CLINICAL
INVESTIGATION

Considerations on clinical trials of leprosy treatment: need of novel drug combinations

Considering that after 30 years of using multidrug therapy (MDT), leprosy eradication has still not been achieved, leprosy treatment must remain on the drug discovery agenda. Due to the complexities inherent in leprosy disease and the many methodological issues involved in clinical trials, the task of translating the bench findings into clinical practice has been arduous. While the effectiveness of reducing the currently recommended MDT remains controversial, a number of highly bactericidal antibiotics and immune-modulatory drugs have emerged as prospective candidates to improve patient adherence and quality of life, reduce adverse effects and prevent resistance. To replace the standard WHO-MDT, the new combination must be the shortest, simplest and, consequently, most affordable treatment possible.

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Keywords: clinical trial • drug combination • leprosy • multidrug therapy • relapse • resistance • surrogate end point • treatment

Leprosy is an infectious disease caused by *Mycobacterium leprae*, a slow-growing bacteria that infects Schwann cells and macrophages. This tropical disease, now considered neglected, is still present in more than 130 countries worldwide. Although its prevalence has been reduced over the past years, the detection rate of cases with permanent disabilities has remained stable at around 0.25/100,000 inhabitants [1].

M. leprae was one of the first agents linked to an infectious disease in the 19th century but the effective antibiotic, intravenous sulphone, only appeared in 1943 [2]. Soon afterwards, a new oral derivate called dapsone (diamino-diphenylsulphone [DDS]) became the standard chemotherapy treatment. Until 1982, sulfone monotherapy was the only validated treatment regimen for leprosy [3]. Upon the appearance of secondary DDS resistance in the 1970s, together with the ready availability of rifampin (RFM), a potent bactericidal drug, the use of combined regimens was recommended [4]. Several treatment combinations, mainly based on previously proven effective tuberculosis therapy, were proposed to combine with DDS, such as RFM, thioamide drugs and isoniazid, which is not active against *M. leprae*. Combined therapy was implemented by several National Programs. For instance, in Paraguay and Malta, Isoprodian® (175 mg of prothionamide, 50 mg DDS and 175 mg isoniazid) and RFM were extensively used with few reported relapse cases [5,6]. However, it was not until 1982 that the WHO Chemotherapy Study Group recommended the combined use of RFM and DDS with or without clofazimine (CLF) [7]. Implementation of this multidrug therapy (MDT), known as WHO-MDT, began in most endemic countries. WHO-MDT is the current standard treatment and continues to be widely administered.

The introduction of MDT at fixed doses brought about important advances in the control of the disease [8]. The accompanying implementation strategies assured drug supply to all endemic countries in the form of specific free-of-cost blister packs

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available for multibacillary (MB) and paucibacillary (PB) leprosy in separate presentations for children and adults. In addition, mobilization was encouraged to ensure that the infrastructure facilitating the delivery of healthcare services would be improved in the countries involved.

Why is there a need to develop new treatment regimens for leprosy?

Although the wide acceptance of WHO-MDT and the use of fixed-dose schemes have greatly contributed to important advances in leprosy treatment and the development of public health policies, their adverse effects have been considerable (see later section 'Principles of leprosy treatment'). In addition, current treatment is exceedingly long and reports of resistance against some of its component antibiotics are increasingly frequent. The general consensus is that new drugs are needed to develop a shorter, single treatment scheme [9–12]. A short scheme will certainly improve patient adherence and their quality of life. Increased compliance to treatments with new drug combinations may bring the additional benefit of reducing bacilli persistence and risk of resistance. However, a search for the terms 'leprosy' and 'treatment' in the WHO International Clinical Trials Registry Platform [201], containing data from the national clinical trial registries of several, often endemic, countries, showed no new studies. The few trials registered were all prior to 2009: a Phase III trial on the long-term use of CLF, the evaluation of the effect of body weight on drug concentrations and the effect of modified WHO-MDT schemes; suggesting a complete lack of initiatives and financing of research in the field.

Principles of leprosy treatment
■ Cure definition parameters
In any clinical trial, the assessment methods used to measure patient responses to the treatment under investigation need to be very well defined and reliable [13]. However, in the case of leprosy many difficulties are encountered. The disease presents as a spectrum of clinical forms that develop according to the particular immunological response to the agent [14]. In addition, the long incubation period required by *M. leprae* equally demands a long period for bacterial clearance. The excellent adaptation to the host favors bacterial persistence in tissues even after completing regular treatment with standard WHO-MDT [15]. On occasions, there is no positive correlation between clinical and microbiological end points. Thus, defining objective and comparable parameters to evaluate the therapeutic effect in all patients is a cornerstone of clinical trials in leprosy.

