provide syringes, needles, water for injection and vials of streptomycin. In addition, streptomycin is associated with risk of acute kidney injury, especially in the elderly, and vestibulo-oto-toxicity. Vestibulo-oto-toxicity may be irreversible, and even though regular audiograms will be performed in this study this side effect may not be detected in time to prevent residual and lasting damage. The current practice under service conditions is not providing audiographic monitoring, and even this monitoring is not able to prevent all cases of ototoxicity. In summary, the concept of a difference in efficacy ≤12% is justified in this trial. Besides, detection of a smaller difference would require an unacceptably large sample size that would make this study impossible to complete within a reasonable period of time. A timely answer to the research question with the proposed sample size and detection limit of difference in efficacy would greatly advance the field, especially at the level of national programs.

4.3 Description of the trial

On the screening day (t-0), all patients (both genders) with clinically diagnosed BUD lesions (categories: I, and II, ≤10 cm cross-sectional diameter) (*Appendix 3*) will be actively and passively selected from the study sites. Health seeking behaviour as well as referral patterns to hospitals for suspected BUD is known to be strongly influenced by beliefs and attitudes (171-174). As described in section 2.2, opinion leaders (village health workers, traditional birth attendants, traditional and herbal healers) as well as other important social leaders (school teachers, chiefs and elders) in each village will play important role in patient recruitment process. The study team (*Appendix 5*) will mobilize them in the communities and try to involve them in case finding and referral to the study sites. Activities in the districts will therefore include providing information about diagnosis and treatment of BUD at schools and durbars. Video, slide shows and role play will be used as tools to increase preparedness of people to identify lesions as

possible BUD and present timely to the health care system. Prior to any diagnostic procedure, pre-consent and pre-assent are required before procedures related to BUD diagnosis can be conducted. None of these study-related activities will be conducted in the community. Only at the study sites, diagnostic and other study-related activities will be conducted after appropriate informed (pre-) consent and/or assent. Potential study participants will be informed about the study protocol. Finally, full informed consents/assents (written and verbal) will be obtained from the potential study participants/parents/guardians/legal representatives. Eligibility tests will be performed and inclusion/exclusion criteria reviewed. Pre-treatment assessments will include: full medical history, clinical assessments and monitoring of vital signs, assessment of the lesion (full description, size measurement, photography, fine needle aspirates and swabs for *M. ulcerans*, drug sensitivity and mycolactone), laboratory investigations (clinical chemistry, full blood count, urinalysis), hearing test, electrocardiogram (ECG), pregnancy test, HIV counseling and testing, and functional limitation assessment.

Patients will be randomized to receive one of the two treatment regimens with antibiotics as follows: (i) *Regimen I (SR8):* Streptomycin (S: 15 mg/kg per day, intramuscularly) in combination with rifampicin (R: 10 mg/kg per day, orally) for 8 weeks; *(ii) Regimen II (CR8):* Clarithromycin (C: 150 mg/kg per day, oral extended release formulation) in combination with rifampicin (10 mg/kg per day, orally) for 8 weeks.

Assessments will be made every week during the first 8 weeks while antimicrobial treatment is given, and monthly after 8 weeks, until 12 months after start of treatment, and at the time of treatment failure. Assessments will include: full medical history, clinical assessments and monitoring of vital signs, assessment of the lesion (full description, size measurement, photography, fine needle aspirates and swabs for *M. ulcerans*, drug sensitivity and mycolactone), laboratory investigations (clinical chemistry, urinalysis, full blood count), hearing test, ECG, pregnancy test, HIV counseling and testing, and functional limitation assessment (see also chapter 6, and table 2, p 57).

4.4 Patients and sample size

The study aims to achieve the highest possible rate of PCR-confirmed BUD, while allowing the results to be able to apply to as many patients with BUD as possible. Based on the PCR-confirmation rate is 80% a total of 415 category I-II BUD ≤10 cm will allow achieving 332 PCR-confirmed category I-II BUD (≤ 10 cm). Using the feed-back of the database the target population of 332 PCR-confirmed cases will be enrolled. Detailed descriptions of the study population, sample size and allocation to treatment regimens is presented in Figure 2.

The sample size is based on the co-primary efficacy population (PCR-confirmed BUD and category I/II lesions ≤ 10 cm), a type I error of 0.025 (one-sided) an 80% chance (power) to get a significant non-inferiority test with a margin set at 12% (considering that a larger difference cannot be accepted as a fair compensation for the advantages of CR8) and expected success rates of 96% for SR8 (4) and 92% for CR8. We expect that at least 80% of participants with disease duration < 6 months will have PCR-confirmed BUD. In the previous studies, confirmation rates varied between 86 and 89% in a total of 160 FNA procedures (93-95, 114). With 166 subjects in each group, the lower limit of the observed one-sided 97.5% confidence interval will be expected to exceed -0.12 (less than 12% in favor of SR8) in 4 trials out of five (80% power) when the success rate for SR8 is 0.96 and the success rate for CR8 is 0.92. Results are based on 10,000 simulations using the Newcombe-Wilson score method to construct the confidence interval (174).

Meetings with traditional & religious leaders, teachers and parents to explain the protocol; durbar / meetings to sensitize the community, and improve case

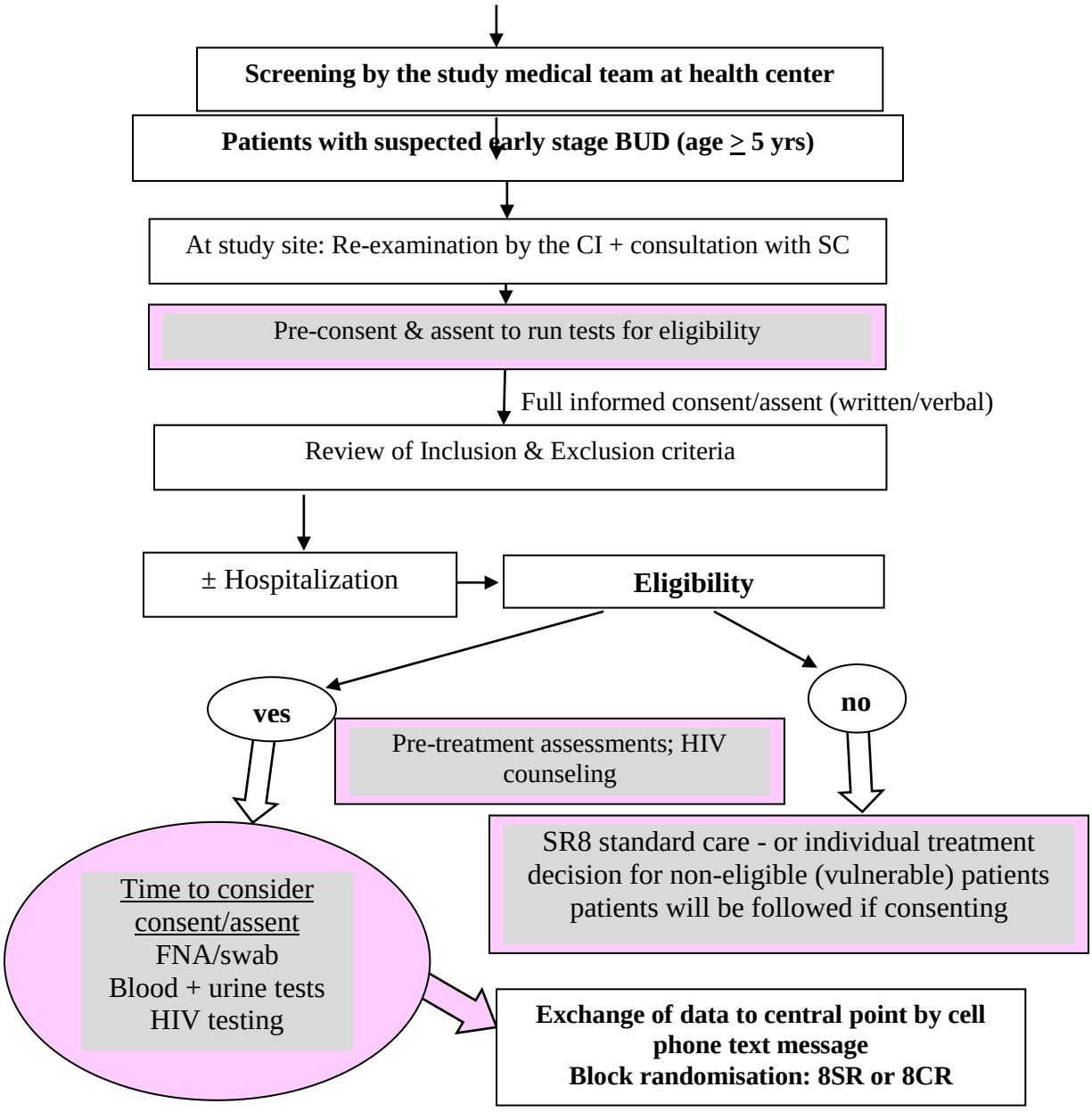
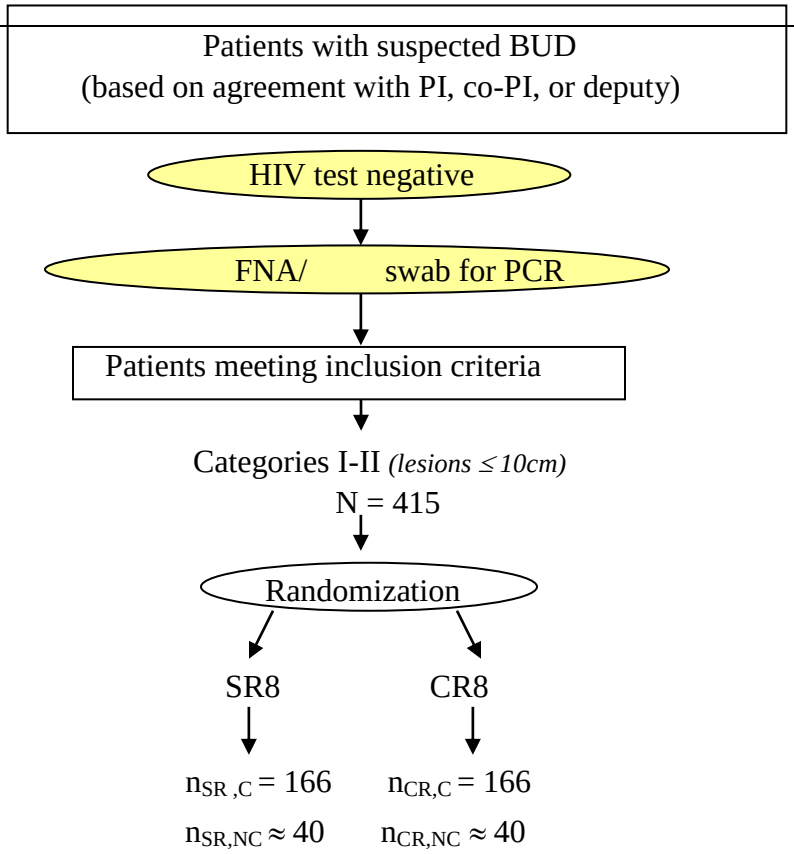


Figure 1 – Flow chart summarizing the overall study design

* The local Co-Principal Investigator (co-PI) will collaborate with the Study Coordinators and the local teams in the collaborating hospitals. The CI will supervise each and every enrollment into the study for which he/she will be responsible. Each inclusion is the result of full agreement with a panel of experts including the Study Coordinators, while if no agreement is reached, the PI or deputy will decide about enrollment. The fields in grey reflect interventions requiring consent and assent of participants and their parents or care takers



Note:

1. * *The target number is PCR-confirmed BUD ≤10cm*
2. *Meaning of subscriptions:*

- *SR and CR = treatment SR-8 and CR-8*
- *C = PCR-confirmed BUD*
- *NC = non PCR-confirmed BUD.*

Figure 2 – Detailed descriptions of the study population, sample size and allocation to treatment regimens

4.5 Inclusion and exclusion criteria

4.5.1 Inclusion criteria

All patients (both genders) with a clinical diagnosis of BUD (categories: I and II, cross-sectional diameter ≤ 10cm) as agreed by study site treatment team led by the lead clinicians will be included in the study.

4.5.2 Exclusion criteria

Those patients included as per the criteria described in 4.5.1 will be excluded from the study for one of the following reasons:

(1) Patients with lesion sizes >10cm in diameter

(2) Children < 5 years, or ≤ 20 kilograms body weight

(3) Pregnancy (self-reported, clinically diagnosed, or urine test (beta-hCG) positive.

(4) Patients with previous treatment of Buruli ulcer, tuberculosis or leprosy with at least one of the study drugs (rifampicin, streptomycin, clarithromycin)

(5) Patients with history of hypersensitivity to rifampicin and/or streptomycin and/or clarithromycin

(6) Patients with previous treatment with macrolide or quinolone antibiotics, or anti-tuberculosis medication, or immuno-modulatory drugs including corticosteroids within one month.

(7) Patients with current treatment with any drugs likely to interact with the study medication, e.g, anticoagulants, cyclosporin, phenytoin, and phenobarbitone. Users of oral contraceptives should be notified that such contraceptive is less reliable if taken with rifampicin; alternative (mechanical) contraceptive methods will be discussed with the study participant (*Appendix 8*).

(8) Patients with co-infection with HIV

(9) Patients with history or having current clinical signs of ascites, jaundice, partial or complete deafness, myasthenia gravis, renal dysfunction (known or suspected), diabetes mellitus, and severe immune compromise (e.g., immunosuppressive drugs

after organ transplant), or evidence of (previous) tuberculosis, Buruli ulcer or leprosy; or terminal illness (e.g., metastasized cancer).

(10) Patients who are unable to take oral medication or having gastrointestinal disease likely to interfere with drug absorption.

(11) Patients with known or suspected bowel strictures who cannot tolerate macrolide antibiotics such as clarithromycin

(12) Patients with mental condition, including addiction with substance abuse (alcohol, qat, etc) likely to interfere with possibility to comply with the study protocol.

(13) Patients who are not willing to give informed pre-consent, and consent (patient and/or parent/legal representative), or withdrawal of consent.

4.5.2 Patient withdrawal

Those patients included as per the criteria described in 4.5.1 and do not have one of the exclusion criteria described in 4.5.2 will be withdrawn from the study for one of the following reasons:

(1) Randomization of ineligible patient

(2) Screening procedure required by protocol not done

(3) Screening or on-study procedure/lab done outside the protocol required time

(4) Incorrect therapy or intervention given to patient

(5) On-study procedure required by protocol not completed

(6) Visit non-compliance

(7) Failure to follow Data Safety Monitoring Plan

(8) Medication noncompliance

4.6 Management of excluded patients

Patients in whom BUD could not be ascertained will receive standard care. If they have BUD but meet exclusion criteria to participate in the study, they will receive medical care in the respective hospitals as deemed best by the attending medical team. They will enjoy all benefits of the ongoing research but their drug treatment allocation will not be based on chance but on choice. Only if treatment for BUD follows, patients will be managed according to the local and National treatment protocol. Participants who appear pregnant as well as children <5 years of age with BUD will be excluded from the randomized study but the attending medical team may decide if that CR8 treatment is the best treatment option. The manager of the National Buruli ulcer Control Program (NBUCP), Accra, Ghana, and the Coordinator of *the Programme National de Lutte contre la Lèpre et l'Ulcère de Buruli* (PNLLUB) (National Leprosy and Buruli ulcer Control Programme), Cotonou, Bénin will be notified throughout the diagnostic and clinical management process.

All tests considered standard (diagnosis testing by PCR, renal function tests if renal impairment is clinically suspected, tests for alternative diagnoses of the diagnosis is clinically uncertain, pregnancy testing for those that need injected S, ECG in those clinically suspected to have a cardiac contraindication for C) and not investigational, will equally be offered to those that meet exclusion criteria to participate in this study.

4.7 Randomization process

The data relevant for randomization will be sent from the study site by text message to a central address, where computer-generated random allocation to receive treatment with either CR8 or SR8 regimen will be given by text message, or sealed envelops will be opened that contain the allocation. Subjects to be enrolled in the study will be allocated to one of the two treatment arms following a computer-generated block-randomisation.

Treatment groups will be balanced every block of 6 patients. Each subject will receive a unique study code number, kept with the PI and the study coordinators.

4.8 Description of the "discontinuation criteria"

4.8.1 For individual subjects

Patients who fulfil all study criteria, give informed consent (or assent ± consent by care takers) and have not yet completed the full treatment course of antibiotic therapy will be discontinued from the study follow up for any one of the following reasons:

(1) Observation of rapid enlargement of the lesion despite drug treatment not attributed to a paradoxical response (e.g., without initial improvement, and/or increase in size of the lesion to twice the original surface area). The outcome is then considered as treatment failure.

(2) Presence of Serious Adverse Event (SAE) or adverse drug reaction of NIH/NCI CTC grade 3 or 4. The outcome is then considered as treatment failure.

(3) Withdrawal of consent or assent.

(4) Need to use any drug with anti-mycobacterial activity or likely to interfere with interpretation of the results during the follow-up period (see under exclusion criteria in section 4.5.2).

(5) Observation of any new condition or situation (including pregnancy, major trauma, or severe immune compromise, e.g., resulting from untreated HIV/AIDS) developing during the trial which, in the judgement of the CI, the study coordinators and/or the co-PI, may interfere with continued participation in the study.

In any case, the time and reasons of discontinuation from the study will be recorded in the CRF, and a complete report will be filed and discussed with the PI.

4.8.2 For part of, or for the entire study

The Data Safety Monitoring Board (DSMB) (*Appendix 9*) will be notified of all important progression of lesions during treatment, as well as of all Serious Unexpected Suspected Adverse

Reactions (SUSAR) and the fate of the study will be discussed by the DSMB, i.e., discontinuation in part or entirely, for any of the following reasons:

- If the first interim analysis shows failure >20% in the CR8 arm after the first 100 patients have been followed for six months, and at least 50% more failure than in the SR8 arm, or the second interim analysis shows failure >20% in the CR8 arm after the first 200 patients have been followed for six months and at least > 40% more failures than in the SR8 arm.
- If the chance of success of the trial is less than 10% once 100 patients were followed for one year.
- Presence of statistically significantly more SUSAR or adverse drug reaction of NIH/NCI Common Toxicity Criteria (CTC) grade 3 or 4 in CR8 relative to CS8 or observation of a number of patients that is considered by the DSMB as too large to be acceptable for toxicity.
- Rapid enlargement of the lesion despite drug treatment (increase in size of the lesion to 150% of the original maximum diameter) in >20% of cases in any (sub-) group. The protocol may need to be amended if lack of efficacy is suspected in such sub-group.

4.9 Procedure after withdrawal from the study treatment

When a patient is withdrawn from the study medication prior to completion of trial treatment, the investigator medical team will provide the best proven treatment for the patient's condition. The patient can continue to be treated with C if decided by the investigator. In such cases, C from a quality-assured commercial source but not from the study drug packs will be used. Additionally, the team will carry out all the safety and efficacy assessments that would have been carried out at the next scheduled visit (unless the patient is lost to follow up*).* No replacement will take place of individuals withdrawn from the study. The "*Study Termination Sheet"* in the Case report Form (CRF) (*Appendix 10*) must be completed.

4.10 Duration of the study

The provisional time-table for the study is as follow:

- Recruitment and treatment of patients 24 months (1 July 2012–1 July 2014
- Follow-up of patients 12 months (ending 1 July 2015)
- Laboratory studies (continued throughout the study)
- Data collection and analysis (continued throughout the study)

5.0 DESCRIPTION OF TRIAL MEDICATIONS

The trial medications will be obtained from an internationally recognized source which follows Good Manufacturing Practice (GMP). Storage and distribution will be handled by the hospital pharmacists in each trial site following the same GMP principles. All the trial medications will be supplied directly by WHO, or as gift by third parties or financial supporters through courtesy of WHO. The medicines will be shipped to Benin and Ghana through the respective WHO offices for onward delivery to the national programs. Standard pharmaceutical procedures and precautions apply to guarantee proper storage and distribution.

5.1 Information on trial medications

5.1.1 Rifampicin

Rifampicin (R) capsules (300 mg) formulated as sulphate salt will be used in the study. R is a semi-synthetic compound derived from Amycolatopsis rifamycinica, acting through inhibition of DNA-dependent RNA polymerase in bacterial cells by binding its beta-subunit, thus preventing transcription to RNA and subsequent translation to proteins.

R is readily absorbed from the gut. The most serious adverse effect is hepatotoxicity; detection of liver test abnormalities helps to detect liver damage early. R is an effective liver enzyme-inducer. It up-regulates hepatic cytochrome P450 enzymes (such as CYP2C9 and CYP3A4), increasing the rate of metabolism of many other drugs that are metabolized by the liver through these enzymes including C. Adverse effects include (Appendix 11):

- Hepatotoxicity: hepatitis, jaundice, liver failure in severe cases
- Respiratory effect: breathlessness
- Cutaneous effect: flushing, pruritus, rash, redness and watering of eyes
- Abdominal effect: nausea, vomiting, abdominal cramps with or without diarrhea
- Flu-like symptoms: chills, fever, headache, arthralgia, and malaise. R has good

penetration into the brain, and this may directly explain some malaise and dysphoria in a minority of users. 5.1.2 Clarithromycin

Clarithromycin (C) tablets (500 mg) as extended release or immediate release formulation will be used in the study at the dose of 15 mg/kg and 7.5 mg/kg for patients aged > 5 and $\leq$ 5 years, respectively. C is a macrolide antibiotic that acts by binding to the subunit 50S of the bacterial ribosome and thus inhibiting the translation of bacterial peptides. It has a fairly rapid first-pass hepatic metabolism, where it is converted to 14-OH clarithromycin. The 14-hydroxy metabolite of C is much less active against *M. ulcerans* that the parent compound and co-administration with R increased the conversion to 14-OH C while on the other hand, blood concentrations of R slightly increase (161). 14-OH C has a half life of 7 hours compared to C (5 hours). C and its metabolites' main routes of elimination are urinary and biliary excretion. Of all the drugs in its class, C has the best bioavailability at 50%, which makes it amenable to oral administration. Most common adverse effects are gastrointestinal: diarrhea, nausea, extreme irritability, abdominal pain and vomiting, facial swelling. Patients with known or suspected bowel strictures cannot tolerate this drug, and they should be excluded from the study. C is best taken with some food. Less common adverse effects include headaches, dizziness/motion sickness, rashes, alteration in senses of smell and taste, including a metallic taste that lasts the entire time one takes it. Although this was not observed in the previous trial, it may aggravate or cause Qtc prolongation (*Appendix 11).* Clarithromycin (C; immediate release formulation) has been studied along with S and R in the previous trial (4).

5.1.3 Streptomycin

Streptomycin (S) is a water-soluble aminoglycoside derived from *Streptomyces griseus*. It kills micro-organisms by interfering with their ability to synthesize certain vital proteins. The nephrotoxic potential of S is very low when compared to other aminoglycosides. However, risk

of ototoxicity is higher. Hearing loss first begins in the high-tone range and can only be detected with an audiogram (*Appendix 11*).