The direct clinical benefits of leprosy treatment are hard to measure via assessment of the health condition of the patient based solely on observation and the interpretation of the disappearance or improvement of skin lesions and peripheral nerve involvement. Further complexity is added by the presence of reactions, such as immunoinflammatory events that may likely complicate the course of the disease and treatment. Reactions may manifest as inflamed skin patches or diffuse nodules with or without nerve tenderness and enlargement, or systemic manifestations, and may occur before, during, and after treatment [16]. Successive reactions may lead to the killing of bacteria by various mechanisms that lead to the production of cytokines and chemokines. During leprosy reactions, the development of an acute inflammatory process, which occurs parallel to the stimulation of cellular immunity induces the production of pro-inflammatory mediators such as interleukins, IFNγ and TNFα. This whole process contributes to the destruction of bacteria and exposure of large amounts of antigens [17], aided by the bactericidal activity of macrophages through the production of inducible nitric oxide synthase [18]. Furthermore, the use of steroids to control the reactions may affect the access of the antibiotics to the inflamed tissues in a way similar to the one affecting the entrance of antibiotics through the blood–brain barrier [19]. Corticosteroids, due to their anti-inflammatory action, inhibit the production of pro-inflammatory cytokines, consequently reducing bacterial destruction. However, no effect has been observed on bacterial clearance or killing by the use of concomitant steroids and MB WHO-MDT [20].

In the absence of a reliable clinical end point, the use of surrogate end points is recommended [21]. Bacterial index (BI) and morphological index (MI) have been used as biomarkers for MB leprosy [22,23]. Data from longitudinal cohort studies and clinical trials have shown that reductions in BI and MI result in direct clinical benefits to the patients in terms of reducing the frequency of reactions [24,25] and, as such have been used to predict the effect of therapy.

Studies of mycobacterial metabolism are used as markers of bacterial viability and, therefore, of the bactericidal effects of drugs [26]. These *in vitro* assays are expensive and time-consuming, requiring large quantities of bacteria that can only be obtained after passage in the mouse foot-pad. The main drawback is that results are only obtained after a year has elapsed [27]. Other biomarkers such as the measurement of anti-phenolic glycolipid-I antibody levels have been proposed as indicators for monitoring treatment since a decline is observed during and after treatment [28]. However, not all patients have antibodies, and discordant findings are frequent, especially among patients in reaction [29].

Which parameter can best reflect the effect of a therapeutic intervention in PB patients in whom these

biomarkers cannot be used? Is the antibiotic effect best measured in these cases only by sign and symptom resolution? Since bacterial eradication from the tissues is extremely hard to measure, there is still a need to define a characteristic that can be objectively measured and evaluated as an indicator of the pharmacologic responses to an intervention in cases with no detectable acid-fast bacilli. New methods to determine the viability of *Mycobacteria* such as the real-time PCR, which permits the quantification of *M. leprae* RNA and DNA obtained from tissue samples and the detection of as few as 30 bacilli should preferably be used to measure therapeutic effect [30].

Overall, to date, no standardized, reproducible and consistent end point has been used to evaluate the meaning of a cure in leprosy. Thus, clinical, laboratory and genetic markers still require validation.

■ Drug combinations

As in other bacterial diseases, drug combinations are recommended in leprosy to increase efficacy and reduce development of resistance and complications. Besides bactericidal drugs, treatments include weak bactericidal or bacteriostatic antibiotics with an additional anti-inflammatory effect, due to the possible development of reactions during the course of the disease.

The duration, safety, efficacy, acceptability, simplicity and cost are all important elements to be taken into consideration in antibacterial treatments. Feasibility seems to have been considered key in deciding the makeup of the current schemes. The supervised monthly 600 mg RFM and 300 mg CLF doses with daily self-administered DDS and 50mg CLF is the standard recommended treatment for MB leprosy. The same scheme but without CLF is administered during a consecutive 6-month period to PB individuals [31]. Interestingly, CLF, a drug with a 70-day half-life, is given daily. RFM, the only bactericidal antibiotic with a 3–5 hour half-life, is administered in monthly doses. However, monthly RFM is recommended during adjunct corticosteroid therapy due to drug interaction between RFM and prednisone [32]. Furthermore, RFM is best absorbed after a period of fasting, whereas food increases absorption of CLF [33], although both drugs are given simultaneously in supervised doses.

Moreover, serious safety considerations need to be considered, specifically regarding DDS, which is the MDT component most often associated with adverse effects [34,35]. In general, side effects of WHO-MDT range from gastrointestinal distress to hemolytic anemia and the DDS hypersensitivity syndrome, which can be severe and even life-threatening [36,37]. The DDS hypersensitivity syndrome is a severe idiosyncratic reaction is now considered to be a drug-induced reaction with

eosinophilia and systemic symptoms [38] and requires immediate discontinuation of treatment. Overall, the lethality rate of DDS hypersensitivity is 10%, resulting in a condition of great concern [37].

■ Relapse

Similar difficulties as to define established cure in leprosy are encountered when defining relapse criteria. Relapse is defined as the occurrence of new signs and symptoms of the disease in a patient who has successfully completed an adequate course of MDT [39]. The relapse rate has also been utilized as an outcome to assess the effectiveness of therapeutic regimens in leprosy.

Most relapse cases are explained by the persistence of live *M. leprae* in various tissues in MB leprosy and in the nerves in PB leprosy [40]; however, in hyperendemic areas, re-infection cannot be excluded. Haldar *et al.* found a 2.6 higher risk of relapse in patients living with active leprosy cases[41], while Rocha *et al.* observed that 31% of the 145 cases with relapse had had relatives who were diagnosed within the 5-year period prior to relapse diagnosis [42].

Various researchers agree there is a subset of MB patients, particularly those at the lepromatous pole of the spectrum and those with a high bacterial burden, who are at substantial risk for relapse [42–45]. Other risk factors include inadequate therapy and immunosuppression [22,46–48].

While relapse in MB patients is relatively easy to clinically recognize by the presence of active skin lesions and an increased BI, in PB cases it is often difficult to distinguish relapse from reversal reaction. As a result, there are wide variations in relapse rate estimations after establishment of 'cure' criteria by the current WHO-MDT policy [22]. These estimations range from zero in the 502 patients of the AMFES cohort in Ethiopia after a follow-up period of up to 8 years after completion of fixed-dose MDT [43], 1.84% in an 18-year follow-up period of 163 patients in India [49] to 2% in patients treated with up to 2 years of WHO-MDT [45,50]. Since most studies report less than 1%, relapse in MB patients is considered to be very low as a result of nearly 30 years of the widespread use of MDT [39]. However, this is virtually a statement of 'absence of evidence', which by no means indicates 'evidence of absence' [51].

In many reports, relapse rates are contradictory and the long surveillance period needed for it to occur in MB leprosy is not always taken into account [52]. Most of these studies were held in reference centers with well-supervised, highly regular MDT [47]. Good patient adherence favors treatment efficacy. However, in the field, defaulter rates and misclassifications of a single or a few skin lesions in MB and PB cases may occur [46]. In addition, insufficient treatment may be given

when DDS is withdrawn after the occurrence of adverse effects, at which time CLF or RFM are then administered as monotherapy [53]. Consequently, operational problems may lead to higher relapse rates.

Since MDT does not destroy all the bacilli, the remaining bacteria will need to be killed and eliminated by an effective immune system, mainly by macrophages [54].The presence of active bacteria is the strongest stimulus to induce and maintain leprosy reactions. Thus, patients with a high bacterial burden, who are both at higher risk of relapse and of suffering recurrent reactions [55], would definitely benefit from a new generation of highly bactericidal MDT.

■ Resistance

Emerging drug resistance has been observed against the RFM and DDS, basic components of the WHO-MDT regimen and the ofloxacin (OFL), which is one of the second-line bactericidal drugs [48,56,57]. Rapid DNA-based molecular assays have been developed to allow DDS-resistant strains (*folP I* gene) along with RFM-resistant (*rpoB* gene) and quinolone-resistant (*gyrA* and *gyrB*) *M. leprae* strains to be detected [58]. The WHO project for Global Surveillance of Drug Resistance came at a most opportune time in 2008 in light of the wide disparity among countries and even among different regions within the same country regarding investigative approaches, management and collection of relapse data and patient samples [59]. At present, little information can be obtained from the vast majority of endemic countries, clearly indicative of relapse under reporting and weak monitoring capacity of the project [60].

Clinical trials with standard WHO-MDT

The initial MB treatment was given during a minimum 2-year period and until skin smears became negative. After proven efficacy was established by various reports (Table 1), the WHO Study Group on Chemotherapy of Leprosy suggested a standard 24-month WHO-MDT regimen [4,8].

Although no clinical trials monitored relapse rates of the recommended regimens due to epidemiological and operational factors, further reduction in the duration of MB treatment was suggested [52]. The long duration of the MB leprosy treatment was neither viable nor affordable for most leprosy control programs thus, in 1998, the Seventh Expert Committee recommended reducing MB-MDT to 12 months [61]. Given the fact that the number of MB cases detected had reduced and that many of the patients were skin-smear negative at diagnosis, this decision was accepted [62].