The drug is supplied as a sterile nonpyrogenic lyophillized cake (1 g /vial) formulated as sulphate salt, and sterile water for intra-muscular injection will be used in the study. The lyophillized cake may reduce to a powder during shipping. After reconstitution the pH range for S for injection should be between 4.5 and 7.0 in a solution containing 200 mg of S activity per ml.

5.2 Treatment groups

A total of 415 BUD patients (lesions ≤10 cm) will be block-randomized to receive one of the two different antibiotic regimens as follow (Figure 2):

(i) *Regimen I (SR8):* Streptomycin (15 mg/kg per day, intramuscularly) in combination with rifampicin (10 mg/kg per day, orally) for 8 weeks

- *166 PCR*-confirmed BUD + approximately 40 non-PCR confirmed BUD

(ii) *Regimen II (CR8):* Clarithromycin (15 mg/kg per day oral extended release formulation) in combination with rifampicin (10 mg/kg per day, orally) for 8 weeks

- *166 PCR*-confirmed BUD + approximately 40 non-PCR confirmed BUD

Administration of each dose of trial medication will be recorded for each patient in *Medication Chart* section in the *CRF*. Any concurrent medication prescribed will be recorded in *Concomitant Medications* section in the CRF.

5.3 Pharmaceutical aspects

Labeling: Bottles and boxes containing the trial medications will be labeled in English by the Sponsor/Company as shown in a copy in the investigator's file. The drugs shipped to Benin will contain information leaflet in French. The information on the label will include: (i) the statement *"For Clinical Trial Uses Only",* (ii) the name, number, or identifying mark of the drug, directions for use, lot number and expiry date, (iii) recommended storage conditions, (iv) the

name and address of the supplier, (v) the protocol number. The contents of the label will be in accordance with all applicable regulatory requirements.

Expiry date: The trial medications will not be used after the expiry date which is specified in the certificate of analysis.

Supply: The trial medications will be gift by third parties through courtesy of WHO. Extended-release clarithromycin will be procured from CentraFarm in the Netherlands and rifampicin will be provided by Sanofi, France. Streptomycin is the standard treatment used in all endemic countries. Trial medications will be shipped to the study sites through the WHO Country Offices in Benin[3] and Ghana[4] to the respective National Buruli Ulcer Control Programs. The national programs will then distribute the medicines to the various study sites. The study coordinators will regularly check the amount and condition of the drugs received by the various pharmacies at the trial sites and return an acknowledgement of receipt to the PI, in close consultation with the national program manager and hospital pharmacists at each location.

Storage: The trial medications will be stored in an air-conditioned room (20-23 °C) at the central pharmacies of the hospitals of the study sites. Drugs dispensed at the health levels will however be stored at room temperature (around 30°C) in airtight containers and protected from light, in a room with limited access. The study coordinators will assure that all participating study sites will have an appropriate stock of the study medications. The local hospital pharmacists will be involved in storage, administration and distribution of the study drugs, in close collaboration with the national Buruli ulcer control program managers.

5.4 Drug accountability

Trial medications will be shipped from the sponsors/companies directly to Ghana and Benin. Upon receiving, the study coordinator (or Program Manager) will complete "*Product Receipt Form"*, which will be maintained in the Investigator's file. Administration of the study drug will be under supervision. The PI and CIs will account for all used and unused trial medications and

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return all remaining study drugs to the NBUCP at the end of the study. *"Drug Accountability Log"* will be maintained in which an account of all capsules/vials/bottles (syrup) received, administered to patients, left over, or lost will be kept on a daily basis. The CI must sign all supplies received and, at the conclusion of the study, complete and sign the *"Certificate of Returns"* with assurance that all used drugs have been returned. These signed documents will be reconciled with delivery against used and returned stocks. The CI will have to account for/explain any discrepancies.

6.0 TRIAL PROCEDURES

6.1 Screening and recruitment

On the screening day (t-0), all patients of both genders with clinically diagnosed BUD will be recruited (actively and passively) from the selected hospitals in Ghana (4 sites) and Benin (1 site) that participate in the trial. During the community education meetings, people living in endemic villages will be informed beforehand of the details and intentions of the study. Culturally sensitive aspects of the protocol, especially testing for pregnancy and for co-infection with HIV will be discussed with opinion leaders in the villages. Potential study participants will also be informed about the importance of early reporting as well as their continuing participation to the end of the study.

After referral to the study site, potential participants will be informed again, and after having cleared all doubts, before any procedure for BUD-related diagnosis, a written informed pre-consent will be obtained from the potential study participant, or pre-assent if the patient is between 10 and 18 years, with pre-consent from a parent/relative/guardian/legal representative for all patients below age 18 years. The lead clinicians at the study sites (*Appendix 5*) are responsible for the informed consent process. The study team members will also be trained to adequately address the issues of pre-consent as well as consent and assent if the candidate participant is a minor.

At the study site (i.e., hospital), patients will be reviewed for possible inclusion/exclusion criteria, and lesions will be clinically reviewed for possible BUD by at least two independent clinicians. The clinical diagnosis will be made by the CI, in collaboration and consultation with the study team or study coordinator(s). Digital photographs and preliminary information based on the clinical history, related to inclusion and exclusion criteria will be collected. Eligibility of the patient will be discussed by cell phone, with digital images sent to the study coordinators or their deputies by cell phone (Short Message Service, multimedia message service or email).

In case that the patient is accepted as BUD suspect eligible for the study, full informed (verbal and written) consent/assent will be obtained both from the candidate study participant and/or caretaker/guardian. Two days minimum time will be provided to consider participation in the study. An independent physician may be consulted if required; the list of these independent physicians is provided for each participating centre separately in *Appendix 6.*

Only after informed consent has been obtained, and forms are signed by the study team member (CI or deputy) including the study participant (sign or thumbprint), further diagnostic protocol-driven steps will be made. Pre-treatment assessments in all eligible patients are included in Table 2.

6.1.1 Demographic, medical history and clinical assessments

Full clinical history, study site information, demographic data and clinical assessment including physical examination, body weight (kg), monitoring of vital signs (body temperature, blood pressure, heart rate) will be performed. Since the treatment does not include a full tuberculosis therapy regimen, the history and physical examination should rule out past or active tuberculosis in study patients. All clinically suspicious tuberculosis cases (cough for 3 weeks or more, loss of weight, night sweats, fever, chest pain) will undergo appropriate tests (sputum for AFB, chest X-ray) and those confirmed to have TB will be treated according to the national TB policies of Benin and Ghana (*Appendix 1).*

6.1.2 Assessment of the lesion

6.1.2.1 Description and size measurement

This will include full description of all lesions (stage: ulcer, non-ulcer; lesion size and category; digital photography) and questionnaire record of Buruli ulcer Limitations Score (*Appendix 16*). Lesion size will be measured using a transparent ruler, with the maximum diameter and the diameter at right angles to the maximum diameter line. The margins of the indurated area of the lesion determined by palpation and marked by non-permanent marker will form the boundaries to be measured. In ulcerative lesions, there will be the margins of the skin defect and the indurated area. Either the ARANZ equipment, or simple acetate sheet drawings will be used for measurements of the lesion (maximally acceptable size for inclusion: $\leq$ 10 cm cross-sectional diameter).

6.1.2.2 Confirmation of M. ulcerans

In non-ulcerated disease, fine needle aspirates (FNA) will be the usual diagnostic procedure for *M. ulcerans* and two samples will be taken per lesion. For ulcerated disease, two cotton wool swabs from undermined ulcer edges will be taken. All sample collection procedures will follow the WHO instructions (93-95, 114). All specimens will be examined for confirmation of *M. ulcerans* by polymerase chain reaction (PCR) using insertion sequence IS2404. In addition, direct smear examination (staining and direct microscopy for Acid-Fast Bacilli), and particularly culture may be included as part of ongoing studies as well as to enhance the chance to confirm BUD.

In exceptional instances, especially when doubt about the diagnosis persists, biopsies (a single 0.3 cm punch biopsy under local anaesthesia) may occasionally be required, and then additional histopathological analyses will be made.

All specimens will be submitted for mycolactone detection as part of formally designed and ethically approved, protocol-driven research (*Appendix 13*) (175).

6.1.2.3 Drug sensitivity test

All *M. ulcerans* isolates will be stored for future study and tested for susceptibility to the trial drugs (R-rifampicin, S-streptomycin, C-clarithromycin). Sensitivity testing will help to understand possible failure and recurrence.

6.1.3 Laboratory investigations

6.1.3.1 Clinical chemistry, full blood count and urinalysis

Clinical chemistry (serum urea, creatinine, total and direct bilirubin, glucose, albumin, globulin, liver transaminase (ASAT/ALT), alkaline phosphatase), full blood count (WBC, RBC, haemoglobin) and urinalysis (detection of leukocytes, nitrite, proteins, glucose using urine stix Combur5®) will be performed at baseline. During follow-up, study sites (hospitals) will carry out the various routine laboratory tests as in standard care. For sites that do not have routine testing available, collaboration with laboratories will be established. For centres in Ghana that do not provide lab tests testing will be done at a central laboratory facility (Komfo Anokye Teaching Hospital, Ghana).

6.1.3.2 Pregnancy test and HIV counseling & testing

For minors, the cultural context in Ghana and Benin is such that, if a non-married minor is unknowingly pregnant, or appears to test positive for HIV antibodies, these test results should always be discussed with this person in the presence of the parents, caretakers or guardians. This implies that all study procedures including HIV and pregnancy testing will be discussed with parents/guardians/caretakers together with the candidate study participant. It will be also made clear that any test result whether expected or not expected will be discussed with the candidate study participant in the presence of the parents or care takers.

Additional pregnancy tests will be carried out during the weekly visits while treatment is given, and the same rules apply. Of note, female participants with child-bearing potential will be notified that the study team provides contraceptives. Condom use is preferred as oral contraceptives are unreliable during co-medication with R due to pharmacokinetic drug interaction (CYP3A4 induction from R). Condoms (preservatives) will be offered free of charge for the duration of drug treatment for those that show interest in having these and the procedure will be such that by covering the package, this will not be discernable by others attending the clinic or Out-Patient Department where potential study participants are received.

(i) Pregnancy test:

Beta subunit hCG urine pregnancy test will be performed in all females of child-bearing potential (menarche until menopause; or roughly age between 10-50 yrs). A Standard

Operating Procedure (SOP) (*Appendix 14*) to counsel participants with positive pregnancy test will be provided.

If pregnancy test demonstrates pregnancy, the test result will first be discussed with the study participant, even if this person is just below 18 years of age but otherwise exposing behavior compatible with adulthood. Pregnant women with BUD may be enrolled in the observational cohort study if the medical team is convinced that drug treatment is indicated.

(ii) HIV Counseling & testing

Participants will be offered Counseling & Testing (C&T) for HIV infection during informed consent to allow for analysis of HIV co-infection as a potential confounder for outcome. C&T is considered potentially beneficial for participants as timely antiretroviral (ART) treatment may be provided in the context of the National Programs in the two countries. There is considerable uncertainty about the optimal treatment for BUD in patients co-infected with HIV. If participants are unwilling to have C&T, they may still be offered such testing during later visits. Blood samples will be collected for HIV-testing after HIV counseling is agreed and consent provided. This offer may be rejected without losing any of the advantages of study participation; treatment will not be affected by opting out for HIV C&T. C&T will be offered without pressure throughout the study to those initially opting out. An SOP (*Appendix 15*) is added with instructions to refer those testing HIV positive to the National HIV/AIDS Programs will be provided.

6.1.3.3 Serum and buffy coat samples for storage

Serum and buffy coat cells will be used for other studies that will help understanding, diagnose and manage BUD. If such tests are needed, the results will be made available and patients will be managed according to the national guidelines. An aliquot of 2 ml serum will be stored for future diagnostic test development, and residual buffy coat will be stored for future host genetic susceptibility research at the treatment sites. The buffy coat specimens for genetic study will be stored anonymously at a central location for a maximum of 10 years after completion of the study, after which these specimens will be destroyed. One aliquot of the serum (1 ml) will be taken to be able to develop a sero-diagnostic test based on genomic information of *M. ulcerans*

(60-62, 80). Another aliquot (1 ml) will be used to detect co-infections relevant to understand response to treatment and abnormal liver tests (HIV, HCV and HBV) in participants separately consenting to have these tests done, after specific C&T. An SOP will be provided. Patients may opt out for this additional blood draw and a separate additional informed consent and/or ticked box need to be filled.

6.1.4 Hearing test

Hearing test will be performed using portable equipment on-site (Interacoustics AS208; Denmark).

6.1.5 Electrocardiogram (ECG)

ECG will be monitored using portable equipment (Cardioscan 2000).

Functional limitation assessment

Participants will be asked to collaborate in answering a questionnaire assessing possible functional limitations resulting from BUD. Functional limitations will be assessed using a Visual Analogue Scale (VAS) for hand function and walking with Likert scale 0-3 (0= no impairment, 1-2 = slight/major impairment of hand function/walking, 3 = inability to walk/use the hand affected). All test results of baseline laboratory and other investigations will be recorded (or attached) in the CRF.

Table 2 – Schedules of procedures before (screening), during and after treatment

	Screening t-0	during treatment Week-2	Week-4	Week-6	Week-8	follow-up Monthly	end 12 months	(>8 wks)	failure
(i) Written & Verbal Informed Consent/Assent; randomization	X							X	
(ii) Inclusion/ Exclusion Criteria	X								
(iii) Demographics	X								
(iv) Previous Medical History	X								
(v) Clinical Assessment	X	X	X	X	X	X	X	X	X
(vi) Adverse Events		X	X	X	X	X	X	X	X
(vii) Vital Signs, Temperature	X	X	X	X	X	X	X		X
(viii) Assessment of lesion* & measuring by digital imaging [a]	X	X	X	X	X	X	X	X	X
(ix) Hearing Test & ECG	X		X		X			X	X
(x) Clinical Chemistry & Full blood count	X		X		X			X	X
(xi) Urinalysis	X		X		X			X	X
(xii) Pregnancy Test [b]	X	X	X	X	X				
(xiii) Counseling & Testing for HIV	X								
(xiv) FNA swab for PCR*, Microbiology and DST [c]	X	X	X	X	X			X	X
(xv) Drug Administration (DOT)		X							
(xvi) Mycolactone [d]	X[e]	X[e]	X[e]					X[e]	X[e]
(xvii)Tissue histopathology								X	
(xviii)Blood for other Tests [e]	X		X		X				
(xix)Drug administration		X7							
(xx)Wound dressing & physiotherapy		X7							
(xxi)Surgery (if needed)								X	
(xxii)VAS, functional limitations [f]	X	X	X			X	X	X	X
(xxiii)BUFLS	X							X	X

NB:

* denote items that equally apply to patients not enrolled in the study.

[a] = ARANZ equipment or acetate sheet drawings will be used [b] = will be done only in female patients aged 10 – 50 years [c] =; [c] = Drug Sensitivity Testing [d] - if test becomes available, with additional sub-study protocol [e] includes buffy coat for DNA studies; serum for future sero-diagnostic test development Visual Analogue Score, [f] Likert scale 0-3 for impaired walking or impaired hand function [g] = Buruli ulcer Functional Limitations Score (179)

6.2 Drug Administration

6.2.1 Allocation of patients and trial medication administration

A total of 415 BUD patients are planned to be enrolled in the study with category I-II BUD (≤10 cm), comprising 332 participants with PCR-confirmed BUD plus approximately 83 non PCR-confirmed BUD. Patients will be block-randomized to receive one of the two different regimens of antibiotic treatment under direct supervision of the medical/nursing team as follows:

- *Treatment regimen I (SR8 regimen):* Streptomycin (S) 15 mg per kilogram body weight *intramuscularly* per day for 8 weeks, in combination with rifampicin (R) 10 mg per kilogram body weight *orally* per day for 8 weeks; or
- *Treatment regimen II (CR8 regimen):* Clarithromycin (C) as an *extended release oral formulation* 15 mg/kg body weight per day for 8 weeks, in combination with rifampicin (R) 10 mg per kilogram body weight *orally* per day for 8 weeks.

The calculated doses of R (approximately 10 mg/kg body weight) are given in Table 3. The dose of S will be 15 mg/kg body weight. The target dose of C will be 15 mg/kg body weight once daily in an extended release formulation (as only one dose is available, all patients will receive one or two tablets according to weight). The tablets/capsules of R, and the tablets/capsules of C will be taken out of bottles assigned to the patient according to the randomization. Remaining tablets/capsules will be kept in the original bottles. The patient will take the medications with one glass of water (150 ml) under supervision of a member of the study team.

After ingestion of R or C dose(s), participants will be observed for 30 min to ensure retention of the drug.

In patients who vomit within a 1 hour observation period, re-dosing of R or C dose(s) at the same (full) dose will be given. Patients who have repeated and persistent vomiting after re-dosing will be withdrawn from the study. S doses will be given concurrently with R oral doses by intramuscular injection (left upper quadrant of the buttock).

Table 3 – Dose of Streptomycin (S), Rifampicin (R) and Clarithromycin (C) according to patient body weights

Body weight of patient (kg)	Streptomycin (1 g) once daily	Rifampicin (300mg/tablet) once daily		Clarithromycin (extended release once daily tablets 500 mg)
	Dose (g)	Dose (mg)	No of tablets	Dose (mg)
21-39	0.50	300	1.00	500 (1 tablet)
40-54	0.75	450	1.50	500 (1 tablet)
>54	1.00	600	2.00	1,000 (1 tablet)

Patients will be either admitted to the hospital during a period of time of antibiotic treatment, or receive directly observed therapy at one of the participating health centers, as deemed appropriate by the co-PI, study coordinators and study site medical team. Both treatment regimens will be fully supervised. The hospital pharmacists at the study sites will supervise, and drug treatment will be fully supervised as in tuberculosis control programs (DOT). Administration of each dose of trial medication will be recorded for each patient in *Medication Chart* section in the *CRF*.

6.2.2 Concomitant therapy

Study participants will be asked to refrain from taking drugs other than those prescribed by the study team. Every study participant will be given an identifier (card) to take with him/her at home. The card will inform other health care providers to consult the study team in case the study participant presents to their health care facility with any ailment during the full duration of the study (12 months). If for any reason, the participant takes concomitant therapy with other drugs, this should be recorded in the hospital files and CRF (*Concomitant Medications* section) indicating the name of the medication, dosage, and dates of administration.

6.2.2.1 Medication(s)/treatment(s) permitted before and/or during the trial

All treatments which are not specified as being contraindicated (section 6.2.2.2 and 6.2.2.3) may be administered as required during the study period. For example, pain can be treated with

routine analgesics and malaria should be treated according to local practice, with the exception that Fansidar® (sulfadoxine/pyrimethamine combination) should be avoided if possible.

All concomitant medication taken during the study must be recorded in the hospital files and transferred to CRF with trade name and generic name, route or formulation, dosing scheme, the indication and start and stop dates of administration.

6.2.2.2 Medication(s)/treatment(s) not permitted before and/or during the trial

All treatments which are specified as being contraindicated should be avoided (section 6.2.2.3). If therapy of these drugs is required for any reason, the patient will be withdrawn from further anti-mycobacterial drug treatment and the appropriate treatment as determined by local treatment guidelines and protocols, and as prescribed by the attending physician will be offered.

6.2.2.3 Medications that need precautions if concurrently administered with the trial drugs

It is especially important that co-administration of some other drugs that are likely to interact with the study medication (R, S, or C) needs precautions as an adverse drug interaction might occur (*Appendix 11*). Patients taking an oral contraceptive should be warned that the contraceptive effect may be compromised by treatment with R and given further counselling on contraceptive methods during and for a month after R treatment.

6.2.2.4 Procedures for monitoring patient compliance

R and C oral medication will be administered in the presence of the investigator medical team or assigned nursing staff and ingestion confirmed. S intramuscular injection will be given by the investigator medical team or assigned nursing staff. This will be recorded in the CRF together with the time and date of dosing. Study medication packs will also be retained to confirm compliance and dose administered.

6.2.2.5 Rescue therapy and treatment modification

If in the judgment of the investigator medical team, the lesion deteriorates any time after the initiation of antibiotic therapy, appropriate therapy will be offered as determined by the CI in consultation with the national study coordinators and the PI.

6.2.2.6 Overdose

Any instance of overdose (suspected or confirmed) will be communicated to the study coordinators within 24 hours and be fully documented as an SAE. Details of any signs or symptoms and their management should be recorded including details of antidote(s) administered. Like all major events, the PI will be informed as soon as possible.

6.2.2.7 Unused medications

At the end of the trial, all unused trial medications will be returned to the stores at NBUCP and PNLLUB. The study coordinators, together with an assigned coordinating pharmacist will review and examine all batches for possible expiry date. If medications appear expired, destruction will be carried out under close supervision of the study coordinators in collaboration with the assigned coordinating pharmacist.

6.3 Assessment during therapy

The following formal investigations will be performed and recorded in CRF:

6.3.1 Clinical assessment and assessment of adverse events

Clinical assessments including vital signs and assessment of Adverse Events (AEs) will be performed every 2-weekly (week-2, 4, 6, 8) during the course of 8-week antibiotic therapy.

6.3.2 Assessment of the lesion

Full description and size measurement of all lesions, identification of *M. ulcerans* by PCR and/or histopathological examination, detection of mycolactone will be performed 2-weekly (week-2, 4, 6, 8) as described in section 6.1.2.1.

If recurrence is suspected, the diagnosis will be confirmed by appropriate tests (section 6.1.2.2). Management of the lesion is guided by consultations with the study coordinators, in telemedicine consultation with surgical experts. Importantly, surgically removed tissues will be closely examined. ARANZ measurements – or conventional acetate sheet measurements, plus digital photographic imaging - immediately before and after surgery will be used to assess extent of surgery. Recurrence will be discriminated from a paradoxical response, and every effort will be made to detect viable, replicating and mycolactone secreting bacilli before the diagnosis ‘recurrence’ is accepted.

After completion of the trial, an expert panel unaware of treatment allocation will use a graded read-out assessment of likelihood of 'true' recurrence, based on all available data – digital images, clinical notes in the hospital file and CRF, histopathology if available, culture (quantitative) PCR, and mycolactone assay results. The final results will be recorded as recurrence to be 'proven', 'likely/probable', 'possible', or 'unlikely/rejected'.

6.3.3 Laboratory investigations

6.3.3.1 Clinical chemistry, full blood count and urinalysis

Drug toxicity was shown to be minor in earlier observational and randomized studies and therefore routine laboratory tests will be limited to only two time points during the course of antibiotic treatment. Renal function test for potential toxicity of S and C (serum urea and creatinine, bilirubin) and liver enzymes for potential toxicity of R and C (transaminases: ALAT/ALT, alkaline phosphatase), full blood count (WBC, RBC, haemoglobin), and urinalysis (detection of leukocytes, nitrite, proteins, glucose using urine stix Combur5®) will be performed at 4 and 8 weeks of therapy for all participants irrespective of treatment allocation.

If toxicity is suspected during the course of therapy, appropriate additional laboratory testing will be performed.

6.3.3.2 Pregnancy test

beta-HCG urine pregnancy test will be performed in all female patients aged 10-50 years, every 2-weekly (week-2, 4, 6, 8) during treatment for all participants irrespective of treatment allocation with specific SOP for those testing positive.

Patients with pregnancy test negative will be offered the option of contraception during the course of the antibiotics treatment. Condom use (to be offered free of charge) is preferred as oral contraceptives are unreliable during co-medication with R. Patients with pregnant test positive will be offered the option to opt out of the study or participate in the observational trial. All pregnant women will also be referred to see the obstetrician or the medical officer responsible for obstetric care at the study site.

6.3.3.3 Serum and buffy coat samples for storage

Additional serum and buffy coat samples will be collected from patients who gave their informed consents/assents as described in section 6.1.3.3. These samples will be stored for future diagnostic test development and host genetic susceptibility, as well as co-infections relevant to the treatment and abnormal liver tests as described in section 6.1.3.3.

6.3.4 Hearing tests

Hearing test will be performed at 4 and 8 weeks of treatment for all participants irrespective of treatment allocation.

6.3.5 Electrocardiogram (ECG)

ECG will be performed at 4 and 8 weeks of treatment for all participants irrespective of treatment allocation.

6.3.6 Performance limitations test

Visual Analogue Scale (VAS) for pain during dressing and three-point Likert scale 0-3 will be recorded at 2 and 4 weeks of antibiotic therapy for physical impairment (walking, hand function).

6.4 Procedures after treatment

Following the 8-week course of antibiotic therapy, follow up of all patients will be performed once every month up to 12 months and when treatment failure occurs. The following assessments will be performed:

6.4.1 Clinical assessment and assessment of adverse events

Full clinical assessment, monitoring of Adverse Events (AEs) including monitoring of vital signs (body temperature, blood pressure, heart rate) will be performed monthly until 12 months of antibiotic therapy and at the time of recurrence (failure).

6.4.2 Assessment of the lesion

Full description and size measurement of all lesions will be performed monthly until 12 months of therapy or at the time of treatment failure as described in section 6.1.2.1. In case of suspected recurrence by appropriate test/procedure as described in 6.3.2.1 will be performed.

6.4.3 Laboratory assessments

Clinical chemistry (serum urea, creatinine, total and direct bilirubin, glucose, albumin, globulin, liver transaminase (ASAT/ALT), alkaline phosphatase), full blood count (WBC, RBC, haemoglobin) and urinalysis (detection of leukocytes, nitrite, proteins, glucose using urine stix Combur5®) will be performed at 12 months and at the time of treatment failure for all participants.

6.4.4 Hearing tests

Hearing test will be performed at the time of treatment failure.

6.4.5 ECG

ECG will be performed at the time of treatment failure.

6.4.6 Performance limitations test

Visual Analogue Scale (VAS) for pain during dressing and three-point Likert scale 0-3 will be recorded monthly until 12 months of antibiotic therapy and at the time of treatment failure for physical impairment (walking, hand function).

6.4.7 BUD functional limitations

BUD Functional Limitation Score (BUFLS) will be recorded at 12 months of antibiotic therapy and at the time of treatment failure (*Appendix 10*).

6.5 Role of surgery

The role of surgery is limited. In this study protocol, surgery will only be considered after completion of antibiotic treatment (*Appendix 12).* As BUD is not a life threatening condition, delayed surgery pose no risk. Rather, delay surgery allows limited and more precise surgery once antibiotics have been completed. Patients with category I-II lesions involving a joint, as well as patients with large necrotic debris may also need additional surgery. Simple removal of necrotic slough and grafting are considered an aspect of normal wound care and this type of surgery does not necessarily influence the primary end point of the study. Importantly, active

Prevention of disabilities will be implemented with physiotherapy, and standardized wound care will be provided for all study participants.

Cases planned for surgery will be discussed with a group of experts (surgery sub-Working Group on Buruli ulcer) by telemedicine in advance. Decision about timing, type and extent of surgery will be made after a telemedicine consultation with surgical experts in each country and outside, in collaboration with the PI and the study coordinators.

After completion of antibiotic treatment, additional surgery (mainly skin grafting) may be required to help speed up healing if the residual surface area of the ulcer after antibiotics treatment is more than 5 cm in diameter. In category I lesions, no additional benefit from surgery is expected in most cases. Larger (category II) lesions may require surgical removal of necrotic tissues followed by skin grafting. All tissues that are surgically removed will be stored for additional tests. All surgical procedures will be documented by a standardized operation report as well as by digital imaging, including photo and film.

Surgical specimens will be divided in aliquots of tissue to be examined for mycolactone concentration, culture, PCR, histopathology and remaining tissues will be stored in polyethylene containers, in 37% formaldehyde in water (formalin) for at least 10 years.

6.6 Patient admission to hospital

For patients with limited size (category I and II lesions), only few hospital admission will not be expected. On the other hand, patients with larger lesions (some category II and III) and those who may require surgery, or patients who live in extremely remote areas, a period of admission of 4-12 weeks may be required. The study will cover any costs involved.

6.7 Specimen handling and ownership

(1) Samples for diagnostic confirmation will be handled according to existing procedures as in the national programs, and standard BU1 forms will be used throughout. The WHO guidelines for FNA swabs will be followed. Immediately

after the FNA (or biopsy procedure), the specimens will be placed in specimen containers with different buffer/transport media suitable for different laboratory tests. PCR-testing will be coordinated by accredited laboratories in Kumasi (KCCR), and Angers, France (GEIHP) that participate in an External Quality Assessment program. In Ghana, the Noguchi Memorial Institute for Medical Research provides local quality control and back-up diagnostic support when needed. Extracted DNA will be processed for PCR quality assurance and sequence analysis done if needed. Diagnostic specimens in PANTA transport medium may be processed for culture at the appropriate laboratories in Ghana and France (specimens from Pobè, Benin)

(2) All *M. ulcerans* isolates will be kept for future study and tested for susceptibility to the trial drugs (R-rifampicin, S-streptomycin, C-clarithromycin). Positive cultures will be sent to WHO Reference laboratory at ITM in Antwerp, Belgium.

(3) Biopsy specimens and blood samples will be sent to a central repository (Groningen, Netherlands) and specimens (swabs and culture isolates) will be stored at WHO reference laboratory in Antwerp, Belgium).

(4) All specimens will be stored using an anonymized database system at Institute of Tropical Medicine, Antwerp, Belgium and University of Groningen Medical Center, Groningen, the Netherland using Material Transfer Agreement (MTA, *Appendix 18*). All specimens will be handled in accordance with SOPs made for each study site. All specimens will be stored for maximum 10 years after completion of the study.

(5) The WHO as the sponsor of the study is the legal owner of all specimens collected during the conduct of the study. A committee will review requests from the Buruli ulcer research community for materials for research purposes according the established agreement (see terms of reference). During preparatory meetings with the communities, we will discuss the storage and future use of specimens to enhance further understanding of the disease.

6.8 Additional studies with additional consent requirement

The side studies listed below will be conducted that require additional consent. All of these tests will be first explicitly discussed with the elders, school teachers and other representatives of the communities, and separated consents will be obtained:

(i) Genetic susceptibility research

(ii) Development of sero-diagnostic tests

(iii) Development of a mycolactone Point of Care test

(iv) Biopsy specimen repository

(v) Mycolactone concentration in surgery specimens; ongoing studies from culture specimens (in collaboration with ITM, WHO Reference laboratory for *M. ulcerans*, Antwerp).

7.0 ASSESSMENT OF EFFICACY

Endpoints of clinical efficacy will be based on improvement of the disease following antibiotic therapy regimens in terms of the following:

7.1 Primary efficacy endpoints and parameters

Complete lesion healing: The primary efficacy endpoint is *complete lesion healing* at 12 months of treatment initiation. The primary efficacy parameter is the *rate of complete lesion healing* at 12 months, defined as the proportion of patients with complete lesion healing in each treatment group at 12 months of treatment initiation without the need for additional excision surgery and without recurrence (relapse) in BUD patients with category I lesions and category II ≤10 cm cross-sectional diameter lesions.

"Complete healing" is defined as healing and re-epithelialisation of ulcerated area with stable (no change during a 3-months observation period) scar formation. Larger lesions (e.g. residual size at 8 weeks > 5 cm) are expected to heal under SR8 or CR8 treatment, but may require additional skin grafting. This type of surgery will not be considered as failure to heal on antibiotic SR8/CR8 treatment, but is considered as advanced 'wound care' (*Appendix 12*). Surgery interpreted as failure is any attempt to obtain cure by attempting to resect (partially) viable tissue. Surgery that will be considered part of wound care involves removal of irreversibly damaged and dead tissue in an attempt to allow lesions to heal faster and possibly, with less scar tissue than if spontaneous epithelialisation of lesions is awaited. There may also still be a palpable lesion.

In order to allow a panel of experts to assess the use of surgery in both treatment arms, assessments of digital photo's and films will be made by an expert panel that is blinded for treatment allocation, in order to make objective comparisons.

7.2 Secondary efficacy endpoints/parameters

7.2.1 Recurrence

The efficacy endpoint is the *recurrence* within 12 months of treatment initiation. The efficacy parameter is the *recurrence rate*, defined as the proportion of patients with recurrence in each each treatment group (SR8, CR8) within 12 months of treatment initiation.

"Recurrence" is defined as complete lesion healing but later, a new or recurrent lesion is detected within the 12 months of treatment initiation that is not judged to be due to paradoxical response to therapy.

Every attempt will be made to detect viable bacilli from lesions suspected to be recurrences. As clarified earlier, after completion of the trial, an expert panel unaware of treatment allocation will use a graded read-out assessment of likelihood of 'true' recurrence, based on all available data: digital images, clinical notes in the hospital file and CRF, histopathology (if available), culture, (quantitative) PCR, and mycolactone assay results. The final results will be recorded as recurrence to be 'proven', 'likely/probable', 'possible', or 'unlikely/rejected'.

7.2.2 Treatment failure

The efficacy endpoint is *treatment failure* within 12 months of treatment initiation. The efficacy parameter is the *rate of treatment failure*, defined as the proportion of patients with treatment failure within the 12 months of treatment initiation.

"Treatment failure" is defined as one of the following criteria

(i) enlargement of the lesion to ≥ 150% maximum surface area compared to baseline of during treatment and/or the observation period (in the absence of a paradoxical reaction); or

(ii) decision by the attending clinician to do debridement plus excision operation (i.e., removal of viable tissue, apart from removal of dead tissues, in an attempt to obtain cure; with bleeding wound edges), or to switch or extend therapy; or

(iii) lack of complete healing within the 12 month observation period, or

(iv) any outcome which is not a success.

7.2.3 Paradoxical response

The efficacy endpoint is the *paradoxical response* within 12 months of treatment initiation. The efficacy parameter is the rate of *paradoxical response*, defined as the proportion with observed paradoxical response within the 12 months follow up.

"Paradoxical response" is defined as increase in inflammatory changes with increase in lesional size, typically after initial improvement during effective reduction of bacterial load.

7.2.4 Lesion healing

1) Lesion surface area

The efficacy endpoint is a *reduction in lesion surface* area on completion of treatment compared to baseline (pre-treatment). The efficacy parameter is the proportion of patients with reduction with respect to baseline (pre-treatment) in lesion surface area at 12 months of treatment initiation.

2) Time to complete lesion healing

The efficacy endpoint is *complete lesion healing.* The efficacy parameter is the time taken for complete lesion healing after treatment.

3) Excess rate

The efficacy endpoint is *complete lesion healing without additional surgery or recurrence.* The efficacy parameter is the proportion of patients with complete lesion healing (Δ% points) without additional surgery or relapse after treatment in each treatment group (SR8, CR8) at 12 months of treatment initiation

4) Interval between healing and recurrence

The efficacy endpoint is *recurrence of the lesion* after initial healing. The efficacy criterion is the time taken from the initial lesion healing until the reappearance/recurrence/relapse of the initial lesion.

5) Proportion of adjunctive surgical therapy

The efficacy endpoint is the *adjunctive surgery operation* (removal of necrosis, split skin grafting). The efficacy parameter is the proportion of patients who undergo *adjunctive surgery operation* (removal of necrosis, split skin grafting).

6) *Time from treatment initiation to surgery*

The efficacy endpoint is the *surgery operation of lesion* in each patient. The efficacy criterion is the time taken from treatment initiation until surgery of lesion in each patient.

7.2.5 Residual functional limitations

The efficacy endpoint is the *occurrence of residual functional limitations.* The efficacy parameter is the proportion of patients with residual functional limitations within the 12 months follow up.

7.3 Criteria of efficacy

Antibiotic regimen(s) will be considered effective for treatment of BUD if, following the therapy, the efficacy parameters are reached;

7.3.1 Primary efficacy criteria

The proportion of complete lesion healing at 12 months without the need for additional excision surgery and without recurrence (relapse) in patients with BUD category I lesions and category II ≤10 cm cross-sectional diameter lesions, is in favor of CR8 or less than 12% point in favor of the SR8 regimen.

7.3.2 Secondary efficacy criteria

7.3.2.1 Recurrence rate

The recurrence rate at 12 months of therapy following treatment with antibiotics is in favor of CR8 or less than 12% point in favor of SR8.

7.3.2.2 Treatment failure rate

The rate of treatment failure at 12 months of therapy following treatment with antibiotics in favor of CR8 or less than 12% point in favor of SR8.

7.4 Data sets to be analyzed

The criteria of efficacy as mentioned in section 8.3 are considered corroborative evidence of efficacy. The primary analysis of the data will be performed for intention to treat (ITT). Additional analysis will include the *"Evaluable Patient Population" or "Per-Protocol Population (PP)"* Patients will be eligible for PP efficacy analysis provided that all the following criteria apply: (i) completion of the course of antibiotic treatment; (ii) no major protocol violation with regard to inclusion/exclusion criteria; and (iii) no prohibited medications taken during the study treatment period.

8.0 ASSESSMENT OF SAFETY AND TOLERABILITY

8..1 Specification of safety parameters

Adverse events (AEs) assessment will be recorded in the CRF. This will include their description, incidence, duration, severity and relationship to trial drugs. The common expected adverse effects following rifampicin (R), streptomycin (S) or clarithromycin (C) treatment are listed in Appendix 11.

8.1.1Adverse event (AE)

Adverse event (AE) is defined as any untoward medical experience in a patient or clinical investigation subject (study participant) administered a pharmaceutical product that does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign, including an abnormal laboratory, or vital signs, hearing

perception, symptoms or disease temporally associated with the use of the trial medication, whether or not it is related to the trial medication.

8.1.2 Serious adverse event (SAE)

SAE is defined as any untoward medical occurrence that occurs at any dose: (i) results in a clinical condition that puts patient at an immediate risk of death (life threatening), or (ii) fatality occurs while on protocol therapy or within 7 days after last dose, or (iii) requires inpatient hospitalization or prolongation of existing hospitalization, or (iv) causes a permanent disability, or (v) causes cancer or is a congenital abnormality/birth defect, or (vi) is another medically important condition.

The term *"life-threatening"* in the definition of *"serious"* refers to an event in which the subject at risk of death at the time of the event: it does not refer to an event which hypothetically might have caused death had it been more severe. Medically important conditions that may not result in death, be life-threatening or require hospitalization may be considered an SAE when, based on appropriate medical judgment, they may jeopardize the subject or may require intervention to prevent one of the outcomes listed in the definition above. Examples of such events are intensive treatment in an Emergency Department or at home for all allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

8.1.3 Adverse effect

Adverse effect is defined as a clinical finding or abnormal laboratory test that first occurs or increases in intensity during antibiotic treatment duration. This will be derived from the signs and symptoms recorded at clinical assessment, plus additional information regarding onset, cessation, and duration of the effects, its intensity, frequency, seriousness, and the attributability to the trial medication (as determined by the physician), as well as any action taken. Laboratory abnormality (outside the normal ranges) that first occurs or increases in intensity during antibiotic therapy period and 1 week thereafter are also evaluated.

8.1.4 Serious Unexpected Suspected Adverse Reaction (SUSAR)

SAE that was not earlier connected to the drug treatment under study but nonetheless suspected to be causally related to the drug or drug combination under study (SUSAR).

8.2 Assessment procedures

Safety and tolerability of antibiotic treatment will be evaluated by clinical assessments and clinically significant laboratory changes [in terms of NIH/NCI Common Toxicity Criteria grades (*Appendix 19*) and deviation from laboratory normal ranges], and the intensity (NIH/NCI CTC grade) version 4.0, causality (relationship to trial medication), seriousness of new adverse events.

The co-PIs and study coordinators in collaboration with the PI will follow-up patients with AEs until the event has subsided (disappeared) or until the condition has stabilized. The value outside the normal or reference range in a routine safety assessment, such as laboratory assessments (clinical chemistry, urinalysis), assessment of vital signs, hearing tests, may signify as adverse finding. Laboratory assessments outside the normal/reference range will be classified as *"relevant but of minor importance", "relevant and of major importance", and "invalid value".* If it is considered to be of major relevance, it would be recorded as an AE. Any abnormality in the findings will be considered of major relevance if it represents an SAE, or leads to premature discontinuation from the study, or requires a therapeutic measure. Liver transaminase > 5x upper limit of normal range is considered AE to rifampicin (R) if no apparent other explanation for the finding emerged. Based on abnormalities on the audiogram the decision to stop streptomycin (S) will be taken in close consultation with the study coordinators, and eventually with the PI; a consultation with an ENT specialist may be necessary. The event will be treated as a SUSAR.

Discontinuation of the entire study critically depends on timely reporting to the DSMB by all workers involved in the study. The DSMB will have planned regular meetings by telephone conference, but will also meet at the request of the PI and CPIs on the ground who identify SAE, and especially, SUSAR, or other undesirable outcomes e.g., unexpectedly high progression of lesions during antimycobacterial chemotherapy.

8.2.1 Adverse events and clinical assessments

The investigator will ask the patient at each evaluation interval whether or not he/she has experienced or is experiencing any unusual medical problem. All Adverse Events (AEs) occurring during the study treatment have to be documented, regardless the assumption of a causal relationship on the AE section in CRF. Documentation of AEs will include: date of onset and offset, pattern, duration, intensity, impact, actions taken, seriousness and outcome. The investigator will also evaluate the probability of a causal relationship (causality) of the adverse event to the trial medication.

Assessment of Intensity: Intensity of each AE will be graded according to the NIH/NCI Common Toxicity Criteria (CTC) grade version 4.0 *(Appendix 19).*

Assessment of Causality: Every effort will be made by the investigators to explain each AE and assess its relationship, if any, to study drug treatment. Causality should be assessed using the following categories: *"unlikely", "likely",* and *"not assessable".* The degree of certainty with which an AE is attributed to trial medication (or alternative causes, e.g., natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the event can be understood in terms of one or more of the following: *(i) known pharmacology of the drug, (ii) reaction of similar nature being previously observed with this drug or class of drug, (iii) the experience having often been reported in the literature for similar drugs or drug-related, (iv) the experience being related by time to drug ingestion terminating with drug withdrawal (the-challenge) or reproduced on re-challenge.*

8.2.2 Clinical laboratory assessments

Safety laboratory parameters will be classified for each value outside normal to (i) abnormal but of minor relevance, (ii) abnormal and of major relevance (serious; i.e., led to discontinuation or required a therapeutic measure), (iii) invalid value; a reason for this judgment must be specified.

8.3 Safety endpoints and parameters

The safety endpoint is the occurrence of adverse events. The safety parameter is the incidence of all Adverse Events (AEs), defined as the proportion of patients who experienced at least one treatment-related AEs, or Serious Adverse Events (SAEs), or grade 3-4 toxicity, during

treatments and following the follow-up period of 12 months, following treatment with CR8 or SR8 regimens.

8.4 Criteria for safety and tolerability

Antibiotic treatment regimen(s) will be considered safe for the treatment of patients with early stage *M. ulcerans* infection if, following the therapy no patients with SAEs and/or NIH/NCI CTC (version 4.0) grade 3 or 4 toxicity is found.

8.5 Data set to be analyzed

"Intent-to-treat (ITT) population" will be used for safety analysis. The ITT patient population is defined as all recruited patients who received at least one dose of antibiotic treatment. Data from all study patients who receive at least one treatment dose of antibiotic drug combination will be used in the safety analysis. The proportion (%) of adverse events and laboratory abnormalities for each CTC grade, as well as that with *"likely"* causality will be calculated with 95% confidence interval (C.I.) and presented in the table format.

8.6 The methods for assessing and analysing safety parameters

8.6.1 Clinical assessments

Clinical assessment will be recorded by the medical study team prior to antibiotic treatment, and during treatment, through physical examination (including blood pressure, pulse rate and respiratory rate), signs and symptoms as well as hearing perception. Timing of clinical assessment is presented in Table 2.

A complete history of familial and acquired hearing impairment and vestibular disorders will be conducted for each patient pre-treatment. Patients will be examined by audiography. A decision to stop streptomycin will primarily be based on results of the audiogram, but dizziness although potentially reversible may be more problematic and may be a more common reason to stop treatment, especially in the elderly. The CI should comment in the respective section of CRF changes compared to baseline and consult with the study coordinator and the PI.

8.6.2 Clinical laboratory tests

Clinical laboratory tests will comprise the following parameters:

Clinical chemistry (total and direct bilirubin, asparate aminotransferase: AST, alanine aminotransferase: ALT), alkaline phosphatase, albumin, globulin, blood sugar, blood urea, creatinine) and full blood count (WBC, RBC, haemoglobin) will be evaluated at pre-treatment, 4, 8 weeks after treatment initiation, and when recurrence occurs, unless toxicity is suspected, in which case additional testing may be ordered as mandated by the clinical judgment during the antibiotic treatment period (refer to section 6.1.3.1).

The investigator must classify each laboratory value outside the normal range: (a) abnormal but of minor relevance (b) Abnormal and of major relevance (serious, led to discontinuation, required a therapeutic measure (c) Invalid value; a reason for this judgment must be specified.

8.7 Adverse events (AEs) reporting

All SAEs will be reported immediately to the national study coordinator, the study manager, and within 24 hours, by phone, fax or email to the PI. The PI will then inform the organisations and companies providing financial and other support (drug companies supporting the study, e.g., Sanofi

; and the other organisations: ALM, WHO, FRF and the coordinator of the EU 7th FP: *Burulivac project* 241500). This will be followed by a written report that gives additional information (using *SAE Report Form*) including a description of the adverse event, onset, date and type, duration, severity, cause-effect relationship with the drug, outcome, measures taken (symptomatic treatment, discontinuation of treatment) and all other relevant clinical and laboratory data.

SUSAR will be reported by the PI to the Local Ethics Committee and Regulatory agencies, as well as to the DSMB. The trial may be put 'on hold' until permission is given by the Local Ethics Committee and Regulatory agencies (if applicable) to continue the study.