Since current recommendations for MDT follow a fixed duration of treatment regardless of skin lesion characteristics or acid-fast bacilli eradication,

BI reduction has been used to compare the relative efficacy of treatment regimens of different duration. In Brazil, a strategic trial compared the WHO-MDT of fixed duration among 213 MB patients. The mean BIs and reaction rates of the patients who received 12 doses (n = 128) were similar to those treated with 24 doses (n = 85) of MDT [63]. This study and others were interpreted to indicate that the reduction to 12 doses did not compromise MDT effectiveness [45,64].

There is some evidence of the reduction of leprosy reactions with the use of WHO-MDT, although some studies are contradictory and incidence rates are very variable. A study in Brazil reported that 35 out of 70 patients had reactions while on WHO-MDT and 77% of the 70 patients had reactions during treatment with 3 months of daily RFM-DDS followed by 21 months of 100 mg daily DDS. The proportion difference was found to be statistically significant and this was considered to be due to the inclusion of CLF in WHO-MDT [65]. However, even with the use of two drugs, with some anti-inflammatory effect reactions continue to complicate the course of the disease during and after completion of both PB and MB MDT schemes [16,66].

To increase compliance and drug supply logistics a further reduction in number of doses and length of treatment, and uniform therapy for all patients was recommended by the WHO Technical Advisory Group at its third meeting [13]. The uniform-MDT regimen consists of 6 months of MB WHO-MDT for all new cases regardless of clinical form or bacterial status. A multicenter trial is in progress in India and China [67]. Other controlled trials have likewise been initiated in India [68] and Brazil [69] (Table 2). Preliminary results suggest that uniform MDT does not adequately treat patients with MB leprosy [10]; and PB patients suffer significantly more adverse effect related to hematological alterations than PB patients treated with WHO-MDT [70].

However, some countries, such as the USA do not follow WHO recommendations and have different guidelines. The US National Hansen's Disease Program recommends the use of daily rather than monthly RFM and for a longer period of time than WHO-MDT: 24 months for MB and 12 months for PB patients [71]. Several researchers also favor this scheme [22].

Revision of various trials developed to define the current WHO-MDT schemes (Tables 1 & 2) reveals a considerable degree of heterogeneity and variability in effectiveness. The study samples are for the most part variable, have very different inclusion criteria, are too small in size and too stratified, all of which reduce the ability to identify outcome differences among the groups studied while making comparisons more difficult to interpret. The interventions, drug dosages and combinations, treatment durations and outcomes are

Table 1. Clinical trials on the development of standard multidrug therapy for leprosy: drugs combinations, doses and duration.

Author (year)	Design (locality)	Final n/ recruited n (% male)	Inclusion criteria	Treatment (n)	Primary outcome (period of evaluation)	Secondary outcomes	Results	Ref.
Pattyn et al. (1975)	Open RCT (Morocco)	93/129 (72)	Adults, untreated, biopsy MI > 0.10, not TB	RFM 450 mg/d (25) vs RFM 900 mg/wk (25) vs CLF 300 mg/wk (21) vs DDS 100 mg/d (22) × 3 mo inpt + DDS 100 mg/d (93)	Biopsy and nasal smear BI and MI, clinical improvement (1, 2, 3, 6 mo)	Weight, ESR, ENL, clinical complications	RFM 450/d = RFM 900/wk > DDS > CLF More ENL, not significant after weekly RFM	[116]
US Leprosy Panel (1975)	Open matched RCT (Philippines)	21/24 (86)	Untreated LL/ BL, wo significant disease or ENL, past the age of puberty, high BI	RFM 600 mg/d (15) vs DDS 100 mg/d (9) 6 d/wk/1 yrs, inpt, fb aceDDS 225 mg/12 wk or DDS 50 mg/d as outpt	Mouse foot-pad inoculations, clinical assessment, BI reduction, LBI (4, 12, 24 wk)	ENL frequency and severity	ML viability reduction: RFM > DDS Bacterial indices, clinical improvement and ENL (48 w): DDS = RFM	[117]
US Leprosy Panel (1976)	Open RCT (Philippines)	36/48 (69)	Untreated LL/ BL, wo significant disease or ENL, past the age of puberty, high BI	1-CLF 200 mg/d 6 d/wk vs 2-CLF 100 mg 3 d/wk vs 3-CLF 300 mg/wk vs 4-CLF 600 mg/2 wk vs 5-CLF 600 mg 2 d/mo × 24 wk, fb 6-CLF 200 mg/d, 6 d/wk, or 7-DDS 100 mg/d × 24 wk	Clinical assessment, BI reduction, LBI (36 and 48 wk), mouse inoculations (8, 16, 24 wk)	ENL frequency and severity, skin pigmentation, toxicity	Bactericidal: 1 = 2 > 3 > 4 = 5; ENL: 1 = 2 = 3 = 4 = 5, 7 > 6; Pigmentation of the skin: 1 >> 2–5 No toxicity	[118]
Yawalkar et al. (1982)	International MC single blinded RCT (Brazil, India, Senegal)	93 (73)	Untreated LL, BI > 3+, no pregnancy, anemia, low platelets, TB, alcoholism, renal/ liver disease	RFM 450 mg/d + DDS 50 mg/d (47) vs RFM 1200 mg/mo (supervised) + DDS 50 mg/d (46) × 6 mo	CI, skin smear and nose-blow BI, MI zeroing, histopathology (2, 4, 6 mo)	ARs ENL	CI: RFM 1200> RFM 450; skin smear MI: RFM 1200 > RFM 450 at 6 mo; nose-blow smear MI: RFM 450 > RFM 1200 MI: RFM 1200> RFM 450; BI and LBI: RFM 1200 = RFM 450; ENL: RFM 1200 < RFM 450	[119]