All adverse events (including laboratory abnormalities) will be recorded in the CRFs. After the trial has been completed or terminated, all recorded adverse events will be listed, evaluated and discussed in the final report.

8.8 The type and duration of the follow-up of patients after adverse events

The patient who develops adverse events will be followed up and treated appropriately until he/she returns to normal condition. Any treatment if necessary, will be recorded in the CRF.

9.0 STATISTICAL CONSIDERATIONS

9.1 Sample size and power

The number of patients of the co-primary population (PCR-confirmed and category I/II lesions ≤ 10 cm) necessary to reach at least 80% chance to get a significant non-inferiority test with a margin of 12% if the expected success rates are 96% in both treatment group is a little less than 60 per treatment group. If the true success rate is actually 92% for both treatment groups then the sample size must reach 100 patients per treatment group to get a power of 80%. Because a 4% difference in efficacy in favor of SR-8 is negligible with regards to the benefit brought by an oral formulation, we do not want to reject an oral formulation in these circumstances and the sample size of the study is set to have 80% chance to get a significant non-inferiority test in the co-primary analyses. In that case, the sample size must reach 166 patients per treatment group (assumptions are $\alpha = 0.025$ one-sided, $1 - \beta = 0.8$, $\pi_{CR-8} = 0.92$ and $\pi_{SR-8} = 0.96$). As a matter of fact the chance to get a significant non-inferiority test is rapidly decreasing as the true difference in proportions of complete healing patients is increasing (power = 66% for a 5% difference, 51% for a 6% difference, 37% for a 7% difference, …, 2.5 % for 12% difference). Moreover, if the difference remains at 4% but the true efficacy of SR-8 is lower than 96% then the power decreases rapidly (e.g. $\pi_{CR-8} = 0.88$ and $\pi_{SR-8} = 0.92$ then $1 - \beta = 0.61$). With a sample size of 332 patients, the power of the co-primary analyses is above 99% if the proportion of patients with complete healing is 96% in both treatment groups and 96% if the proportion of patients with complete healing is 0.92 in both groups. The primary analysis performed on clinically confirmed BUD will be more powerful than what is mentioned above if the expectations are the same for non PCR-confirmed BUD. Because the percentage of non PCR-confirmed BUD should be relatively small, both primary analyses are highly correlated and the difference in results should be small. The overall study design is described in Figure 2.

9.2 Populations of interest

9.2.1 Primary safety population

The primary safety population is composed of all randomized patients taking at least one dose. This population will be used for comparison purposes with respect to the comparator.

9.2.3 Primary efficacy population: Small lesion clinically confirmed population

The primary efficacy population is composed of all randomized patients 1 (lesion of category I or category II but ≤ 10 cm) with a clinically confirmed BUD who took at least one dose of study medication. This efficacy population is appropriate to assess effectiveness.

9.2.4 Co-primary efficacy population: Small lesion PCR confirmed population

The co-primary efficacy population is composed of all randomized patients (lesion of category I or category II but ≤ 10 cm) with a PCR confirmed BUD. This population excludes randomized patients without PCR confirmation of BUD. It is not possible for ethical reasons to wait for the PCR confirmation before randomization and start of the treatment. Thanks to randomization the treatment groups of the first primary efficacy population should be comparable on known and unknown parameters, but results may be altered by the presence of patients clinically diagnosed with BUD without PCR confirmation. In the co-primary efficacy population, treatment groups should remain comparable because diagnostic tests (FNA, swab) are made before randomization and PCR is an objective criterion. This co-primary population is more appropriate to assess efficacy rather than effectiveness.

9.2.5 Secondary efficacy populations

Secondary populations are defined as follows:

(1) *Per protocol population:* Per protocol population is composed of patients of the co-primary population excluding patients with major protocol deviations. A list of major violations is provided in the list below and the status of patients will be determined during the blind review of data before the break of the randomization code.

Major violations include:

1. Consent not obtained or obtained after procedures started
2. Inclusion and exclusion criteria not followed
3. Randomization of ineligible patient
4. Screening procedure required by protocol not done
5. Screening or on-study procedure/lab done outside the protocol required time
6. Incorrect therapy or intervention given to patient
7. On-study procedure required by protocol not completed
8. Visit non-compliance
9. Failure to follow Data Safety Monitoring Plan
10. Medication noncompliance

9.3 Primary question

The primary question is to know whether within the primary efficacy populations the difference in proportions of complete lesion healing at 12 months, without the need for additional excision surgery and without recurrence (relapse), is in favor of CR8, equal, or in favor of SR8 but smaller than an unacceptable difference of 12%.

9.3.1 Primary analyses

The first primary analysis will be performed on the primary population (see definition at section 9.2.3), the primary endpoint (see definition at section 8.1.1) and the primary test (see section 9.2.1.1)

The co-primary analysis will be performed on the co-primary efficacy population (small lesion PCR confirmed population); the primary endpoint and the primary test.

(1) *Primary test:* The primary test is the Blackwelder test using a margin of 12% and a type I error of 0.025 one-sided.

(2) *Rules for success:* The trial is fully demonstrative in terms of efficacy/effectiveness if the primary analysis and the co-primary analysis are positive (i.e. if the non-inferiority tests are significant in both populations). As a matter of fact, the benefit / risk

evaluation of the new formulation will depend on the primary efficacy analyses but also on the safety analyses as well as all other secondary analyses.

(3) *Adjustment of the type I error:* There are no multiplicity issues because both primary analyses must be positive. In that case the overall type I error will be smaller than 0.025. The interim analyses planned in section 19.A.1 are all futility analyses performed by an independent statistician. In the absence of stopping rules for success there is no need for adjustment of alpha.

(4) *Handling of missing values (observation):* Any missing observation (missing primary endpoint) at 12 months is a failure whatever the reason.

9.3.2 Sensitivity analyses

(1) *Per protocol analyses:* The Blackwelder test will be applied to the per protocol set of patients (per protocol within the co-primary efficacy population i.e. the PCR confirmed population).

(2) Generalizability of results: A logistic model including the treatment, the center and the treatment by center interaction will be fitted. If the interaction term is significant ($p < 0.05$) then the magnitude of the difference between treatment groups depended significantly upon the center. In that case, results cannot be generalized to all centers. Centers responsible for the interaction will be searched and the possible causes for the deviation will be scrutinized. In case of interaction, the magnitude of the treatment effect cannot be generalized to the entire population. If there is a signal of interaction ($p < 0.10$) it will be search whether it is a qualitative interaction (marked inversion of the treatment effect) or a quantitative interaction (difference in the magnitude of the treatment effect).

9.3.3 Choice of the margin

The margin was set at 12%. A difference of 12 percent in efficacy is clinically relevant in such a disease. The benefit of an oral treatment exceeds the loss of efficacy which is at worst 12%,

knowing that if the worst difference is true the chance to get a significant test is less than 2.5% because both co-primary analyses need to be significant.

9.4 Secondary questions

9.4.1 First secondary question

What are the *recurrence rates* within 12 months of treatment initiation with each antibiotic regimen (CR8 and SR8) in both categories and within each of the two categories (I, or II: ≤10 cm lesion)? Estimation of rates and 95% confidence intervals will be calculated within each cell of the treatment by category table. A logistic model will be used to assess the significance of the treatment effect, the category effect and the category by treatment interaction.

9.4.2 Second secondary question

The second secondary question is threefold. What are the *treatment failure rates, the rates of residual functional limitations and the incidence of paradoxical response* within 12 months of treatment initiation with each antibiotic regimen (CR8 and SR8) and within each category. Estimation of failure rates and 95% confidence interval will be calculated within each cell of the treatment by category table and for the marginal total (both categories). Logistic models will be used to assess the significance of the treatment effect, the category effect and the category by treatment interaction.

9.4.3 Third secondary question

The third secondary question of clinical interest concerns each assessment criterion. Does the *complete healing rate, the recurrence rate, the failure rate, the residual functional limitation rate and the incidence of paradoxical response* within 12 month after the start of treatment depend upon the treatment after adjustment for the significant prognostic factors such as clinical form, (nodule, oedema, plaque or ulcer), category of lesion, age of the patient, gender, and comorbidities.

To answer these questions the various rates in the two treatment groups, in PCR confirmed BUD and non PCR confirmed BUD, will be estimated. A complete logistic model (all main effect of covariates and interactions) will be fitted and non significant terms including the interaction terms (treatment by category, treatment by PCR confirmation and treatment by category by PCR confirmation) will be discarded through a backward selection of explanatory terms. The treatment factor will be kept in the model whatever its significance level. In the absence of interaction, the rate of interest will be estimated within each treatment group and the treatment effect adjusted for the mean of significant quantitative covariables and the observed proportions for the category coded 1 of significant binary categorical covariables will be estimated and tested. In case of a signal of interaction between treatment and a covariate the treatment effect will be estimated and tested within each category of the factor involved in the interaction.

9.4.4 Fourth secondary question

Is surgery in patients of category II (lesion $\leq$ 10cm) within the margin or less extensive in patients treated with CR-8 than in patients treated with SR-8?

To answer this question, the margin is set at 20%. The data will be log-transformed. A general linear model will be fitted using the treatment, the lesion size at baseline, the PCR confirmed BUD factor and interaction between treatment and PCR confirmed BUD factor as well as between the treatment and the lesion size at inclusion as explanatory terms. The non significant terms will be removed from the model through a backward selection process, except for the treatment factor which will be forced to remain in the model. In the absence of interaction, the 95% confidence interval of the difference in the means of log transformed data between treatment groups adjusted for significant covariates will be calculated. If the ratio of geometric means (SR8 at the denominator) obtained by back transformation of the upper limit of the confidence interval is smaller than 1.25 then the non-inferiority of CR8 with respect to SR8 will be retained. If the distribution of residuals is far from normality, a non-parametric approach based on Hodge-Lehman estimator will be used as in pharmacokinetic studies (see Hauschke's approach). In the presence of a significant interaction between treatment and PCR-confirmed BUD then the confidence interval will be calculated for each diagnosis status. In the presence of a significant

interaction between treatment and the lesion size at baseline, the confidence interval will calculated at the first quartile, the median and the third quartile of the distribution of the lesion size at baseline.

9.4.5 Fifth secondary question

The fifth secondary question is to know whether:

(i) The time to complete healing;
(ii) The need of adjunctive surgical therapy (removal of necrosis or split-skin grafting) ;
(iii) The occurrence of paradoxical response;
(iv) The duration of daily dressings; and
(v) The speed of healing calculated from assessments during follow-up are dependent upon the treatment, and other prognostic factors such as clinical form, (nodule, oedema, plaque or ulcer), category of lesion, age of the patient, gender, and co-morbidities

A Cox model will be fitted to predict the time to complete healing using all covariables of interest as well as interactions. Then the most parsimonious model will be retained through a backward selection of significant terms except for the treatment that will be forced to remain in the model. The effect of each factor will be estimated through the hazard ratio. The median time to complete healing will be estimated in categories of interest using a parametric Cox model (Weibull function). In case of interaction between the treatment and another factor the number of categories for the estimations of medians will be increased accordingly.

A general linear model will be fitted on the duration of daily dressing using the same covariables and data of the all lesion population. If the distribution of the residuals of the model is very skewed on the right then the logarithm of duration will be calculated. The complete model will be fitted and the most parsimonious one will be obtained through a backward selection process except for the treatment that will be forced to remain in the model. The arithmetic or geometric mean duration of daily dressing will be estimated for each category of interest (treatment group) and the magnitude of the treatment effect adjusted for significant covariates will be estimated.

A random coefficient linear model will be fitted on the rate of healing at the successive follow-up visit using the data of the all lesion population. The complete model and most parsimonious one

will be fitted. The linear (speed) and quadratic (speed up or down) trend over time will be estimated and if the main effect and interaction between time and treatment on the quadratic trend is not significant the term will be removed from the model. The magnitude of the treatment effect adjusted on significant covariates will be estimated.

A logistic model will be fitted on the need of adjunctive surgical therapy and on the occurrence of paradoxical response. The approach is the same as that presented in section 9.4 except for response variables.

9.4.6 Sixth secondary question

The sixth secondary question is to know whether the overall acceptability (efficacy, tolerance, burdensome) of CR8 is sufficiently better than that of SR8 to decrease the discontinuation rate.

The rate of withdrawals will be estimated for both treatment groups and compared through a chi square test. If the number of withdrawals is not very small, a multinomial logistic model will be fitted to estimate the effect of treatment and other prognostic factors on the response. The response will be categorized in 3 nominal classes: withdrawal for lack of efficacy or safety (clearly linked to treatment effect), other reasons of withdrawal more or less identified and completers. The magnitude of the effect of significant factors and treatment will be estimated by the odds ratio with respect to "other causes".

9.4.7 Seventh secondary question

Does CR8 lead to a better observance of treatment than CR8?

To answer this question the number of missed medications will be estimated in the two arms. A generalized linear model using a link function equals to $\eta_i = Log_e\ \mu_i$ will be used and the variance will be $Var(Y_i) = \Phi\mu_i$ where Φ is the over (under) dispersion factor. The model will include the treatment and the other above mentioned covariables. The treatment effect will be estimated and tested through the most parsimonious model. The data of all lesion population will be used.

9.4.8 Eighth secondary question

What is the proportion of complete healing in patients who met an exclusion criterion? And in what respect the response rate differs from the other randomized patients including those with a non PCR confirmed BUD?

The population of interest is the primary safety population. Apart from the estimation per treatment group of the proportion of patients with a complete healing in this cohort of patients as well as in the subgroups of randomized patients and in PRC confirmed BUD group, a logistic model will be fitted to identify determinant factors of complete healing. The candidate explanatory variables are the treatment, the stratum and other prognostic factors such as clinical form, (nodule, oedema, plaque or ulcer), category of lesion, age of the patient, gender, and comorbidities. The most parsimonious model will be obtained through a backward selection of variables. The magnitude of the effect of retained factors will be estimated by the odds ratio along with its 95% confidence interval.

9.5 Questions regarding safety

- What is the incidence rate per treatment group and the relative risk of any adverse event of any grade in the cohort of randomized patients?
- What is the incidence rate per treatment group and the relative risk of any adverse event of any grade in the secondary safety population (all patients taking at least one dose)?
- What is the incidence rate per treatment group and the relative risk of serious adverse events or grade 3-4 toxicity in the cohort of randomized patients?
- Is the difference in grade 3-4 toxicity between both treatment groups in the cohort of randomized patients is equivalent to SR-8 or in favour of CR-8? (10% margin)
- What is the incidence rate per treatment group and the relative risk of all serious adverse events or grade 3-4 toxicity in the secondary safety population (all patients taking at least one dose)?
- Which factors explained significantly the occurrence of adverse events of interest?
- Does intensity of the most severe event of each patient depends upon the treatment?

- Does the percentage of premature withdrawal for lack of tolerance depend upon the treatment?

Apart from descriptive statistics and interval estimation of the relative risk concerning CR-8 with respect to SR-8, a logistic model will be fitted to identify factors that significantly explain the various responses mentioned above (one model per binary response). As mentioned above the complete model will be presented as well as the most parsimonious one obtained through a backward selection of terms, except for the treatment factor that is constrained to remain in the model. The odds ratio will be used to estimate the magnitude of the effect of significant factors as well as treatment. For the non-inferiority test, the Blackwelder test will be performed.

9.6 Safety and tolerability

9.6.1 Before treatment

To compare the comparability of treatment group on signs and symptoms, as well as baseline signs descriptive statistics will be estimated and compared through a t-test, a Wilcoxon test or a chi square test as appropriate.

9.6.2 After treatment

The incidence of adverse events will be tabulated by severity and relationship to trial drugs. The number of patients with maximum NIH/NCI CTC grade will be summarized by treatment group. If appropriate, changes from baseline will be displayed graphically by treatment schedule.

(1) Number of patients with adverse event(s) that are related causally to drug administration (likely) will be displayed in table by treatment group.

(2) Frequency and intensity of NIH/NCI CTC grades (1, 2, 3, 4) of each adverse event will be displayed in table by treatment group.

9.7 The level of significance to be used

The significance level of 0.05 two sided will be used in all statistical tests.

9.8 Trial monitoring

Monitors will be employed that check all CRF data with the source data, verify the conduct of the trial according to protocol and applicable regulation (including GCP), and verify that rights of participants are respected. Monitors will be recruited and trained in Ghana and Benin.

9.8.1 Interim analysis

A Data Safety and Monitoring Board (DSMB) will be appointed that oversees the trial and evaluates interim analysis conducted after the first 100 patients have been followed for six months, and after the first 200 patients have been followed for six months. In addition, the PI, one of the CIs, or the national study coordinators or study manager may request for an interim analysis.

9.8.2 Stopping rules

Based on a previous study, the relapse rate after medical treatment is negligible. In large lesions, healing may take more than 12 months. If the first interim analysis shows an observed failure rate larger than 20% in the CR8 arm after the first 100 patients have been followed for six months, and at least 50% more failures than in the SR8 arm, or if the second interim analysis shows an observed failure rate larger than 20% in the CR8 arm after the first 200 patients have been followed for six months and at least > 40% more failure than in the SR8 arm, the DSMB should advise the study team and PI to stop the study. Moreover, if the chance of success of the trial is less than 10% once 100 patients were followed for one year then the study will be stopped for futility.

10. QUALITY CONTROL AND QUALITY ASSURANCE

Quality assurance and quality control systems with written SOPs will be maintained to assure that the trial is conducted and data are generated, recorded, and reported in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirement(s). Prior to the enrolment of any patient/subject at a site, the CI will review the protocol and all trial related procedures, information of test medications, procedures for obtaining informed consent,

procedures for reporting adverse experiences and procedures for completing the CRFs. Site monitoring visits will be scheduled by the PI and the national study coordinators on a regular basis. During these visits, information recorded in the CRFs will be verified against source documents for accuracy and completion. These reviews will address the informed consent procedure, product accountability and storage, trial documents and trial progress. They will verify that the CI follows the approved protocol or amendments (if any), he/she will observe trial procedures and will discuss any problems with the PI, the study coordinators and other study team members if necessary. Monitoring visits will be recorded in the *Monitoring Log* at the CI's site, and at the end of the trial a copy of the completed form will be returned to the DSMB and to the members of the WHO Study Group on Drug Treatment.

Personal patient/subject data will be kept confidential. CRFs or other documents submitted to the Project Study Group will identify a patient/subject by initials and number only. The CI will keep in investigator's files a *Patient/subject Identification List + Screening/Enrolment Log* (including complete name, age and Hospital I.D. number). To allow compliance with GCP rules and principles, each subject will be asked for consent regarding access to the source documents for monitoring, audit, and inspections. The agreement covering the use of the data or analysis has to be documented in writing, together with the written informed consent for trial participation.

External Quality Assessments are mandatory for all diagnostic laboratories involved.

10.1 Essential documents for trial initiation

No study medication will be shipped to the trial site before the CI has provided to UMC Groningen the following "essential documents":

(1) Written ethics committee (IEC/IRB) note on the review of the clinical trial protocol and informed consent form (and of other trial related documents required locally).

(2) *Signed Agreement on Study Conduct* according to the protocol (SOP)

(3) *Signed Agreement* between the financial supporters and the sponsor – WHO

(4) *Curriculum vitae* of PI, co-PIs and study coordinators (updated, dated, and signed).

(5) Regulatory authority(ies) authorisation/approval/notification of the trial, if required.

(6) Current laboratory normal ranges and documentation of laboratory certification (if applicable).

To fulfil the ICH-GCP, the UMC Groningen will be responsible to maintain *Trial Master Files* (TMF) for the 'essential documents' (as specified by GCP) but all data will be equally shared with the co-PI, the WHO, and the National BU Control Programmes of Benin and Ghana. The PI, on behalf of the WHO as the sponsor of the study may keep the *Investigator's File*, containing several relevant documents for at least 15 years after completion of the study, to allow regulatory bodies to review the data should the need arise to do so (see Investigator's file form or ICH GCP). Investigator's file will be kept and updated by the investigator.

10.2 Archiving

The PI, in close consultation with WHO, will arrange for the retention of the patient/subject identification list for at least 15 years after completion or discontinuation of the trial. The CI is required to retain all subject files and source documents for 10 years. The PI at the UMC Groningen will inform the CI(s) as to when these documents no longer need to be retained.

No document pertinent to the trial will be destroyed without prior written agreement by the WHO. Should the co-PI wish to assign these records to another party, or move them to another location, written agreement will be obtained from the PI at the UMC Groningen, the Netherlands or the WHO. The originals of protocol, CRFs, and *Product Accountability List* will be archived by the PI.

11. ETHICAL ASPECTS

The study will be conducted according to Good Clinical Practice (Declaration of Helsinki and ICH Guidelines).

11.1 Approval by the local Independent Ethics Committee and Institutional Review Board (IEC/IRB) and regulatory bodies

The protocol of this study will need approval from the local IEC/IRB. In 2010, the UMCG METc (Ethics Committee, University Medical Center Groningen) studied the protocol and approved its content, although formal approval is essentially not possible in the Dutch legal system (WMO) for study protocols involving individuals that are non residents of the Netherlands. The Ethics Committee of the WHO will be asked for approval. Next, the Ghana Health Service Ethics Committee, Ghana Health Research Unit, Accra, and the Food & Drug Authority in Accra, Ghana and the Ministère de la Santé - République du Bénin will be asked for approval. The PI will submit the study protocol to other authorities as required.

The approved protocol together with a specimen of the intended "written informed consent form" will be sent to the manager of the NBUCP and the coordinator of PNLLUB, the study coordinators, and to the DSMB of the study prior to sending the case record forms (CRFs) to the study site(s). At all times, the PI will inform the manager of the NBUCP and the coordinator of the PNLLUB, the study coordinators, IEC/IRB, UMCG and the members of the DSMB, and the Drug Treatment Working Group, GBUI, WHO, as well as the financial supporters and pharmaceutical product manufacturers:

CentraFarm; Sanofi, ALM and RFF, on any SUSAR (Serious Unexpected Suspected Adverse Events). No protocol amendments will be undertaken without prior approval by the IEC/IRBs and UMCG.

11.2 Recruitment and pre-consent and pre-assent

Patients will be actively and passively recruited in the community and health facilities at the different study sites using information, education and communication materials developed by WHO. In the endemic communities in the districts served by the study centres, opinion leaders, notably, chiefs and elders; religious leaders; health workers – formally trained or not: village health workers, traditional birth attendants, fetish priests, herbal practitioners and other traditional healers; and school teachers - will be approached to discuss the study protocol. Special attention

will be paid to all culturally sensitive aspects of the protocol – notably, pregnancy testing in minors, and HIV testing. Health seeking behaviour as well as referral patterns to hospitals for suspected BUD is known to be strongly influenced by beliefs and attitudes (170-173). Ignorance about novel treatments, efficacy, fear for surgery but also anticipated and experienced stigma, curse and cultural prejudice are all influencing health seeking behaviour including delay in seeking effective and timely health care. On the other hand, recent observations in Benin show a significantly increased tendency to seek help early-on, with significantly less anticipated stigma and reduced referring to curses and witchcraft compared to the time before antimicrobial treatment had become standard of care (unpublished data on file).

Activities in the districts will therefore include providing information about diagnosis and treatment of BUD at schools and durbars. Information and knowledge transfer are only part of what will be included in these outreach activities. Also cultural and religious barriers will be addressed in a culturally sensitive way. With the comic (cartoon) books in English and French produced by WHO, video (recently produced by WHO, for lay people that generally reflects the notion of hope for quick healing without sequelae, expressing a message of hope rather than fear), slide shows with images of early lesions of BUD, and role play, we plan to enhance preparedness of people to identify lesions as possible BUD and present timely to the health care system. In schools, teachers, children and their parents or caretakers will be informed about early signs of BUD, primarily using the comic books that are also appropriate for parents of school children. The emphasis is on the advantages to present early for treatment; and to explain how early detection and treatment helps to obtain fast cure without sequelae.

At the village level, no testing and therefore no pre-consent is applicable. Potential candidates suspected to have BUD will be invited to present to the study centres. They will be referred to the hospital for further assessment, for possible participation.

Prior to any diagnostic procedure, pre-consent and pre-assent are required before any test covered by the study protocol can be conducted. None of these study-related activities will be conducted

in the community, only at the study sites diagnostic and other study-related activities will be conducted after appropriate informed (pre-)consent and / or assent.

11.3 Handling of Pre-consent, Consent and Assent procedures: responsibilities

The lead clinicians at the study sites as listed above in the document are responsible but the team members will all be trained to adequately address the issues of pre-consent (for screening activities to identify possible study participants) as well as consent and assent if the candidate-participant in a minor. All members of each study site team that have successfully followed and completed the courses with certificate in GCP principles, are engaged in this process but the lead clinician is responsible for the conduct of this process. The lead clinician in turn reports to the study coordinator, as well as (co-)PIs as the overall responsible executives for the proper conduct of the study. The monitors are involved in checking whether all procedures have been recorded correctly, and the (international) auditors have a role in checking at surprise visits whether all activities are conducted in accordance with GCP and GMP rules, and whether data entry is handled correctly.

11.4 Informed consent and assent

Only individuals that are clinically confirmed with BUD and who are consenting and/or assenting to participate in the trial will be enrolled in the study and randomized. As BUD predominantly affects children, it is also essential to enroll children. Potential participants will be allowed at least 48 hours to consider participation. They will have the right to consult an independent physician, specified in the information sheet. For each centre, a doctor not involved in the study will be identified for this purpose. Participants and/or their parents or guardians are allowed to withdraw consent or assent at anytime, without specified reason. Their rights to benefit from treatment will remain unchanged as a result of their withdrawal from the study.

11.5 Patient information and informed consent

The informed consent will be developed according to the current edition of the Helsinki declaration, and administered before protocol-specified procedures and interventions are carried out.

Patient information and consent form:

(1) The written consent form will comply with local regulations and be in a language fully understandable by the prospective study participant. Every effort should be made to avoid words and phrases that are against cultural norms or that may hinder information and the granting of consent. There will be a pre-consent procedure that covers actions necessary to start the screening procedure; this will be covered in a separate formal procedure.

(2) Each patient will receive an information form and will be explained in presence of witness if required. Sufficient time (at least 48 hours) will be given to the patient to decide whether or not to participate in the study.

(3) Patients and their care takers will be given the opportunity to inquire about details of the study and any question regarding the study will be answered.

(4) CI will ensure that the consent form has been signed and dated by the patient, care taker, physician and witness.

(5) CI will ensure that the written information and the consent form will be revised when required by an amendment to the clinical trial protocol.

(6) A copy of the actually used patient information and (pre-)consent and (pre-) assent form will be given to the trial monitor.

(7) The information sheet will make it clear that participants may choose to withdraw from the trial without the need to provide reasons to do so, and that such withdrawal of consent will not have any adverse impact on the medical treatment.

(8) An independent physician not associated with the study will be available in each participating hospital/study site, to answer questions, should the patient and/or guardian so wish.

Only if the trial subject wishes, an independent witness will attend the information session, sign and date the consent form prior to entry of the patient to the study. If the subject is illiterate, “informed verbal consent” will be sought in the presence of an impartial witness or a family member. The witness to the grant of consent will either be an escorting relative or someone among the staff at the health facility of the study who is not a member of the study team. The

witness will be required to countersign the consent form and indicate the date. Each signed informed consent form must be filed together with the respective CRF by the CI for inspection by the regulatory authorities (clinical monitor and Regulatory Compliance persons).

By signing this protocol, the CI assures the WHO, and the PI that informed consent will be obtained and that the consent form and consent information to be used will be submitted to ICE/IRB who are in charge of approving the conduct of the study.

11.6 Confidentiality

Obviously, a high degree of confidentiality during the conduct of the study is paramount and this should be ensured for maximal recruitment and co-operation from the study participants and members of the community.

The investigator and other members of the study team, as well as the monitors and auditors will keep confidential any information related to this study and all data and records generated in the course of conducting the study, and will not use the information, data, or records for any purpose other than those specified in this study protocol, or its amendments.

12. DATA HANDLING AND RECORD KEEPING

All trial data will be recorded on the CRFs supplied by the PI. Only the investigators and authorised co-workers, according to the list of *Authorised Signatory Form* (ASF, a list of all authorised and trained personnel involved in the study), are authorised to make entries on the CRFs. CRFs should be completed in English.

Entries into the CRFs should be made with a black or blue ink ballpoint pen to ensure legibility. Corrections should be made by drawing a single line through the original entry, entering the new value and placing initials and date next to the new entry (follow the procedure in the appropriate SOP). Completed CRFs will be dated and signed by the investigator or authorised study personnel.

All clinical and epidemiological data will be entered into the Patient Record Form (source data) from where transcription will be made into the CRFs. The patient hospital record forms information is made available to the monitors, auditors, as well as legislative and legal authorities. Original lab data will be attached in hospital files (source data) and only the results will be transferred into CRFs. CRFs will be kept in locker assigned for this study.

After completion of the CRFs by the investigator, a clinical monitor will review all CRFs for completeness and accuracy before sending them to the study coordinator and the PI. The data will be entered into a database where computer checks are used to identify selected protocol violations and data errors. Alternately, this can be done manually. Requests for clarifications or correction will be sent to the investigator, if necessary. Statistical analysis will be carried out after all enquiries have been done and data has been locked.

13. FINANCING AND INSURANCE

Financing is addressed in a separate attachment; a list of reimbursement fees will be provided separately. All costs for study participants, including diagnostic tests, admission fees, travel costs, costs incurred by medicinal products, dressing materials, physiotherapy, and surgery if needed, will be carried by the study team. Clinical trial liability insurance will be provided to cover any physical harm caused to persons taking part in the biomedical research attributable to the research (see appendix 17 and attached insurance proposal).

14. SHARING OF RESULTS AND PUBLICATION POLICY

The progress of the study will be shared with all the stakeholders at regular intervals. During the annual meetings on Buruli ulcer which is organized by WHO, the progress of the study will be shared with participants.

It will be the responsibility of the PI to produce a report to the members of the study team, the members of the Drug Treatment Group, Technical Advisory Group, GBUI WHO for review and comments. The PI, Co-PIs and the study coordinators will agree on the contents of any publication. All CIs shall co-author publications based on this study; their position on the author list follows their relative contribution to the study. The PI ultimately decides after consultation with the entire study team. Draft versions of abstracts or manuscripts must be made available to the co-authors before presentation of results or submission for publication. At least 45 days should be allowed for review and comment of an abstract and full paper. WHO as the sponsor as well as funding sources and financial supporters have the right to comment on drafts of the report but the decision to publish remains with the study team chaired by the PI. There is no role of the funding sources in the study design, data collection, data analysis, data interpretation, writing the report, or in decisions about submission of results for publication.

15. PROTOCOL AMENDMENT AND TRIAL REGISTRATION

After the protocol has been signed, any amendment of the protocol has to be agreed among WHO and the study team in the form of a written amendment, in as much as the changes proposed are relevant for its members. Any change will be signed and dated by the PI, co-PI and the study coordinators, the manager of the NBUCP and the coordinator of the PNLLUB, the CIs, and attached to the original protocol (following the procedure in relevant SOPs).

All amendments will be notified to the Ethics Committees (IRBs/ECs) and should be submitted for Ethics clearance, both at the WHO, Geneva; and at the HRU, Accra, Ghana, and the Ministère de la Santé - République du Bénin.

It is the responsibility of both the PI, the managers of the NBUCP in Ghana and the Coordinator of the PNLLUB in Benin, and the study coordinators to decide whether or not a formal vote of the IRBs/ECs is required for a given amendment, and whether the trial will be put on hold until the receipt of the results of the formal IEC/IRB vote. This as well as the need for modification of

the informed consent, CRF, etc. will be indicated in the amendment. The CI will acknowledge in writing receipt of the amendment. All major amendments including the involvement of additional districts, a justification and approval will be sought from the ethical review bodies.. The trial will be registered in accordance with the internationally accepted standards for studies involving human subjects (176). Reporting will also be done in accordance with current guidelines (177;178).

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APPENDIX 1: DIAGNOSIS OF TB DISEASE

http://www.cdc.gov/tb/publications/factsheets/testing/diagnosis.pdf

TB *Elimination*

Diagnosis of Tuberculosis Disease

When Should You Suspect Tuberculosis (TB)?

TB is a disease caused by *Mycobacterium tuberculosis*. TB disease should be suspected in persons who have the following symptoms:

- Unexplained weight loss
- Loss of appetite
- Night sweats
- Fever
- Fatigue

occupation) that may increase the patient's risk for exposure to TB or to drug-resistant TB. Also, clinicians should determine whether the patient has medical conditions, especially HIV infection, that increase the risk of latent TB infection progressing to TB disease.

2. Physical Examination

A physical exam can provide valuable information about the patient's overall condition and other factors that may affect how TB is treated, such as HIV infection or other illnesses.

3. Test for TB Infection

If TB disease is in the lungs (pulmonary), symptoms may include:

- Coughing for ≥ 3 weeks
- Hemoptysis (coughing up blood)
- Chest pain

If TB disease is in other parts of the body (extrapulmonary), symptoms will depend on the area affected.

How Do You Evaluate Persons Suspected of Having TB Disease?

A complete medical evaluation for TB includes the following:

1. Medical History

Clinicians should ask about the patient's history of TB exposure, infection, or disease. It is also important to consider demographic factors (e.g., country of origin, age, ethnic or racial group,

The Mantoux tuberculin skin test (TST) or the special TB blood test can be used to test for *M. tuberculosis* infection. Additional tests are required to confirm TB disease. The Mantoux tuberculin skin test is performed by injecting a small amount of fluid called tuberculin into the skin in the lower part of the arm. The test is read within 48 to 72 hours by a trained health care worker, who looks for a reaction (induration) on the arm.

The special TB blood test measures the patient's immune system reaction to *M. tuberculosis*.

4. Chest Radiograph

A posterior-anterior chest radiograph is used to detect chest abnormalities. Lesions may appear anywhere in the lungs and may differ in size, shape, density, and cavitation. These abnormalities may suggest TB, but cannot be used to definitively diagnose TB. However, a chest radiograph may be used to rule out the possibility of pulmonary TB in a person who has had a positive reaction to a TST or special TB blood test and no symptoms of disease.

5. Diagnostic Microbiology

The presence of acid-fast-bacilli (AFB) on a **sputum smear** or other specimen often indicates TB disease. Acid-fast microscopy is easy and quick, but it does not confirm a diagnosis of TB because some acid-fast-bacilli are not *M. tuberculosis*. Therefore, a **culture** is done on all initial samples to confirm the diagnosis. (However, a positive culture is not always necessary to begin or continue treatment for TB.) A positive culture for *M. tuberculosis* confirms the diagnosis of TB disease. Culture examinations should be completed on all specimens, regardless of AFB smear results. Laboratories should report positive results on smears and cultures within 24 hours by telephone or fax to the primary health care provider and to the state or local TB control program, as required by law.

6. Drug Resistance

For all patients, the initial *M. tuberculosis* isolate should be tested for drug resistance. It is crucial to identify drug resistance as early as possible to ensure effective treatment. Drug susceptibility patterns should be repeated for patients who do not respond adequately to treatment or who have positive culture results despite 3 months of therapy. Susceptibility results from laboratories should be promptly reported to the primary health care provider and the state or local TB control program.

Additional Information

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APPENDIX 2: DESCRIPTION OF STUDY SITES

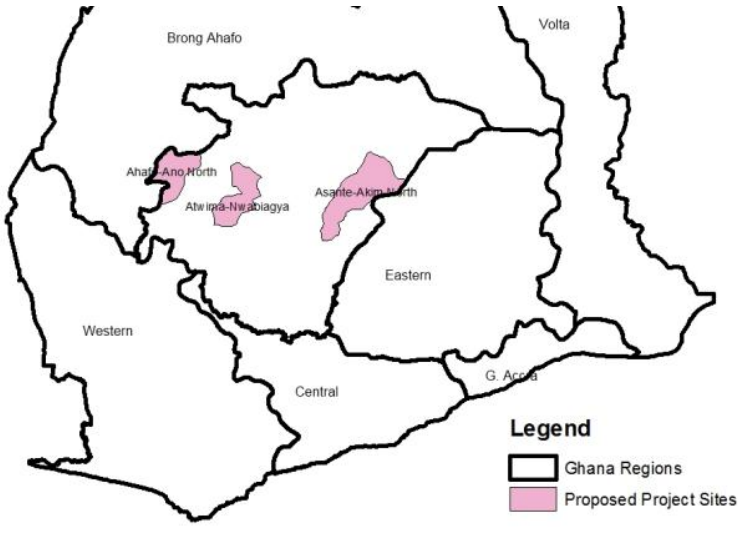
The Asante Akim District: lies in the tropical rain forest although there are some transitional zones due to farming and logging activities. The climate is tropical; temperature variation is 20 ^{o}C - 36 ^{o}C with monthly rainfall varying from 2.0 mm in February to 400 mm in July. It has 2 rainy seasons; a major one extending from April to August and a minor one from October to November. The local economy is based on cash crops like cocoa, coffee and oil palm, although subsistence farming is the main occupation. This district is divided into four sub-districts and has a population of 126,477. Agogo Presbyterian Hospital is a well established hospital that covers the Asante-Akim North district. It has a modern facility for managing Buruli ulcer sponsored by ANESVAD. This is the largest hospital with facilities for training nurses and doctors doing their housemanship. It is a major hospital for treatment of Buruli ulcer. It has been the site of previous studies in Buruli including the previous trial. It has a dedicated Buruli ulcer clinic that is held on Wednesdays with patients coming from the surrounding villages.

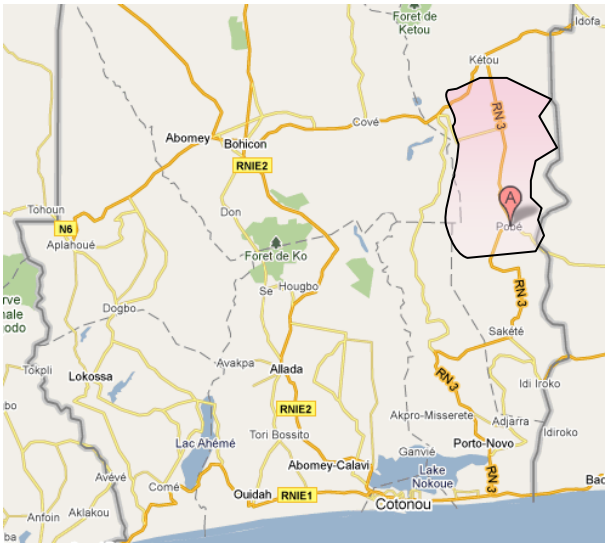
Ahafo Ano North District: of Ashanti Region lies within the tropical rain forest belt of West Africa and has 5 sub-districts. The population is about 99,900 living in 135 villages. Most villagers are cocoa farmers and it is one of the poorest districts in the Ashanti Region. M. *ulcerans* disease occurs mainly in villages along the rivers- Tano, Abu, Kwasu, and Abonsu. Most villages are without electricity most of the roads are poor. The Tepa Government Hospital is the main hospital that serves the district. Outreach educational activities are carried out by the district health directorate to identify early Buruli ulcer cases which are referred to the Buruli ulcer clinic held on Thursdays. The Ahafo Ano North district is surrounded by the Asutifi and Asunafu District in the Brong Ahafo region. Cases of Buruli ulcer in these 2 nearby districts are often referred to the Tepa Government Hospital for treatment.

Atwima District: of the Ashanti Region lies 30 km from the Ashanti capital Kumasi. with a population of 204,601. Buruli ulcer endemic villages are mainly sited around the river Offin. The main hospital serving this district is the Nkawie Government Hospital.

This hospital is a major treatment site of Buruli ulcer. ANESVAD has recently built an ultra modern facility for management of Buruli ulcer with provision of a surgical theatre, wards, consulting rooms, and physiotherapy. There is a dedicated Buruli ulcer clinic held on Fridays with patients attending from surrounding villages in the district.

Upper Denkyira District lies in the forest zone in the central region of Ghana. It is subdivided into two parts- east and west. Buruli endemic communities are distributed around the river Offin. The main hospital is the Dunkwa Government Hospital that has been the focus of treatment of the Buruli ulcer cases in the Central Region of Ghana. ANESVAD recently refurbished and provided theatre facilities, wards, consulting rooms and physiotherapy for management of Buruli ulcer. Outreach educational programmes are organized to identify the Buruli ulcer cases and referred to the Dunkwa Government Hospital for treatment. This programme has been effective in identifying early cases.





APPENDIX 3: CLASSIFICATION OF LESION HISTOPATHOLOGY

a. *Non-reactive lesion:* extensive subcutaneous fat necrosis (with or without ulceration), with no granuloma formation. Inflammatory response very slight in most. Acid-fast bacilli (AFB) usually seen.

b. *Reactive lesion:* epitheloid and giant-cell granulomas of the tuberculoid type present in subcutaneous fat, with minimum necrosis, A.F.B seen in very small numbers.

c. *Reactive lesion, presumed Buruli:* as b, but no AFB seen.

d. *Miscellaneous lesions involving subcutaneous fat:* not Buruli ulcer.

APPENDIX 4: ROLES AND RESPONSIBILITIES OF INVESTIGATOR TEAM

The World Health Organization will be the principal sponsor and coordination body for the study.

The *Principal Investigator* (PI) takes direct responsibility for completion of the study, in close collaboration with the coordinators, National Program Managers, the WHO, the WHO-TAG Drug Treatment Working Group members and the DSMB members

The *Co-Investigators* work at their study site. They conduct and organise the activities of the trial at study sites, help recruiting study participants with their study team; help organise training sessions; organise all the activities of the trial at their study site, work in close cooperation with the study coordinators, and finally report to the PI

The *Study Coordinators* organise all activities in the various study sites, organise training sessions for recruitment and selection of study participants; they help safeguarding the principles of Good Clinical Practice; they work in close cooperation with the co-investigators, and finally report to the PI

The members of *Data Safety Monitoring Board* are independent; they are appointed based on their specific expertise as senior members of the scientific and clinical community; and oversee the trial and conduct interim analyses at predefined as well as occasional points in time

The members of the *WHO-TAG Drug Treatment Working Group* help in the design, funding, evaluation, monitoring and auditing of the study, the final reporting to WHO and the research community in general.

APPENDIX 5. SUMMARY CVS OF THE LEAD CLINICIANS

NAME & TITTLE	PLACE OF WORK	CURRENT POSITION	QUALIFICATION	SCHOOL &YEAR
Dr William Thompson	Agogo Presbyterian Hospital	Medical Superintendent	Bsc, MB ChB	School of medical sciences K.N.U.ST (1988)
Dr Mark Forson	Tepa -Government Hospital	Medical Superintendent	B.Sc MBChB	School of medical sciences K.N.U.ST (1999)
Dr. Kwabena Sarpong	Dunkwa Municipal Hospital	Municipal Director of Health Services	Bsc Med Science, MB ChB, MPH	University of Ghana Medical School, Korle-Bu (2002)
Dr Peter Awuah	Nkawie Hospital	Medical Superintendent	MB ChB	University of Ghana Medical School, Korle-Bu (1982)
Dr Annick Chauty	*Centre de Dépistage et Traitement de l'Ulcère de Buruli BP191 POB7-BENIN*	Director	Doctor of Medicine	Faculté de Médecine de Nantes, France (1976)
			Diploma in Tropical Medicine	Université René Descartes Paris V,

APPENDIX 6. LIST OF INDEPENDENT DOCTORS THAT PARTICIPANTS MAY CONSULT

DISTRICT	NAME	E-MAIL	TEL
ASHANTI AKIM NORTH **(GHANA)**	DR JACQUES KEMABIA	jkemabia@yahoo.fr	+233 244519437
ATWIMA NWABYIGYA **(GHANA)**	DR GEORGE GYAU	georgeyaw@yahoo.com	+233 244701947
DUNKWA ON OFFIN **(GHANA)**	DR ABRAHAM MARTEY	marteyabraham@yahoo.com	+233 244170159
AHAFO ANO NORTH **(GHANA)**	DR FRANK FIIFI OCHERE	Fiifi_o@yahoo.com	+233 247002915
POBE **(BENIN)**	DR CRESPIN SOGLOHOUN	cresph2004@yahoo.fr	+229 95050459

APPENDIX 7. TARGETED COMMUNITIES FOR MOBILIZATION OF STUDY PARTICIPANTS

Region	District	Community	Preparatory visits (October)	3-monthly visits (5-days each) for 3 years
Ashanti, Ghana	Ahafo Ano North	Asuhyiae	Week 1	
		Manfo		
		Achiawkrom		
		Achina		
		Nfanibu		
		Dwaaho	Week 2	
		Konkori		
		Dotoam		
	Asutifi	Nkaseim		
		Woromso		
		Konkontireso	Week 3	
		Huridiem akurakan		
		Subriso		
		Kenyasi No 1 & 2		
		Achirensua		

Region	District	Community	Preparatory visits (October)	3-monthly visits (5-days each) for 3 years
Ashanti, Ghana	Atwima Nwabiagya	Nkawie	Week 1	
		Toase		
		Mim		
		Kyerease		
		Nerebehin		
		Nkorang	Week 2	
		Gyankobaa		
		Abuakwa		
		Koforidua		
	Atwima Mponua	Ntobroso		
		Akyease	Week 3	
		Adobewora		
		Ayinamso		
		Agogoso		
		Akropong		

Region	District	Community	Preparatory visits (October)	3-monthly visits (5-days each) for 3 years
		Ananekrom	Week 1	
		Nshyieso		
		Sereboso		
		Wenamda		
		Bebuso		
		Afrisere	Week 2	
		Nsonyameye		
		Dukusen		
		Abrade		
		Makyire		
		Kowireso	Week 3	
		Mankala		
		Bebome		
		Kwameaddo		
Ashanti, Ghana	Asante Akim North	Pewodee		

Region	District	Community	Preparatory visits (October)	3-monthly visits (5-days each) for 3 years
		Dunkwa	Week 1	
		Pokukrom		
		Mbradan		
		Buabinso		
		Atechem		
		Denyase	Week 2	
		Kyekyewere		
		Akyempim/Breman		
		Compound		
		Toll Bridge		
		Sofo Akurase	Week 3	
		Mfantseman		
		Buabin		
		Ntontom		
Central, Ghana	Upper Denkyira	Agyempoma		

BENIN			Preparatory visits (October)	3-monthly visits (5-days each) for 3 years
l'OUEME	BONOU	1. Bonou centre		
		2. Affamey		
		3. Assrossa		
		4. Atchonsa		

		5. Dame-Wogon		
		6. Agbona		
	ADJOHOUN :	7. Adjohoun centre		
		8. Azowlisse		
		9. Akpadanou		
		10. Kode		
	DANGBO	11. Dangbo,Zoungue		
	ADJA OUERE :	12. Adja-Ouere		
		13. Tatonoukon		
	POBE	14. Issaba		

APPENDIX 8: LIST OF DRUGS WHICH ARE LIKELY TO INTERACT WITH THE STUDY MEDICATIONS

(A) List of Drugs Which Are Likely To Interact With Rifampicin

These medicines may increase the chance of liver damage if taken with rifampicin:

Acetaminophen (with long-term, high-dose use)
Amiodarone
Anabolic steroids
Androgens
Antithyroid agents
Carbamazepine
Carmustine
Chloroquine
Dantrolene
Daunorubicin
Disulfiram
Divalproex
Etretinate
Gold salts
Hydroxychloroquine
Isoniazid
Mercaptopurine
Methotrexate
Methyldopa
Naltrexone (with long-term, high-dose use)
Phenothiazines
Plicamycin
Valproic acid

Rifampicin may decrease the effects of these medicines:

Anticoagulants
Aminophylline
Amprenavir
Antidiabetics, oral
Chloramphenicol
Corticosteroids
Delavirdine
Digitalis glycosides
Disopyramide
Efavirenz
Fluconazole
Indinavir
Itraconazole
Ketoconazole
Methadone
Mexiletine
Nelfinavir
Nevirapine

Oxtriphylline	Theophylline
Quinidine	Tocainide
Ritonavir	Verapamil
Saquinavir	

Rifampicin may decrease the effects of these medicines. These medicines may also increase the chance of liver damage if taken with rifampin:

Estramustine	Oral contraceptives
Estrogens	Phenytoin

(B) List of Drugs Which Are Likely To Interact With Streptomycin

Aminoglycosides (other than streptomycin)	Deferoxamine
Anti-infectives	Gold salts
Capreomycin	Hydroxychloroquine
Carmustine	Inflammation or pain medicine, except narcotics
Chloroquine	Lithium
Cisplatin	Methotrexate
Combination of pain medicine containing acetaminophen and aspirin	Penicillamine
Cyclosporine	Plicamycin
Streptozocin	Quinine
Tiopronin	

The ototoxic effects of the aminoglycosides, including streptomycin, are potentiated by the co-administration of ethacrynic acid, furosemide, mannitol and possibly other diuretics.