The authors also compared the effect of regimens with ofloxacin but these were not considered for this table as the interest is on WHO-multidrug therapy dose reduction from 2- to 1-year duration.

Allocation to DDS 100 mg/d + RFM 600 mg/d + RFM 500 mg/d + PRT 500 mg/d × 2 yr regimen was stopped after only 12 pts because of hepatotoxicity.

aceDDS: Acedapsone; AFB: Acid-fast bacilli; AR: Adverse reaction; BI: Bacteriological index; BL: Borderline lepromatous; CLF: Clofazimine; CI: Clinical improvement; d: Day; DDS: Dapsona; ENL: Erythema nodosum leprosum; ESR: Erithrocyte sedimentation rate; ETH: Ethionamide; fb: Followed by; Int: Indeterminade; inpt: Inpatients; INZ: Isoniazide; LBI: Logarithmic biopsy index; LL: Lepromatous; MB: Multibacillary; MC: Multicenter; MI: Morphological index; ML: *Mycobacterium leprae*; mo: Months; NA: Not available; outpt: Outpatients; PRT: Prothionamide; pts: Patients; RCT: Randomized controlled trial; RFM: Rifampin; RR: Reversal reaction; sdo: Single dose; TB: Tuberculosis; TCZ: Thiacetazone; wk: Week; wo: Without; yr/s: Year/s; yo: Years old; zeroing: Become zero.

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Author (year)	Design (locality)	Final n/ recruited n (% male)	Inclusion criteria	Treatment (n)	Primary outcome (period of evaluation)	Secondary outcomes	Results	Ref.
Reyes-Javier (1983)	Open trial (Philippines)	25 (88)	Skin smear MI > 10%, BI > 4+, not pregnant, anemia, low platelets, TB, alcohol, renal/liver disease	RFM 1200 mg/mo supervised + CLF 100 mg 3 d/wk + DDS 50 mg/d × 6 mo	Dermatological and peripheral nerves examination, skin smear and skin biopsy BI and MI (2, 4, 6 mo)	Tolerability	Moderate/marked CI: 96%; skin smears MI approximately 0% at 6 mo 15% reduction in BI Moderate/marked histological improvement: 48%	[120]
Pattyn et al. (1989)	Open trial (Anjouan – Comores and Burundi) 1981–1984	216/231 (NA): 109 untreated 107 treated DDS/DDS– CLF, 16 DDS resistant	Included if agreed to stay in or near the center for 12 mo, or capable of coming to the center daily	RFM 600 mg/d + ETH 500 mg/d + DDS 100 mg/d (except Sundays) × 8 wks, fb RFM 600 mg/w + ETH 500 mg/d + DDS 100/d × 44 wk vs RFM 600 mg/d + ETH 500 mg/d + CLF 100 mg/d × 8 wk + 44 wk RFM-ETH-CLF as above	Clinical, neurological and histopathological improvement, disability, skin smears and biopsy BI decrease, solidly staining AFB in biopsy (12 mo for 2–6 yrs)	Side effects, reactions, relapse	No relapses, hepatitis: 5% RFM/ETH/DDS = RFM/ ETH/CLF Reactions: RFM/ETH/DDS = RFM/ ETH/CLF Disability score worsening: RFM/ETH/ CLF > RFM/ETH/DDS	[121]
Pattyn et al. (1989)	Open trial, randomly allocated (Zaire, Rwanda) 1981–1984	289/356 (100) untreated or previously treated < 5 yrs, vs all pts treated ≥ 5 yrs	MB = BI > 2+ (three sites), or skin, agreement to stay near center for 6 mo, able to visit daily	RFM 600 mg/d+ ETH 500 mg/d + DDS 100 mg/d × 6 mo (6 d/ wk) fb DDS 100 mg/d × 6 mo, vs idem fb ETH 500 mg/d + DDS 100 mg/d × 6 mo vs previously DDS treated pts ≥ 5 yrs: same schemes but CLF 100 mg/d for DDS	As above (12 mo for 2–6 yrs)	Relapse, ENL, RR	No relapse Hepatotoxicity: 5% RFM/ETH/DDS = RFM/ ETH/CLF	[122]