(C) List of Drugs Which are Likely to Interact With Clarithromycin

Acenocoumarol
Alfentanil
Astemizole
Carbamazepine
Cisapride
Clindamycine
Cyclosporine
Digoxin
Disopyramide
Ergot alkaloid medicines and ergotamine derivates
Fluconazole
Hexobarbital
Indinavir
Lincomycine
Midazolam
Omeprazole
Phenytoin
Pimozide
Quinidine
Rifabutin
Ritonavir
Statine medicines
Tacrolimus
Terfenadine
Theophylline
Triazolam
Warfarin
Zidovudine

APPENDIX 9: CURRICULUM VITAE, MEMBERS OF THE DATA SAFETY MONITORING BOARD

World Health Organization

Protocol

RANDOMIZED CONTROLLED TRIAL COMPARING EFFICACY OF 8 WEEKS TREATMENT WITH CLARITHROMYCIN AND RIFAMPICIN VERSUS STREPTOMYCIN AND RIFAMPICIN FOR BURULI ULCER (*M. ULCERANS* INFECTION)

BENIN AND GHANA

Principal Investigator

Tjip S. van der Werf, Departments of Internal Medicine, and Pulmonary Diseases & Tuberculosis, University Medical Centre Groningen, University of Groningen, the Netherlands.

Co-Principal Investigators

1. **Richard Phillips,** Department of Internal Medicine, Kwame Nkrumah University of Science & Technology, Komfo Anokye Teaching Hospital, Kumasi, Ghana
2. **Annick Chauty,** Centre de Diagnostic et de Traitement de l'Ulcère de Buruli, Pobe, Benin

The DSMB responsibilities include:

- evaluate the progress of the trial, including periodic assessments of data quality and timeliness, recruitment, accrual and retention, participant risk versus benefit, performance of the trial sites, and other factors that can affect study outcome;
- consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- review study performance, make recommendations and assist in the resolution of problems reported by the Principal Investigator;
- protect the safety of the study participants;
- report to WHO on the safety and progress of the trial;
- make recommendations to the WHO, the Principal Investigator, and, if required, to the GHS-ERC, Food and Drug Board (FDB) concerning continuation, termination or other

modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;

- if appropriate, review interim analyses in accordance with stopping rules, which are clearly defined in advance of data analysis and have the approval of the DSMB;
- ensure the confidentiality of the study data and the results of monitoring; and,
- assist WHO and study team by commenting on any problems with study conduct, enrollment, sample size and/or data collection.

1. *David Ofori-Adjei*, Noguchi Memorial Insitute for Medical Research, Accra, Ghana
2. *William Faber*, AMC, University of Amsterdam, Netherlands
3. *Sara Eyangoh*, Pasteur Institute, Yaounde, Cameroon
4. *Alan Knell*, United Kingdom
5. *Paul Saunderson*, American Leprosy Missions, Greenville, USA

Curriculum Vitae David Ofori-Adjei

David Ofori-Adjei (born April 15, 1949) is a prominent Ghananian physician and medical researcher. A graduate of the University of Ghana Medical School[1] and University of Edinburgh he has written 78 original research papers in international journals. His main areas of research are clinical pharmacology, pharmacogenetics, infectious diseases such as malaria, schistosomiasis, Buruli ulcer, and HIV/AIDS and public sector pharmaceutical management. He was elected to the Council of the Division of Clinical Pharmacology of the International Union of Pharmacology and Clinical Pharmacology in 2000. Ofori-Adjei has promoted the Rational Use of Drugs in Ghana and the development of the National Essential Drugs List with Therapeutic Guidelines since the late 1980s.[He served on the United States Pharmacopoeia Convention and the International Health Advisory Panel for ten years.

He is currently the Director of the Noguchi Memorial Institute for Medical Research and Editor-in-Chief of the Ghana Medical Journal.[2]

References

1. ^ "David Ofori-Adjei—biilding bridges for health care and research in Ghana". The Lancet Infectious Diseases, Volume 3, Issue 4. April 2003. pp. 251–254.

http://www.thelancet.com/journals/laninf/article/PIIS1473-3099%2803%2900583-8/fulltext.

2. ^ "Professor David Ofori-Adjei". Africa Institute of Biomedical Research and Technology. http://www.aibst.com/index.php/about-aibst/index.php?option=com_content&view=article&categoryId=45&id=113.

Curriculum vitae Professor W.R. Faber, MD PhD

21.02.1940	Born in Schiedam
11.03.1966	Physician's degree, University of Leiden
14.03.1966 - 15.02.1967	Training for overseas appointment in Tropical Medicine and Hygiene, Surgery and Obstetrics
15.03.1967 - 01.09.1969	Medical Officer of the Uganda Government
01.12.1969 - 01.08.1970	Assistent Physician at the Department of Medicine, "De Lichtenberg", Amersfoort
01.10.1970 - 01.08.1971	General practitioner in Beetsterzwaag and Boornbergum
01.09.1971 - 01.05.1975	Training for specialist in Dermatology and Venereology at the Department of Dermatology, Binnengasthuis, University of Amsterdam (chairman Prof. Dr. R.H.Cormane)
01.05.1975	Registered as specialist in Dermatology and Venereology. Appointment as staff member at the Department of Dermatology, University of Amsterdam
01.10.1975 - 01.09.1977	Chef de Clinique of this Department
01.09.1977 - 01.01.1981	Consultant for this Department at the Wilhelmina Gasthuis
06.07.1978	Thesis: "Leprosy: Clinical and Immunological Studies"
1981-2001	Dermato-venereologist at Hospital Eemland, location "De Lichtenberg", Amersfoort; later Meander Medical Center Part-time staff member at the Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam
01.11.1987	Appointment in Tropical Dermatology at the Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam
15.03.1995	Professor in Tropical Dermatology, University of Amsterdam
01.10.2000	Full time appointment Department of Dermatology, Academic Medical Center
01.03.2005	Part time appointment Department of Dermatology, Academic Medical Center
01.06.2008	Emeritus Professor

Present position: part time appointment member Department of Dermatology, Academic edical Center

Relevant Research Experience

- Immunological research in leprosy at the Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam (4 years)
- Head of the immunofluorescence laboratory, Binnengasthuis, University of Amsterdam, Amsterdam (8 years)
- Studies in disorders of fibrinolysis in vascular diseases of the lower extremities, Hospital Eemland, location "De Lichtenberg", Amersfoort (10 years)
- Research in tropical dermatology and leprosy, University of Amsterdam, Amsterdam (since 1974)
- Immunological investigations in leprosy, with emphasis on leprosy reactions
- Multidisciplinary investigations of neuropathic foot complications in leprosy patients
- Investigation of aspects of wound healing under tropical climatic conditions

Curriculum Vitae Sara Irène EYANGOH - NGO NIOBE

Adress: Mycobacteriology Unit, Centre Pasteur of Cameroon,
Po Box: 1274 Yaoundé - Cameroon
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E-mail : eyangoh@pasteur-yaounde.org

Current Position

- Head of Mycobacteriology unit
- Head of the National Reference Laboratory for the National Programmes against Tuberculosis and Buruli ulcer in Cameroon

Universities Degrees and other Qualifications

- PhD Microbiology "Molecular epidemiology of tuberculosis in Cameroon", University of Paris VII, France, 2003
- PhD in Biochemistry " Génotyping of apolipoprotein E in patient with diabetes and hypertension" University of Yaoundé I, 1999, Cameroon
- Master in Tropical Infectious Diseases, Agence Universitaire de la Francophonie, 1999
- Organizer of the international course on Microbiology of *M. ulcerans* since 2006, funding by WHO and International network of Pasteur Institute
- Teacher in international course "Molecular Tools and TB epidemiology" Pasteur Institute Paris, since 2006 , appointed technical organizer in 2010 and 2011
- WHO GLI (Global Laboratory Initiative) Member (2011-2013)

Areas of Professional activities

- Microbiological and epidemiological aspect on Tuberculosis and Buruli ulcer

- Improvement of laboratory diagnosis of Tuberculosis, Buruli ulcer and other mycobacterial diseases in humans and animals
- Organization and monitoring of laboratory network and building capacities

Some Relevant Publications:

- Marion E, Landier J, Boisier P, Marsollier L, Fontanet A, Le Gall P, Aubry J, Noumen-Djeunga B, Umboock A, **Eyangoh S**. Expansion of Buruli ulcer disease in Cameroon Emerg Inf Dis 2011;17:3 551-553
- **Eyangoh S**. Buruli ulcer Disease: challenges and opportunities for Institut Pasteur International network. BMC Proceedings. 2011 5(suppl 1):L5
- E. Marion, **S. Eyangoh**, J. Doannio, J. Aubry, J. Landier, A. Fontanet, C. Rogier, V. Cassisa, J. Cottin, A. Marot Y. Kamdem, P. Legras and L. Marsollier. · Seasonal and regional dynamics of M. ulcerans transmission in environmental context: deciphering the role of water bugs as hosts and vectors .PLoS Negl Trop Dis. 2010 Jul 6;4(7):e731.
- **S. Eyangoh**, G. Torrea, M.C Tejiokem, Y. Kamdem, F. Fostso Piam , J. Noeske , A. Van Deun. 2008. HIV-related incremental yield of bleach sputum concentration and fluorescence technique for microscopic detection of tuberculosis. Eur. J. Clin. Microl. Infect. Dis 5:
- R. Pouillot, G. Matias, C.Mbondji, F.portaels, N.Valin, F.Ngos, A. Ndjikap, L.Marsollier, A. Fontanet and **S.Eyangoh**. 2007. Risk factors for Buruli Ulcer:A case –Control Study in Cameroon. *PloS Neglected Disease.* 1(3):e101.
- **S. Ngo Niobe-Eyangoh**, C. Kuaban, P. Sorlin, J. Thonnon, V. Vincent and M. C. Gutiérrez. 2004. Molecular characterization of closely related strains isolated in Cameroon. J. Clin. Microbiol. 42 (11).5029-5035.
- **S. Ngo Niobe-Eyangoh**, C. Kuaban, P. Sorlin, P. Cunin, J. Thonnon, C.Sola, N. Rastogi, V. Vincent and M. C. Gutiérrez. Genetic Biodiversity of *Mycobacterium tuberculosis* complex strains from patients with pulmonary tuberculosis in Cameroon. 2003. J. Clin. Microbiol. 41(6): 2547-2553.

Curriculum vitae, Dr Alan J Knell MD PhD FRCP

Dr Knell has had extensive experience in tropical medicine including Buruli ulcer during his work and several different journeys around the world. He has served in the e-learning activities of the Wellcome Tropical Institute on malaria and other tropical diseases, with Prof Eldryd Parry. He also served on the DSMB of the BURULICO Drug Trial, published in 2010 (Lancet).

He has published numerous articles and chapters in books, one of which was on Buruli ulcer.

Educational track record and academic and professional positions:

MB BChir [Cambridge] 1964

MRCP [London] 1969: FRCP 1979

MD [Cambridge] 1980

1972 to 1987: Consultant Physician, South Warwickshire Hospitals, UK

1980: Visiting Lecturer, University of Zimbabwe

1982 to 1985: annual visits to teach at Komfo Anokje Teaching Hospital, Ghana; sponsored by the British Council Fellow of the Royal Society of Hygiene and Tropical Medicine

1987 to 1990: Deputy Director, Wellcome Tropical Institute, London 1990 to 1992: Senior Consultant Physician. Tawam Hospital, Al 'Ain, UAE

1992 to 1997: Senior Consultant Physician, Sultan Qaboos University Hospital, Muscat, 'Oman

1997 to 2002: Locum Consultant Physician, Selly Oak Hospital, Birmingham; then Dumfries General Hospital, finally Neville Hall Hospital, Abergavenny; UK

Curriculum vitae: 2011 email: psaunderson@leprosy.org

Dr Paul R. Saunderson, Medical Director, American Leprosy Missions

Contact details: Home address: Østrem, 6013 Ålesund, Norway. Phone: +47 7016 9988

Date of birth: 21st February, 1952 **Sex** Male **Nationality** British

Education (UK): Bedford School, Bedford, England (1959-69)

1969	Cambridge University: Open scholarship in Natural Sciences to read Medicine	
1976	Clare College, Cambridge and Guy's Hospital, London	MA, MB, BChir
1978	Royal College of Physicians, London	MRCP(UK)
1978	Royal College of Obstetricians and Gynaecologists, London	DRCOG
1984	London School of Hygiene & Tropical Medicine	DTM&H
1993	Keele University, England	MBA
2004	Cambridge University, England	MD

Honorary positions:

2008-present	Research Director, The Leprosy Mission International, London, UK
2006-present	Member: WHO Technical Advisory Group for Buruli ulcer
2004-present	Member: ILEP Technical Commission (ILEP is the International Federation of Anti-Leprosy Associations)
1996-present	Reviewer for a number of leprosy and international health journals.
2004-2009	Member: WHO Technical Advisory Group for Leprosy

Employment since 1981:

2006-present	Scientific Director, Leonard Wood Memorial, Cebu, Philippines
2000-present	Medical Director, American Leprosy Missions, Greenville, South Carolina.
1998-99	Consultant in Leprosy/TB Control, Training Division of ALERT, Ethiopia.
1994-97	Director of the Leprosy and Tuberculosis Control Division, ALERT, Ethiopia.
1988-92	Leprosy and Tuberculosis Adviser, Western Region, Uganda.

1985-7 Medical Superintendent and Project Director at Kagando Hospital.
1981-5 Medical Officer at Kagando Hospital (Church of Uganda), Western Uganda.

Employment 1976 - 81:
Internships in medicine, surgery, obstetrics and paediatrics in the British National Health Service.

Teaching experience:
At ALERT, the *All Africa Leprosy, Tuberculosis and Rehabilitation Training Centre*, I was involved in teaching the general principles of management, and the epidemiology of leprosy and tuberculosis. Since 1999, I have been involved in writing training materials and guidelines for ILEP and WHO.

Research experience:
My research experience in Ethiopia (1994-99) focused on neuropathy and other complications of leprosy. As Medical Director of American Leprosy Missions, Scientific Director of the Leonard Wood Memorial Research Institute, and Research Director of The Leprosy Mission International, my role is to facilitate collaborative research in leprosy, Buruli ulcer and tuberculosis.

Selected publications:
1. Richardus JH, Saunderson P, Smith WCS. Will new tuberculosis vaccines provide protection against leprosy? Editorial. Int J Tuberc Lung Dis 2011; 15(2): 143.

2. W Cairns S Smith and Paul Saunderson. Leprosy. Clinical Evidence 2010; 06:915.

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4. Saunderson PR. ILEP Technical Guide: Training in Leprosy. London: ILEP, 2003.

5. Saunderson PR, Groenen G. Which physical signs help most in the diagnosis of leprosy? A proposal based on experience in the AMFES project, ALERT, Ethiopia. Lepr Rev 2000; 71:(1) 34-42.

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7. Saunderson PR. An economic evaluation of alternative programme designs for tuberculosis control in rural Uganda. Soc Sci Med 1995; 40: 1203-1212.

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APPENDIX 10: CASE REPORT FORM (CRF)

Please refer to a separate document.

APPENDIX 11: ADVERSE EFFECTS FOUND WITH TRIAL MEDICATIONS

(A) Streptomycin

More common adverse effects: Any loss of hearing, clumsiness or unsteadiness, dizziness, increased or decreased frequency of urination or amount of urine, increased thirst, loss of appetite, nausea and vomiting, numbness, tingling or burning of face or mouth, muscle twitching or convulsions (seizures), ringing or buzzing or a feeling of fullness in the ear.

Less common: Any loss of vision, skin rash, itching, redness or swelling.

The presence of other medical problems may affect the use of streptomycin:

a. Kidney disease: patients may have increased streptomycin blood levels and increased chance of side effects

b. Loss of hearing and/or balance (eighth-cranial-nerve disease): High drug blood levels may cause hearing loss or balance disturbance

c. Myasthenia gravis or

d. Parkinson's disease: Streptomycin may cause muscular problems resulting in further muscle weakness

(B) Rifampicin

Less common: Chills, difficult breathing, dizziness, fever, headache, itching, muscle and bone pain, shivering, skin rash and redness.

Bloody or cloudy urine, increased frequency of urination or amount of urine, loss of appetite, nausea or vomiting, sore throat, unusual bleeding or bruising, unusual tiredness or weakness, yellow eyes or skin.

The presence of other medical problems may affect the use of rifampicin:

a. Alcohol abuse (or history of) or

b. Liver disease—There may be an increased chance of side effects affecting the liver in patients with a history of alcohol abuse or liver disease**.**

(C) Clarithromycin

Less common: loss of appetite, nausea, dyspepsia; changes in taste and smell sensation; headache; ringing or buzzing in the ears (tinnitus), dizziness; liver enzyme elevation; allergic skin reactions including Stevens-Johnson syndrome; blood dyscrasias; reversible loss of hearing. In elderly (especially, female) patients, long QT-syndrome sometimes complicated with Torsade de Pointes has infrequently been reported – while in half of cases, co-medications (especially, cizapride) have played a role.

APPENDIX 12: GUIDELINES FOR WOUND CARE AND TIMING OF LIMITED SURGERY AND SKIN GRAFTING

Management	Procedure	Expected outcome/primary end point
Wound care	Dress wounds based on standard guidelines (type of dressings, frequency)	
POD	Implement standard physiotherapy procedures to prevent disability	Prevent and treat functional limitations
Surgery	Remove necrosis after completion of antibiotics treatment if present Consider skin graft (between weeks 10 and 14) if remaining ulcer is more than 5 cm in diameter after 10 weeks of antibiotics – to be discussed with external consultants that are blinded for treatment allocation	Early closure of the ulcer after antibiotics treatment

APPENDIX 13: PROTOCOL FOR MYCOLACTONE DETECTION

Biopsy Extraction, Purification, and TLC Protocol

EXTRACTION:

For Biopsy material:
1. Place the biopsy sample in a 1 mL Potter-Elvehjem or Dounce tissue grinder. Add 500 μL of 2:1 chloroform/methanol. Homogenize the tissue.

2. Transfer the solvent from the grinder into to a separate 2 mL Eppendorf tube. Rinse the Potter-Elvehjem apparatus with 600 μL of 2:1 chloroform:methanol, and transfer this rinse to the Eppendorf tube. The total volume should be about 1 mL.

For swabs:
1. Swirl swab in 500 ul 2:1 chloroform methanol, roll swab against tube side to remove all solvent and transfer to Eppendorf tube. Repeat this step with an additional 500ul and add to Eppendorf tube. Total volume will be 1 ml.

3. Add 200 μL of water, shake vigorously (or vortex, if available). Allow the layers to settle (centrifugation, if available, will accelerate settling of the layers).

a. If centrifugation not used: the lower organic layer should become clear, and the upper layer might remain slightly cloudy. Foamy amorphous material will likely be present at the interface of the layers.
b. If centrifugation used: the layers should both turn clear, and a film of amorphous material will be present at the interface of the layers.

4. Using a Pasteur pipette, collect the lower organic layer and transfer it to another Eppendorf tube. Dry this sample under a nitrogen or air stream to obtain the crude residue.

PURIFICATION:

1. Prepare SiO_2 plug for purification (Diagram 1):

    c. Place a small wad of cotton into a Pasteur pipette and tamp it down with a piece of wire until it is tightly secured.
    d. Add ~200 mg of silica gel to the top of the cotton plug (or to a height of around 1.5 cm).