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Author (year)	Design (locality)	Final n/ recruited n (% male)	Inclusion criteria	Treatment (n)	Primary outcome (period of evaluation)	Secondary outcomes	Results	Ref.
Cellona et al. (1990)	Open RCT (Thailand, Philippines, Korea) 1979–1988	358 (78): Thailand: 97; Philippines: 128; Korea: 133	15–60 yo, IB > 4+, no life-shortening diseases Group 1: new pts; Group 2: relapsed and previously DDS	Group 1 (202): DDS 100 mg/d × 5 yrs (all) + RFM 1200 mg sdo (97) vs + RFM 600 mg/d × 4 wk (32) vs + RFM 1200 mg sdo + CLF 100 mg 3 d/wk × 24 wk (39) vs + RFM 1200 sdo + PRT 375 mg/d × 8 wk (34) Group 2 (156): CLF 100 mg 3 d/wk × 5 yrs (127) +RFM 600 mg/d × 4 wk (83) vs + RFM 600 mg/d × 2 wk (16) vs+ RFM 1200 mg/mo × 24 wk (28) vs RFM 600 mg/d × 4 wk fb RFM 600 mg 2 d/mo × 5 yrs + PRT 375 mg/d × 8 wk, fb TCZ 150 mg/d × 5 yrs (29)	BI, clinical improvement, histopathological changes, ENL, toxicity, mouse foot-pad drug sensitivity tests (6, 12, 18, 24, 36, 48, 60 mo)	Acceptability, field practicability, cost	BI, clinical and histopathology improvement: no significant differences among regimens in group 1 and 2 ENL frequency and severity: group 2 CLF << RFM/PRT/TCZ < group 1 Toxicity: only with PRT and RFM	[123]
Thomas et al. (1990)	Open RCT (India)	177/210 (87)	≥12 yo LL (78%) BL (18%) Int (4%) BI ≥ 2.5+	RFM 12 mg/kg/d + INZ 300 mg/d + CLF 100 mg/d + DDS 10 mg/kg/wk × 3 mo fb DDS + CLF 5 yrs (88) vs CLF 100 mg/d + DDS 10 mg/kg/wk × 5 yrs (89)	Clinical progress, bacteriological investigation, correlation of clinical score and BI (3, 12, 60 mo)	Drug regularity, reactions	RFM/INZ/CLF/ DDS = CLF/DDS	[124]
Jadhav et al. (1992)	Open RCT (India)	88 (82)	Recently diagnosed MB BI+	RFM 600 mg/d × 9 mo fb RFM 600 mg/mo + DDS 100 mg/d + CLF 50 mg/d × 2 yrs (47) vs RFM 600 mg/mo + CLF 300 mg/mo + DDS 100 mg/d + CLO 50 mg/d × 2 yrs (41)	Mean BI reduction (6, 12, 18, 24 mo)	Neurological assessment, hepatitis, ENL	BI mean reduction: daily RFM > monthly RFM ($p < 0.01$) ENL: daily RFM > monthly	[125]

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too diverse to be reliably analyzed. Significantly, there is, in fact, no clear evidence that the treatments evaluated are any different from each other in most of the trials reported.

Other authors have also pointed to the difficulties encountered in attempting to draw precise conclusions from these studies. After evaluating interventions for leprosy according to type of evidence, consistency, quality, directness and effect size, Smith and Saunderson classified studies about the effects of treatment on clinical improvement and/or relapse rates as either low or very low [72]. Thus, although widely accepted by most leprosy control programs, evidence demonstrates that any estimate of their effect is uncertain and that further research might change the estimate of that effect.

Clinical trials with non-WHO-MDT drugs

Several drugs not included in standard WHO-MDT have been recommended for treatment of leprosy patients. Screening of antibiotics known to be safe and well tolerated for their *in vitro* bactericidal effect against *M. leprae* [73,74], such as some fluoroquinolones, tetracyclines and macrolides, made possible the formulation of new regimens that have been tested in several trials (Table 3). Franzblau and White compared the *in vitro* activities of 20 fluoroquinolones against *M. leprae* and reported that OFL had excellent bactericidal activity, whereas pefloxacin and temofloxacin could have potential for treating clinical leprosy [73]. Further studies comparing different antibiotics reported that sparfloxacin and temofloxacin were highly bactericidal, while OFL, pefloxacin and lomefloxacin were the least bactericidal among the quinolones [75]. Thus, microbiological surrogate markers of disease resolution were not all consistent prior to clinical trials that followed (Tables 3 & 4).

As for the MDT trials, most of the studies demonstrating the effect of the new regimens consist of case reports, case series and field trials [52,76,77]; however, many have weak evidence of efficacy, leading to contradictory findings [78–80]. Nonetheless, suggestions of new regimens were made. At present, OFL, clarithromycin, and minocycline (MIN) are already being used as second-line antileprosy drugs for cases of adverse reactions or resistance to the standard medications [12]. However, MIN, like other tetracyclines, is not indicated for children or pregnant women.

The short course chemotherapy for tuberculosis was found to require two or more bactericidal agents [81]. Applying this concept to leprosy, treatment reduction included the use of additional bactericidal antibiotics, OFL and MIN, to RFM. Thus, single-dose RFM, OFL and MIN (ROM) was recommended to treat single skin lesion PB cases [82]. This recommendation assumes that the host response, considered efficient

Table 1. Clinical trials on the development of standard multidrug therapy for leprosy: drugs combinations, doses and duration (cont.).							
Author (year)	Design (locality)	Final n/ recruited n (% male)	Inclusion criteria	Treatment (n)	Primary outcome (period of evaluation)	Secondary outcomes	Results	Ref.
Subcommittee on Clinical Trials of the Chemotherapy of Leprosy/ THELEP (1996)	Open RCT (Bamako [Mali], Chingleput [India] 1977–1983	203/215 Bamako 90/99 (100%) Chingleput 113/116 (90)	Untreated MB	DDS 100 mg/d + RFM 600 mg/d + CLF 100 mg/d × 2 yrs (39) vs RFM 1500 mg/sdo + DDS 100 mg/d × 2 yrs (44) vs RFM 1500 mg/ sdo + DDS 100 mg/d × 2 yrs + CLF 100 mg/d × 3 mo (38) vs DDS 100 mg/d + RFM 600 mg/d + PRT 500 mg/d × 2 yrs (12 ×) vs DDS 100 mg/d × 2 yrs + RFM 900 mg/wk + PRT 500 mg/d × 3 mo (43)	Detection of persisting *ML*, bacteriological status (skin-smears and nose-blows BI, LBI, number of *ML/g* tissue), clinical and histopathological changes, adverse effects (3, 12 and 24 mo)	Hematologic changes, hepatic function, urinary tract function, weight loss, ENL, voluntary withdrawal	Persisting *ML* 7.8% (did not vary with center, regimens or duration of treatment), BI decrease: mean annual: 75%, LBI by a mean of 87% and the *ML/*tissue a mean rate of 69%, number of AFB: 69% ENL less severe and frequent in DDS/ RFM/CLF × 2 yrs in Chingleput	[126]

The authors also compared the effect of regimens with ofloxacin but these were not considered for this table as the interest is on WHO-multidrug therapy dose reduction from 2- to 1-year duration.
Allocation to DDS 100 mg/d + RFM 600 mg/d + PRT 500 mg/d × 2 yr regimen was stopped after only 12 pts because of hepatotoxicity.
aceDDS: Acedapsone; AFB: Acid-fast bacilli; AR: Adverse reaction; BI: Bacteriological index; BL: Borderline lepromatous; CLF: Clofazimine; CI: Clinical improvement; d: Day; DDS: Dapsona; ENL: Erythema nodosum leprosum; ESR: Erithrocyte sedimentation rate; ETH: Ethionamide; fb: Followed by; Int: Indeterminade; inpt: Inpatients; INZ: Isoniazide; LBI: Logarithmic biopsy index; LL: Lepromatous; MB: Multibacillary; MC: Multicenter; MI: Morphological index; *ML*: *Mycobacterium leprae*; mo: Months; NA: Not available; outpt: Outpatients; PRT: Prothionamide; pts: Patients; RCT: Randomized controlled trial; RFM: Rifampin; RR: Reversal reaction; sdo: Single dose; TB: Tuberculosis; TCZ: Thiacetazone; wk: Week; wo: Without; yr/s: Year/s; yo: Years old; zeroing: Become zero.