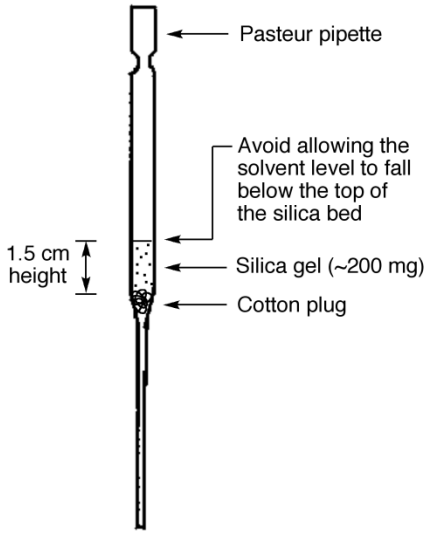

Diagram 1. Silica gel plug inside a Pasteur pipette for purification of biopsy extract

    e. Add ~2 mL of 25% ethyl acetate in hexanes to the top of the silica gel. With a gloved finger placed over the open end of the pipette, invert/shake the column several times until all the silica becomes suspended in the solvent. Allow the suspended silica to settle back down atop the cotton plug.
    f. Using a pipette bulb, force the solvent through the pipette column, stopping just before the solvent level enters the silica bed.

2. Place the bottom of the Pasteur pipette column into a test tube. Dissolve the residue from step 4 in 500 μL of 25% ethyl acetate in hexanes. Apply this solution to the top of the silica gel bed prepared in Step 5. Using a pipette bulb, force the liquid into the silica bed, stopping just before the solvent level enters the silica bed. Add 2 mL of 25% ethyl acetate in hexanes to the column and force the solvent through the plug, stopping just before the solvent level enters the silica bed. Repeat the 2 mL flush with 25% ethyl acetate in hexanes twice more, collecting all elutions into the same test tube.

3. Switch the collection vessel to a 2 mL Eppendorf tube. Add 2 mL of 10% methanol in ethyl acetate to the column. With a pipette bulb, force the solvent through until 1.5-2.0 mL of solvent is collected into the Eppendorf tube. Dry this solution under a stream of nitrogen or air to obtain extract for TLC.

THIN LAYER CHROMOTOGRAPHY (TLC)

4. Obtain a TLC plate. With a pencil, mark a baseline approximately 0.7 cm from the bottom of the plate (see Diagram 2).
    g. Note: for the demonstration, we will likely use TLC plates that are 2 x 5 cm. Longer plates (~ 10 cm) often give better separation of the spots.

5. Dissolve extract for TLC (from Step 7) in 15 uL chloroform. Using a capillary tube, apply the entire extract to a small TLC silica gel 60 plate (no fluorophore, glass-backed plate*). Also spot 1 uL each of the 0.2 mg/mL and 0.02 mg/mL standards onto the plate.

    * The spots on the TLC plate are best visualized through the glass side of a plate, and the plate can be easily photographed when the plate is placed silica-side-down on a 365 nm UV light source. If photography is not required or glass plates are not available, aluminum- or plastic-backed plates can be used, although photographing the plates may be difficult.

6. Add chloroform:methanol:water (90:10:1) to the bottom of a small glass jar/chamber to a height of approximately 0.5 cm. This will serve as the solvent system. Line the TLC chamber with a piece of filter paper to wick solvent, gently swirl the chamber, then insert the TLC plate. Close the lid of the chamber and develop the TLC plate until the solvent front reaches about half a centimeter below the top of the plate. Remove the plate and immediately mark a line along the solvent front with a pencil.

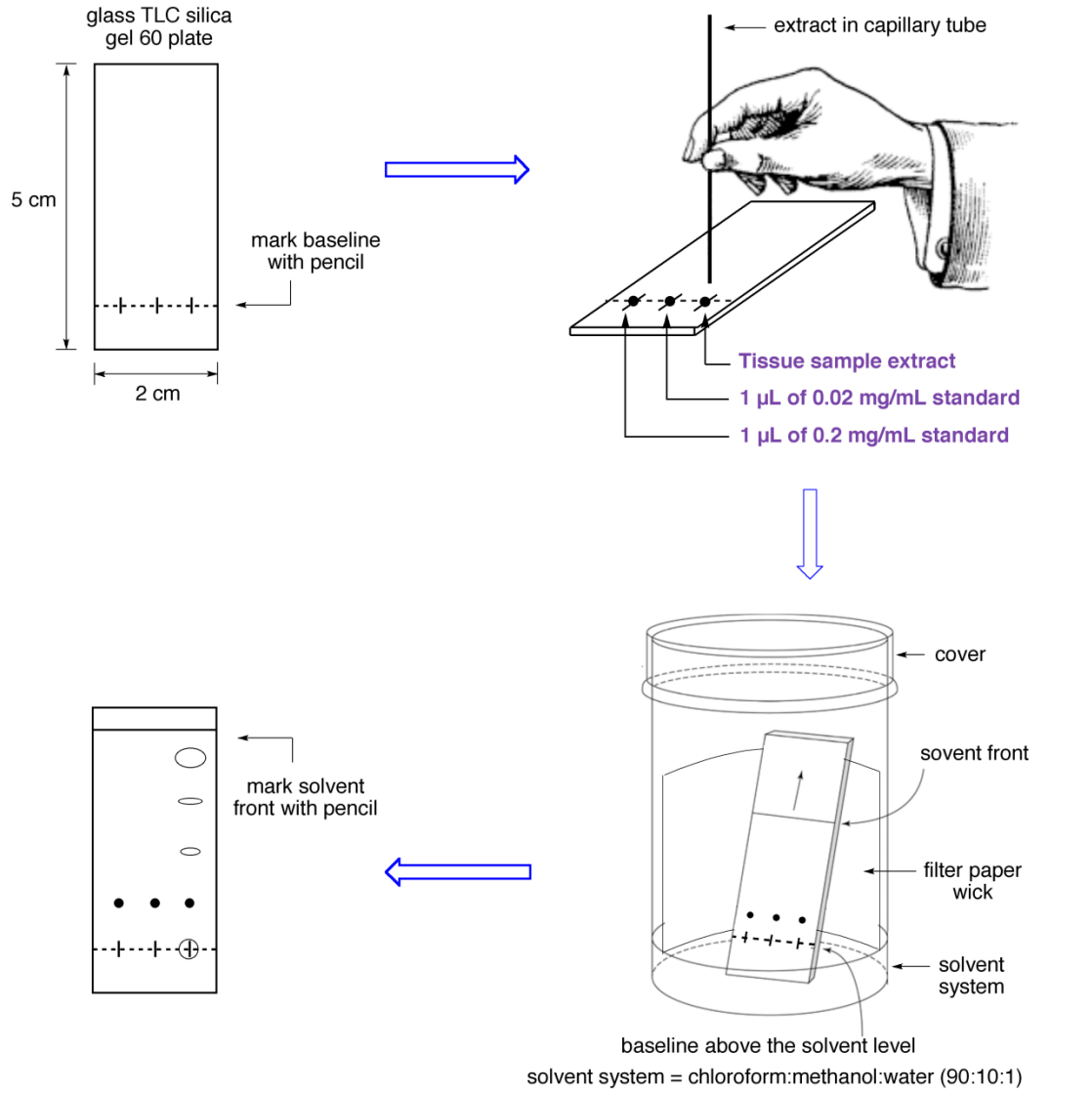

Diagram 2. Thin layer chromatography procedure.

VISUALIZATION

7. Briefly warm the eluted TLC plate with a heat gun or hot plate to evaporate any solvents. While the plate is warm, immerse it quickly into a solution of 0.1 M 2-naphthalene boronic acid in acetone, then remove immediately. Heat the plate to ~ 100 °C on a hot plate or over a heat gun for 5-10 seconds. With a paper towel and acetone, carefully wipe the glass side of TLC plate to remove excess stain. Be careful not to touch or damage the silica gel on the front side of the plate.

8. Visualize the spots by irradiating the plate with an 8 W hand-held UV lamp (365 nm filter). Lower watt lamps can be used, although the mycolactone spot may be dimmer. Mycolactone, if present, should appear as a yellow-green spot with $R_f \approx 0.23$.** It is easiest to see the mycolactone spot when the plate is viewed in the dark.

**Note: R_f = (migration distance of spot / migration distance of solvent front)

9. If you wish to photograph the results for documentation, place the TLC plate silica side down on 365 nm UV light. The plate can be color-photographed in the dark.

APPENDIX 14: STANDARD OPERATING PROCEDURE FOR COUNSELING MINOR FEMALE PARTICIPANTS TESTING POSITIVE FOR PREGNANCY

A pregnancy test (beta-HCG in urine) will be offered in all female patients of child-bearing potential between menarche and menopause, i.e., basically, between aged between 10 and 50 years. The test is necessary to prevent unintended use of drugs during pregnancy that may potentially harm the fetus. Streptomycin is not acceptable during pregnancy, because of potential hearing and renal damage to the fetus; clarithromycin is a class C drug and there is limited data on safety for the fetus if this drug is administered during pregnancy.

Standard Operating Procedure:

1. The female minor study participant as well as her parents, caretakers and/or the partner/husband are informed that we will test for pregnancy, even if she is not aware that she might be pregnant. Only after fully informed consent and assent, such tests will be carried out.

2. The female study participant is informed that, should the test be positive and prove that she is pregnant without knowing, this information will be shared with her, and her parents, caretakers or the partner / husband.

3. Should the urine test show that she is pregnant, she will be informed in a private conversation with herself, her husband/partner, and/or her parents or caretakers with the CI, or his representative, who is like the CI fully trained as a study team member representing the CI.

4. She will first be invited to share her feelings about this news with her parents, caretakers or the partner / husband. Next, she will be encouraged to explain how she and her partner/husband and parents/caretakers plan to deal with it.

5. For further counseling and management, she will be referred to the gynecology service in the hospital.

6. If the study team is convinced that drug treatment for BUD is required even though the patient appears pregnant, she may be invited to participate in the observational cohort study.

7. The basic rule for study team members involved in this counseling after pregnancy testing is COMMUNICATING WELL; see below

COMMUNICATING WELL

A useful way of remembering some of these ideas is to remember the acronym: **WELL**

W = Welcome your patient

- Ensure privacy and confidentiality
- Greet the patient in a friendly manner (for example: “hello Mr/Mrs… please come in”)
- Offer him/her a seat
- Ask his/her name
- Show empathy (I understand how you feel)

E = Encourage your patient to talk

- Ask general questions "what is your problem", "what are you concerned about"
- Allow your patient to answer
- Nod, agree or say "tell me more about that" to help your patient explain

L = Look at your patient

- Make sure that your facial expression is warm and friendly
- Maintain eye contact with your patient as he/she speaks
- Observe his/her feelings, as well as his/her general medical condition

L = listen to your patient

- Listen carefully to what your patient has to say and do not interrupt him/her.
- Show the patient that you are interested in what he/she is saying by reflecting back what you have heard. That is, repeat what he/she has said in another way to clarify the answers.

By communicating well with people, we can improve a patient's understanding of his/her problem. With better understanding of his illness the patient is more likely to complete a full course of treatment and be cured.

APPENDIX 15: STANDARD OPERATING PROCEDURE FOR COUNSELING & TESTING FOR HIV INFECTION

All candidate study participants will be offered Counseling & Testing (C&T) for HIV infection. This offer may be rejected without loosing any of the advantages of study participation; treatment will not be affected by opting out for HIV C&T. Study participation is however prohibited for those who refuse to be tested, but they may however still participate in the observational cohort study should drug treatment for BUD be necessary. C&T will be offered without pressure throughout the observational study to those initially opting out.

Standard Operating Procedure for those testing HIV positive:

1. Potential study participants as well as their parents, caretakers and/or the partner/ husband/wife are informed that we will test for HIV.

2. Only after fully informed consent and assent, such tests will be carried out.

3. The candidate study participant is informed that should the test be positive and prove that HIV co-infection is present, this information will be shared with the candidate and the parent, caretakers or the partner/husband/wife, if the candidate is minor.

4. Should the test show that the candidate-participant has HIV infection, this information will be shared in a private conversation with the candidate and the CI, or one of the senior, fully trained study team members representing the CI.

5. The patient will first be invited to share his/her feelings about this diagnosis. Next, he/she will be encouraged to explain how he/she plans to deal with it.

6. If the candidate is minor, the information will be shared in the presence of partner/spouse, and parents or caretakers.

7. For further counseling and management he/she will be referred to the National HIV/AIDS Programs.

8. If the study team is convinced that drug treatment for BUD is required, he/she may be invited to participate in the observational cohort study.

9. The basic rule for study team members involved in this C&T for HIV is COMMUNICATING WELL; see below.

COMMUNICATING WELL

A useful way of remembering some of these ideas is to remember the acronym: **WELL**

W = Welcome your patient

- Ensure privacy and confidentiality
- Greet the patient in a friendly manner (for example: "hello Mr/Mrs… please come in")
- Offer him/her a seat
- Ask his/her name
- Show empathy (I understand how you feel)

E = Encourage your patient to talk

- Ask general questions "what is your problem", "what are you concerned about"
- Allow your patient to answer
- Nod, agree or say "tell me more about that" to help your patient explain

L = Look at your patient

- Make sure that your facial expression is warm and friendly
- Maintain eye contact with your patient as he/she speaks
- Observe his/her feelings, as well as his/her general medical condition

L = listen to your patient

- Listen carefully to what your patient has to say and do not interrupt him/her.

- Show the patient that you are interested in what he/she is saying by reflecting back what you have heard. That is, repeat what he/she has said in another way to clarify the answers.

By communicating well with people, we can improve a patient's understanding of his/her problem. With better understanding of his illness the patient is more likely to complete a full course of treatment and be cured.

APPENDIX 16: BURULI ULCER FUNCTIONAL LIMITATIONS SCORE – QUESTIONNAIRE

The BUFLS questionnaire was published in 2004 (179), and again in a 2005 paper (169). The questions asked are - 'do you experience any / moderate / severe (absolute) difficulties - in performing the task listed, as a result of BUD that you had?' Answers are in Likert scale: 0-1-2-3 - with: 0 = no difficulty; 1 = mild difficulty; 2 = moderate difficulty; and 3- impossible for me now that I had BUD. Basically it consists of 18 questions with answers ranging from 0-3.

APPENDIX 1
ITEMS ON THE BURULI ULCER FUNCTIONAL LIMITATION SCORE

Type of activity	Activity	Extremity involved
Preparation of food/eating	Fetching water from pump	Lower and upper
	Pound fufu (/manioc*)	Lower and upper
	Pouring water from a bottle into a glass	Upper
	Cutting vegetables with a knife	Upper
Clothing/personal care taking	Putting on T-shirt	Upper
	Wash yourself	Upper
	Cleaning yourself after using the toilet	Upper
Working	Using a cutlass	Lower and upper
	Heave loads on head	Lower and upper
	Carry harvest home	Lower and upper
	Opening bottle with screw top (/corked bottle*)	Upper
	Tie a knot	Upper
Mobility	Walking level ground	Lower
	Walking uphill	Lower
	Walking downhill	Lower
	Running	Lower
	Squatting	Lower
	Kneeling	Lower
	Standing up from floor	Lower and upper

(171)

* As asked in Benin.

(6)

APPENDIX 1

Correlation matrix of activities

Area	Activity	Upper extremity						
		Opening bottle with screw top	Cutting vegetables with a knife	Wash yourself	Cleaning yourself after going to the toilet	Putting on T-shirt	Tie a knot	Puring water from a bottle into a glass
Upper extremity	Cutting vegetables with a knife	0.757						
	Wash yourself	0.527	0.400					
	Cleaning yourself after going to the toilet	0.508	0.448	0.618				
	Putting on T-shirt	0.586	0.675	0.570	0.544			
	Tie a knot	0.621	0.716	0.680	0.716	0.893		
	Pouring water from a bottle into a glass	0.456	0.543	0.412	0.342	0.401	0.508	
Lower extremity	Running	−0.056	0.001	0.179	0.163	0.121	0.131	0.102
	Walking level ground	−0.176	−0.161	0.100	0.042	−0.171	−0.103	−0.166
	Walking uphill	−0.222	−0.196	0.010	−0.021	−0.213	−0.161	−0.172
	Walking downhill	−0.196	−0.179	0.058	0.013	−0.189	−0.128	−0.127
	Squatting	−0.148	−0.137	0.167	0.088	−0.147	−0.064	−0.034
	Kneeling	−0.064	−0.044	0.214	0.169	−0.062	0.007	0.016
Both extremities involved	Fetching water from pump	0.342	0.276	0.535	0.352	0.386	0.375	0.185
	Pound fufu	0.474	0.407	0.514	0.408	0.436	0.489	0.434
	Using a cutlass	0.489	0.432	0.530	0.348	0.462	0.518	0.454
	Carry harvest home	0.261	0.280	0.361	0.355	0.380	0.425	0.262
	Heave loads on head	0.401	0.411	0.503	0.412	0.440	0.493	0.470
	Standing up from floor	0.007	0.034	0.294	0.311	0.010	0.117	0.040

		Lower extremity						Both extremities involved				
		Running	Walking level ground	Walking uphill	Walking downhill	Squatting	Kneeling	Fetching water from pump	Pound fufu	Using a cutlass	Carry harvest home	Heave loads on head
Lower extremity	Walking level ground	0.569										
	Walking uphill	0.629	0.743									
	Walking downhill	0.641	0.774	0.889								
	Squatting	0.494	0.627	0.415	0.412							
	Kneeling	0.483	0.637	0.422	0.427	0.762						
Both extremities involved	Fetching water from pump	0.404	0.463	0.437	0.431	0.195	0.269					
	Pound fufu	0.325	0.161	0.093	0.200	0.154	0.157	0.569				
	Using a cutlass	0.297	0.201	0.146	0.183	0.115	0.126	0.518	0.614			
	Carry harvest home	0.580	0.409	0.458	0.420	0.269	0.323	0.611	0.501	0.394		
	Heave loads on head	0.299	0.178	0.118	0.217	0.204	0.143	0.605	0.675	0.583	0.430	
	Standing up from floor	0.277	0.480	0.286	0.251	0.571	0.619	0.184	0.289	0.092	0.273	0.161

APPENDIX 17: INSURANCE COVERAGE PROPOSAL

Draft translation from French:
Example of a coverage for a different clinical trial

The physical and moral persons who take the initiative of the biomedical research on human beings, named sponsor and/or financial donors as well as the principle and co-principle investigators, the study coordinators and other medical staff, and the persons responsible for the follow-up of research.

Protocol

Text gathering all the descriptive elements of a biomedical research and that specify the conditions in which the research must be conducted and managed.

Physical injury

All physical and mental "injury" suffered by a study participant as well as non-material prejudices arising from it.

***Defence* fees**

Charges and fees of the survey, the expertise, the instruction, the lawyer, the compliance with the enforcement of legal court decisions, as well as legal fees.

End of research

The biomedical research will be considered as closed from the moment when, in all the investigation sites, the last patient will receive the last medical act/observation planned by the protocol.

Object of the guarantee

The contract guarantees the Policyholder against the financial consequences of the civil liability that could be their responsibility due to physical harm caused to persons taking part in the biomedical research attributable to the research.

Exclusions

Besides the exclusions stated in the general conditions, the financial consequences of the civil liability attributable to the following cases are excluded :

- The research is not conducted under the direction or surveillance of a physician who can justify appropriate experience.
- The consent of persons who are taking part in the research is not collected.

APPENDIX 18: MATERIAL TRANSFER AGREEMENT

"Randomized controlled trial comparing efficacy of 8 weeks treatment with clarithromycin and rifampicin versus streptomycin and rifampicin for Buruli ulcer (*M. ulcerans* infection)" – Benin and Ghana (Trial ID number: T9-370-115)

- *Principal investigator: Professor Dr. Tjip van der Werf, University of Groningen, Netherlands*
- *Sponsor: World Health Organization (WHO)*

Schedule of Particulars

Subject to the terms and conditions of this **Agreement** (which expression means this Schedule and the Annexes I to III attached hereto), the **Provider** agrees to provide, and WHO accepts, the **Materials and Information** specified below to be used for such Purposes of Use as specified below.

In this **Agreement**, the following expressions shall have the following meanings:

1. **"Collection Site"** is the study site (hospital) in Benin and Ghana where patients are recruited and specimens are taken for the above clinical trial.
   (i) Agogo Presbyterian Hospital, Agogo, Ghana
   (ii) Tepa Government Hospital, Tepa, Ghana
   (iii) Nkawie Government Hospital, Nkawie, Ghana
   (iv) Dunkwa Government Hospital, Dunkwa, Ghana
   (v) Centre de dépistage et de traitement de l'ulcère de Buruli "Raoul et Madeleine Follereau" de Pobè, Benin

"Provider" is the local laboratory/center in Benin and Ghana that will receive **Specimens** from the **Collection Sites** and shipping them to the "**Repository".**

(i) Kumasi Center for Collaborative Research in Tropical Medicine (KCCR), School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (Contact person: *Ellis Owusu-Dabo*)

(ii) Laboratoire de Parasitologie – GEIHP, Centre Hospitalier Universitaire d'Angers 4, rue Larrey, 49033 Angers cedex 01, France (Contact person : *Laurent Marsollier*)

2. **"Repository"** is the place where the **Specimens** are stored and later distributed third parties.

   (i) Institute of Tropical Medicine, Antwerp, Belgium[1] for microbiological samples (Contact person: Professor Francoise Portaels)

   University of Groningen, Netherlands for blood and tissue samples samples (Contact: Professor Tjip van der Werf)

3. **"WHO"** is the World Health Organization, 20 Avenue Appia, CH-1211 Geneva 27, Switzerland which is the Sponsor of the study and legal owner of the Specimens.

4. "Specimens"

The **Specimens** sent by the **Provider(s),** stored by the **Repository** on behalf of **WHO,** hereunder, are as follows (see Annex II for a more detailed description):

- **Specimens** (blood, tissue and culture isolates) from patients diagnosed with Buruli ulcer (BU);
- The relating clinical, haematological, immunological, microbiological and histopathological;
- The relating and duly signed informed consents.

5. "Information"

Any information, unpublished or otherwise, owned by the **Provider** and shared with **WHO** during the exchange of this **Agreement** relating to the **Specimens,** their production, properties and/or experimental results observed using the specimens or any derivatives therefrom.

6. "Purposes of Use":

The **Specimens** are provided to contribute to a reference collection of human specimens aimed at facilitating Buruli ulcer research in the following areas:

(i) Laboratory confirmation of patients enrolled in the clinical trial;

(ii) Analyses of the isolates with the aim of determining drug resistance;

(iii) Research, development, testing and/or evaluation of new diagnostic tests and vaccines; for Buruli ulcer (BU);

(iv) Pathogenesis of Buruli ulcer and impact of new prophylactic/therapeutic strategies on Buruli ulcer.

(v) Host genetic susceptibility.

[1] (WHO Collaborating Centre for the Diagnosis and Surveillance of Mycobacterium ulcerans Infection: http://apps.who.int/whocc/Detail.aspx?cc_ref=BEL-40&cc_code=bel&cc_city=antwerp&

The **Repository** will distribute the **Specimens** on behalf of **WHO** to third parties upon request and subject to approval by **WHO**, for research areas specified above.

7. "Restrictions on Use":

The **Specimens** shall not be used for any purpose other than the **Purposes** of Use.

8. "Period of Agreement":

The **Specimens** will be kept for a least ten (10) years after which the remaining Specimens and Information may be destroyed upon the direction of **WHO** unless justification for extended storage is provided.

9. "Materials Charges":

Specimens will be provided free of charge. **WHO** will reimburse the **Providers**, within reasonable amounts, any expenses incurred in shipping the specimens to the **Repositories.**

10. "Annexes":

The Annexes attached hereto, i.e. the **General Conditions** (Annex I); the **Materials** description (Annex II) the **Material Request Form** (Annex III) and the **Material Release Form** (Annex IV), form an integral part of this **Agreement.**

Signed for and on behalf of WHO		**Signed for and on behalf of the "Provider"**
Name:	**Dr Lorenzo Savioli**	**Name:**
Title:	**Director, NTD**	**Title:**
Date:		**Date:**

Annex I

GENERAL CONDITIONS

1. Use

1.1 **Materials and Information** are supplied by the **Provide**r to **WHO** solely for the **Purposes of Use** and subject to the **Restrictions on Use** as set out in the **Schedule of Particulars.**

1.2 **WHO** shall allow only parties who are referred to in the Purposes of Use and who are bound by similar obligations of confidentiality and restrictions on use as contained in this Agreement to have access to **Materials and Information.** In this connection, WHO shall require the Repository to distribute the **Materials and Information** only in accordance with the directions and instructions from WHO, to third parties, having completed the **Material Release Form** attached hereto as Annex IV, whose request for use of the **Materials** has been approved by WHO, after having considered the recommendation of the **BU Clinical Trial Specimen Bank Review Committee.**

1.3 WHO shall require any party handling and/or using the **Materials and Information** to comply with all relevant national and local laws, rules and regulations applicable to the use of infectious substances and other biological **Materials.**

2. Confidentiality

2.1 The Information may incorporate confidential information of the **Institute.** Accordingly, if and to the extent any such Information is clearly marked by the I**nstitute** as "confidential", **WHO** shall during the term of this **Agreement** and for a period of five years following its termination, treat such Information confidential and only disclose it under like obligations of confidentiality and restrictions on use as those contained herein. W**HO** shall be deemed to have fulfilled its obligations, if it exercises at least the same degree of care in maintaining confidentiality as it would in protecting its own confidential information.

2.2 However, the above mentioned obligations of confidentiality shall not apply to information which

(i) can be shown to have been known to **WHO** at the time of its acquisition from the **Institute**; or

(ii) is acquired from a third party, not in breach of any obligation of confidentiality to the **Institute**; or

(iii) is independently devised or arrived at by, on behalf of, or for **WHO** without access to the **Information;** or

(iv) enters the public domain otherwise than by breach of the undertakings set out in this **Agreement.**

3. Rights

Subject to the other provisions of this Agreement, WHO shall be entitled to control, use, distribute, transfer and dispose of the **Materials and Information** for the **Purposes of Use** in any manner as WHO may in its sole discretion deem appropriate.

4. Identifiers

The **Institute** will remove all identifiers from the Materials and Information which could enable such **Materials and Information** to be traced back to any individual. The **Institute** will, however, provide **WHO** with clinical, haematological, immunological, microbiological and histopathological Information which will be recorded by or for WHO (see Annex II).

5. Warranties and Liabilities

5.1 The Provider makes no warranty of the fitness of the Specimens for any particular purpose or any other warranty, either express or implied.

5.2 **WHO** agrees the Provider has no control over the use that is made of the **Materials** or the **Information** by **WHO**, or parties collaborating with WHO in accordance with the terms of this Agreement. Consequently, **WHO** agrees the **Provider** shall not be liable for such use.

6. Miscellaneous

6.1 Nothing in this **Agreement** shall be interpreted as establishing a partnership between the parties or establishing one party as the agent of the other or conferring a right on one party to bind the other, except as may be specifically set out herein.

6.2 Any dispute relating to the interpretation or application of this Agreement shall, unless amicably settled, be subject to conciliation. In the event of failure of the latter, the dispute shall be settled by arbitration. The arbitration shall be conducted in accordance with the modalities to be agreed upon by the parties or, in the absence of agreement, with the rules of arbitration of the **International Chamber of Commerce.** The parties shall accept the arbitral award as final.

6.3 This **Agreement** sets forth the entire understanding between the parties and supersedes any prior agreements, written or verbal. It shall only be capable of change by written amendment executed by duly authorized officers of the parties.

This Agreement is duly signed on behalf of the parties as follow:

Signed for and on behalf of WHO		**Signed for and on behalf of the Provider**
Name:	**Dr Lorenzo Savioli**	**Name:**
Title:	**Director, NTD**	**Title:**
Date:		**Date:**

Annex II

MATERIALS DESCRIPTION

At least **450 patients** diagnosed with different clinical forms of Buruli ulcer (nodule, plaque, oedema, ulcer). The types and quantities of specimens according to the different clinical forms are as follows:

	Nodule	**Plaque**	**Oedema**	**Ulcer**
Plasma				
Serum				
Buffy coat				
Biopsy				
Culture				

1. The blood samples (plasma, sera and buffy coat) must have been kept at cold chain and transferred at a minus 70°C temperature.
2. The microbiological **Materials** (swabs and culture isolates) must have been kept in a cold chain and be stored and transferred at a minus 80°C temperature.
3. Biopsy **Materials** must have been stored in 10% formaldehyde.

The following documents must be attached to each **Specimen**:

1. The case report forms which summarize the main results of the clinical, biological and histopathological examinations of the participants.
2. The informed consent forms allowing the researchers to perform "further research" and duly signed by the participant.
3. In the case of children under 18 years old, the assent forms allowing the researchers to perform "further research" and duly signed by the participant.

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Annex III

For administrative use

MATERIAL REQUEST FORM
WHO
BURULI ULCER CLINICAL TRIAL
SPECIMENS
BANK

A. Requesting Party	
B. Contacts	
1. Name of the contact person	
2. Shipping Address	
3. E-mail address	
5. Tel	
7. Fax	

C. Specimens requested: Please indicate the appropriate quantities of samples requested by type and clinical form.

	Nodule	**Plaque**	**Oedema**	**Ulcer**
Plasma				
Serum				
Buffy coat				
Biopsy				
Culture				

D. The **Materials** are provided to contribute to a reference collection of human specimens aimed at facilitating Buruli ulcer research in the following areas: 1) laboratory confirmation of patients enrolled in the clinical trial; 2) analyses of the isolates with the aim of determining drug resistance; 3) research, development, testing and/or evaluation of new diagnostic tests and vaccines; for Buruli ulcer (BU); 4) pathogenesis of Buruli ulcer and impact of new prophylactic/therapeutic strategies on Buruli ulcer. 5) genetic susceptibility research. Describe the research for which you are requesting Clinical Specimens (give details and continue on a separate sheet, if necessary). Please reference any previously published or abstracted information concerning this assay:

E. The Specimens (hereinafter referred to as "the Material") and any information relating thereto (hereinafter referred to as "the Information") are provided on the following conditions:

1. The entity requesting and receiving the **Material and the Information,** hereinafter referred to as "the Receiving Party", will not permit the **Material and/or the Information**, or any part thereof, to come into the possession or control of any other entity or person, except those who are engaged in the above-mentioned research areas, under the supervision of the Receiving Party and who have accepted the same obligations in respect of the **Material and the Information** as set forth in this document.

2. The Receiving Party will use the **Material and the Information** exclusively for the purpose of the described above. Without the prior written authorization of WHO, the Receiving Party will not sell, or have sold, furnish or have furnished, such **Material and/or Information** to any third party. Except as explicitly provided in this **Material Request Form** (including Section D above), the Receiving Party will not, furthermore, use, or have used, such **Material and/or Information** in any way for the commercial production or sale of any products, or otherwise for commercial purposes.

3. Other than explicitly provided herein, this **Material Request Form** will not be construed as conveying to the Receiving Party any rights or title to the **Material and/or the Information.** The **Receiving Party** will treat the **Material and Information** as strictly confidential and proprietary to **WHO,** and/or persons or entities collaborating with WHO, and will disclose such **Material and Information** only under the same obligations of confidentiality and restrictions on use as those contained herein.

   Obligations of confidentiality will not apply to Information which the Receiving Party can show was in the public domain at the time of its acquisition hereunder, or becomes part of the public domain otherwise than by breach of the undertakings set forth in this **Material Request Form.**

4. The **Material** is not appropriate, nor intended, for use in humans.

5. **WHO** and persons and entities collaborating with WHO make no warranty of merchantability or fitness of the **Material or the Information** for any particular purpose or any other warranty, either express or implied.
   The **Receiving Party** agrees that **WHO** has no control over the use that is made of the **Material** and the Information by the Receiving Party. Consequently, the Receiving Party agrees that WHO shall not be liable for such use.
   Thus, the Receiving Party agrees to assume full responsibility for, and to hold WHO harmless from, any and all claims and liabilities resulting from or otherwise related to, the possession and use of the **Material** and/or the Information, as well as of **Materials** incorporating the **Material.**
6. The Receiving Party will ensure that the Material will at all times be used and handled in compliance with all relevant national and local laws, rules and regulations applicable to the use of biological Materials. The Receiving Party furthermore undertakes to comply with the Guidance on regulations for the Transport of Infectious Substances 2009-2010 – WHO/HSE/EPR/2008.106.

[6] http://www.who.int/csr/resources/publications/biosafety/WHO_HSE_EPR_2008_10.pdf

7. Any information provided by the **Receiving Party** to **WHO** under, or in connection with, this **Material Request Form**, will – if marked 'confidential' – be treated by WHO as confidential and proprietary to the Receiving Party, for a period of five years after the disclosure of such information to WHO. In this connection, **WHO** will only use and disclose such information (under similar obligations of confidentiality and restrictions on use as those contained herein) for the purpose of evaluating such information and determining (in WHO's sole discretion) the merit of releasing **Material** for the **Purposes** described above
   However, there will be no obligations of confidentiality and restrictions on use, to the extent that **WHO** is clearly able to demonstrate that the aforementioned information or any part thereof:

   (i) was known to WHO prior to their disclosure by the Receiving Party hereunder; or

   (ii) has been independently devised, or arrived at, by WHO without access to the disclosure made by the Receiving Party hereunder; or

   (iii) was in the public domain at the time of disclosure hereunder, or becomes part of the public domain through no fault of WHO; or

   (iv) becomes available to WHO from a third party, who is not in breach of any obligations of confidentiality owed to the Receiving Party

8. Prior to publication or presentation of any results using the **Material and/or Information**, the Receiving Party will provide WHO with a copy of such intended publication or presentation for the purposes of ensuring that it contains no disclosure of confidential and/or proprietary Information. Any objection to publication or presentation for the aforesaid reason will be notified by WHO to the Receiving Party within a period of sixty days of receipt of the draft copy. In the absence of such an objection within that sixty-day period, the publication or presentation may proceed. All such intended publications and presentations will contain an acknowledgement of WHO, the WHO BU Clinical Trial Specimen Bank, the Collection Sites and the Repository. The Receiving Party agrees to consult WHO with regard to giving appropriate acknowledgement as aforesaid, before such publication is published or presentation is made.

9. On completion of the research, the Receiving Party shall notify the result of the use of the **Materials and/or Information** to **WHO.**

10. The **Receiving Party** shall notify **WHO in** writing of any invention, improvement, modification, discovery or development made by the Receiving Party with respect to the **Material and/or Information.** The Receiving Party furthermore agrees that WHO and parties collaborating with WHO shall in any event entitled to receive samples of any **Material** derived from the **Material** for their own research and evaluation purposes.

11. The **Receiving Party** will ensure that any product resulting from the use of the **Materials and/or Information** will be safe and effective and manufactured in accordance with Good Manufacturing Practices.

12. The **Receiving Party** will use all reasonable efforts to ensure that the commercial exploitation of any product resulting from the use of the **Material** will be designed to achieve the following objectives in the following order of priority:

(i) the general availability of the product

(ii) the availability of the product to the public health sector in developing countries on preferential terms.

In connection with the foregoing, the **Receiving Party** will use all reasonable efforts to ensure that the product will be made available at cost to the public sector in developing countries that have reported probable BU cases to **WHO.**

On the request of **WHO,** the **Receiving Party** will present supporting documentation adequately justifying the proposed pricing structure.

13. On completion of the research, using the **Material**, the **Receiving Party** will cease to use any remaining quantities of the Material and the Information for any purpose and, at the direction of **WHO,** either destroy, or return to the Repository, all such remaining quantities of the M**aterial** and any and all copies of the Information.

14. Completion of the research, using the **Material** will not relieve the Receiving Party of any obligations under this **Material Request Form.**

15. Without the prior written consent of the other party, neither party will, in any statement, or **Material** of an advertising or promotional nature refer to the relationship of the other party to their collaboration pursuant to this **Material Request Form,** or to the relationship of the other party to the **Materials, Information** or any product resulting from their use. Any dispute relating to the interpretation or application of this Agreement will, unless amicably settled, be subject to conciliation. In the event of failure of the latter, the dispute will be settled by arbitration. The arbitration will be conducted in accordance with the modalities to be agreed upon by the parties or, in the absence of agreement, with the rules of arbitration of the International

Chamber of Commerce. The parties will accept the arbitral award as final.
If the foregoing terms and conditions are acceptable, please complete and return this document to the address mentioned below. Please note, however, that your signature of this document does not automatically imply that you will receive the **Material and the Information**, nor that you will receive the **Materials** in the quantity requested by you. Once your request has been approved by WHO, arrangements will be made for dispatch of the **Material** to you (of which arrangements you will be notified). You may wish to take a photocopy of this form for your records.

I certify that I have read and I accept the conditions listed above:

Signature of Receiving Party:	Date:
Name of Receiving Party:	
Title of Receiving Party:	
Name of Institute:	

I warrant that I, as the Responsible Administrative Authority of the Receiving Party, have the full authority to execute this Agreement and to thereby bind the Receiving Party:

Signature of the Responsible Administrative Authority:

Please return this form (**two** copies with original signatures) to: **Dr Kingsley ASIEDU,**
Global Buruli Ulcer Initiative
Department of Control of NTDs
World Health Organization,
1211 Geneva 27, Switzerland.
FAX: +41 22 791 4777

Approved quantities of specimens by WHO:

	Nodule	**Plaque**	**Oedema**	**Ulcer**
Plasma				
Serum				
Buffy coat				
Biopsy				
Culture				

Comments

Signature:
Name:
Title:
Date:

Annex IV

MATERIAL RELEASE FORM
WHO
BURULI ULCER CLINICAL TRIAL
SPECIMENS
BANK

A. Requesting Party	
B. Contacts	
1. Name of the contact person:	
2. Shipping address:	
3. E-mail address:	
4. Tel:	
5. Fax (optional):	

Approved quantities samples

	Nodule	**Plaque**	**Oedema**	**Ulcer**
Plasma				
Serum				
Buffy coat				
Biopsy				
Culture				

Comments:

WHO

Signature:
Name:
Title:
Date:

WHO – BU Specimen Bank
Review Committee

The aim of the review committee is to assist WHO to manage the use of human specimens (blood, biopsies, cultures) that will be collected and stored from the clinical trial: "Randomized controlled trial comparing efficacy of 8 weeks treatment with clarithromycin and rifampicin versus streptomycin and rifampicin for Buruli ulcer (*M. ulcerans* infection)"

In this context, specimens shall also referred to as Material or Samples, and Institute refers to the repository.

I. Roles

The roles of this committee are:

- To review the scientific merit of the requests from researchers for specimens and make recommendations to WHO;
- To assist WHO-GBUI in managing the specimens bank.

II. Composition

The committee will consist of three experts.

1. Professor Paul Johnson, (Austin Health, Melbourne, Australia)
2. Dr Richard Phillips (Komfo Anokye Teaching Hospital, Kumasi, Ghana)
3. Professor Pamela Small (University of Tennessee, USA)

III. Procedure

Requests may be sent either to the contact persons at the "Institute" or WHO, who will in turn provide a Material Request Form (MRF) to the "recipient" (by e-mail).

The committee will review requests by e-mail or via teleconference (if needed) and make recommendations for consideration by WHO. A note for the record shall be kept for deliberations on each request.

In case of approval, WHO shall specify the quantity of specimens that will be sent to the recipient, fill and sign the Material Release Form (last page of the Material Request Form). One original copy will be sent to the recipient and another copy forwarded to the Institution.

IV. Information management

All information on each specimen will be handled in strict confidence and will not be discussed, published, or otherwise distributed outside the members of the Review Committee.
The safe and reasonable handling of the material, as well as the legal and ethical rights and responsibilities of recipient, are detailed in the MRF, which is the only means by which researchers can obtain the Material.

World Health Organization

APPENDIX 19: NIH/NCI COMMON TOXICITY CRITERIA – VERSION 4.0

Toxicity type, and grading will be reported according to standards derived from NIH/NCI toxicity grading (https://webapps.ctep.nci.nih.gov/ctcv2/plsql/ctc000w$.startup)

List of possible side-effects of treatment with streptomycin, rifampin and/or clarithromycin, based on Common Toxicity Criteria (CTC), version 2					
	Grade				
Allergy:	0	1	2	3	4
Allergic reaction / hypersensitivity (including drug fever)	None	Transient rash, drug fever < 38°C (<100.4°F)	Urticaria, drug fever > 38°C (>100.4°F), and/or asymptomatic bronchospasm	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema	Anaphylaxis
Allergy/Immunology other (specify,..)	None	Mild	Moderate	Severe	Life-threatening or disabling
Auditory/hearing:					
Hearing	Normal	Hearing loss only when tested	Tinnitus or hearing loss, not interfering with daily activities	Tinnitus or hearing loss, interfering with daily activities	Severe unilateral or bilateral hearing loss (deafness)
Auditory/Hearing other (specify,….)					
Blood:					
Blood disorder (specify,…)	None	Mild	Moderate	Severe	Life-threatening/disabling

Constitutional symptoms:					
Fatigue (lethargy, malaise)	None	Increased fatigue over baseline, but not altering normal activities	Moderate (causing difficulty performing some activities)	Severe (loss of ability to perform some activities)	Disabling daily activities
Rigors, chills	None	-	Present	-	-
Constitutional symptoms other (specify,..)	None	Mild	Moderate	Severe	Life-threatening or disabling
Dermatology/ skin					
Bruising	None	Localized or in dependent area	Generalized	-	-
Flushing	Absent	Present	-	-	-
Rash/desquamation	None	Macular or popular eruption or erythema without associated symptoms	Macular or popular eruption or erythema with prutritus or other associated symptoms covering < 50% of body surface or localized desquamation or other lesions covering < 50% of body surface area	Symptomatic generalized erythroderma or macular, popular or vesicular eruption or desquamation covering > 50% of body surface area	Generalized dermatitis
Urticaria (hives, wheels)	None	Requiring no medication	Requiring PO or topical treatment or IV medication or steroids for < 24 hours	Requiring IV medication or steroids for > 24 hours	-
Discoloration of skin, urine, saliva, sweat, sputum or tears	None	Present	-	-	-
Dermatology/skin other					

(specify,….)					
Gastrointestinal					
Anorexia	None	Loss of appetite	Oral intake significantly decreased	Requiring IV fluids	-
Colitis	None	-	Abdominal pain with mucus and/or blood in stool	Abdominal pain, fever, change in bowel habits	Perforation or requiring surgery or toxic mega colon
Dehydration	None	Dry mucous membranes and/or diminished skin turgor	Requiring IV fluid replacement		
Diarrhea	None	Increase of < 4 stools / day over pre-treatment	Increase of 4-6 stools/day, or nocturnal stools	Increase of > 7 stools/day or incontinence; or need for parenteral support for dehydration	
Dyspepsia/heartburn	None	Mild	Moderate	Severe	-
Nausea	None	Able to eat	Oral intake significantly decreased	No significant intake, requiring IV fluids	-
Vomiting	None	1 episode in 24 hours	2-5 episodes in 24 hours over pretreatment	> 6 episodes in 24 hours or need for IV fluids	-
Gastrointestinal other, (specify,…)	None	Mild	Moderate	Severe	Life-threatening or disabling
Hepatic:					
Liver dysfunction/failure	Normal	-	-	Dysfunction present	Encephalopathy or coma
Jaundice	None	-	-	Present	
Hepatic other	None	Mild	moderate	severe	Life-threatening or

(specify,….)					disabling
Neurology:					
Ataxia (incoordination)	Normal	Asymptomatic but abnormal on physical exam, and not interfering with function	Mild symptoms interfering with function, but not interfering with activities of daily living	Moderate symptoms interfering with activities of daily living	Disabling
confusion	Normal	Confusion or disorientation or attention deficit of brief duration ; resolves spontaneously with no sequelae	Confusion or disorientation or attention deficit interfering with function, but not interfering with activities of daily living	Confusion or delirium interfering with activities of daily living	Harmful to others or self; requiring hospitalization
Neurology other (specify,…)	None	Mild	Moderate	Severe	Disabling
Pain					
Abdominal pain or cramping	None	Mild pain not interfering with function	Moderate pain; (= pain or analgesics interfering with function, but not interfering with activities of daily living)	Severe pain; (=pain or analgesics severely interfering with activities of daily living)	Disabling
Arthralgia (joint pain) or bone pain	None	Mild pain not interfering with function	Moderate pain	Severe pain	Disabling
Headache	None	Mild pain not interfering with function	Moderate pain	Severe pain	Disabling
Pain other					

(specify,….)					
Renal/genitourinary dysfunction (specify.....)	None	Mild	Moderate	Severe	Life-threatening or disabling
Sexual dysfunction (specify,….)	None	Mild	Moderate	Severe	Disabling

World Health Organization

APPENDIX 20: PROVISIONAL BUDGET ESTIMATE

Activities	Year 1	Year 2	Year 3	Total
Procurement, shipment and distribution of medicines	7,000	-		7,000
Personnel costs	64,320	67,536	70,913	202,769
Meetings	34,000	6,000	15,000	55,000
Training and active case-finding	36,200	36,200	30,200	102,600
Patients care costs	167,698	167,698	117,698	453,093
Institutional support	18,000	18,000	13,500	49,500
Communication	8,800	5,000	5,000	18,800
Equipment, transport and logistics	90,400	5,000	2,500	97,900
Monitoring and operational costs	51,000	52,000	44,850	147,850
Ethics and publications	7,000	-	2,000	9,000
Sub-total 1	**484,418**	**357,434**	**301,661**	**1,143,512**
Contingency 5%	*24,221*	*17,872*	*15,083*	*57,176*
Sub-total 2	**508,639**	**375,305**	**316,744**	**1,200,688**
WHO Programme Support Costs (13%)	*66,123*	*48,790*	*41,177*	*156,089*
Grand-total (USD)	**574,762**	**424,095**	**357,920**	**1,356,777**
Average per year				**452,259**

Author contributions

Click here to download Necessary additional data: author contributions.xlsx