Table 2. Clinical trials on the development of standard WHO multidrug therapy for leprosy: definition of dose and duration.

Author (year)	Design (location)	Final n/recruited n (% male)	Inclusion criteria	Treatment (n)	Primary outcome (period of evaluation)	Secondary outcomes	Results	Ref.
Ponnighaus et al. (1995)	Not-blinded randomized (Malawi)	305 (NA)	Untreated patients BI ≥ 2+, or treated MB relapse cases	MDT × 18 mo (157) vs MDT × 30 mo (148)	Relapse, BI slit-skin smear (variable)	Reaction disability	No relapse at mean follow up 33.9 mo (18 do), 38.2 mo (30 do)	[64]
Kroger et al. (2008)	International MC open trial	1008/2912 (59)	Newly diagnosed untreated PB (1777) and MB (1135), not PN	MB WHO-MDT × 6 mo	Relapse, clinical assessment, impaired nerves (1, 2, 3 y)	Hepatitis reactions	Relapse: 6/218, CI: PB > MB (p < 0.001) Four deaths due to hepatitis	[67]
Fajardo et al. (2009)[†]	Initially double-blind RCT (Philippines)	189/230 (77.8)	BI and LBI ≥ 2+ at ≥ 1 sites, 15–65 yo, no prior RFM or FLQ, nor long reaction	WHO-MDT × 1 y (64) vs WHO-MDT × 2 y (28)	Relapse (annually for 12 y)	Clinical assessment, BI and mouse foot-pad inoculation	One relapse case in 1 y WHO-MDT	[45]
Rao et al. (2009)	Open comparative trial	64/127 (69)	PB (32) MB (32)	PB WHO-MDT (14) MB WHO-MDT (22) vs MB WHO-MDT (28)	Clinical and histopathological assessment, skin smears (6, 12, 18, 24 mo)	Reactions, adverse events, tolerance	CI: PB U-MDT > PB WHO-MDT (0.2195) MB WHO-MDT >> MB U-MDT (0.0064)	[68]
Shen et al. (2012)	Open MC clinical trial (China, India)	89/166 (75)	Newly diagnosed, positive BI, not pregnant	MB WHO-MDT × 6 mo	Relapse, reaction mean BI, from 2003 to 2007 (6, 18, 30, 42 mo)	–	BI reduction: 0.06/mo Reaction: 29%	[127]
Penna et al. (2012)	MC open RCT (Brazil)	613/859 (59.4)	Newly diagnosed PB/MB, returning defaulters and relapse treated >5 y earlier, aged >5 to <66 yo	PB/MB WHO-MDT (290) vs MB WHO-MDT × 6 mo (323)	Clinical assessment, reaction, impaired nerves, from 2007 to 2012 (6, 12, 18, 24 mo)	Slit-skin smear in MB pts	Reaction in patients with BI < 3: U-MDT > MB-MDT (p = 0.04) at 6–18 mo	[69]

†The authors also compared the effect of regimens with ofloxacin but were not considered for this table as the interest is on WHO-MDT dose reduction from 2- to 1-year duration.

BI: Bacteriological index; CI: Clinical improvement; do: Doses; FLQ: Fluoroquinolone; LBI: Logarithmic biopsy index; MB: Multibacillary; MC: Multicenter; MDT: Multidrug therapy; mo: Months; NA: Not available; PB: Paucibacillary; PN: Pure neural; pts: Patients; RCT: Randomized controlled trial; RFM: Rifampin; U-MDT: Uniform MDT; WHO-MDT: Standard multidrug therapy; y: Years; yo: Years old.

Table 3. Clinical trials with monotherapy for leprosy.

Author (year)	Design	Number of patients (% male)	Clinical form (n)	Drugs	Scheme	Improvement criteria (period)	Outcome	Ref.
Grosset et al. (1990)	Randomly allocated into two groups	17/21 (76)	LL (19) BL (2)	PFL OFL	sdo PFL 800 mg fb PFL 800 mg/d from d 7–56 fb 4 mo PFL 800 mg/d + WHO-MDT (n = 9) vs sdo OFL 400 mg (n = 8) fb OFL 400 mg/d for 50 d fb 4 mo OFL 400 mg/d + WHO-MDT	Nude mouse inoculation, changes in skin lesions, reactions, AR (d 7, 14, 28, 56 + monthly until d 180)	CI: PFL = OFL BI reduction in PFL (p < 0.05) MI reduction: PFL >> OFL best after MDT (p < 0.001) Sdo: no effect bac act: PFL = OFL Adverse effects: mild Monotherapy = MDT	[128]
Gelber et al. (1992)	Open clinical trial	8 (88)	LL (5) BL (3) Relapses (2)	MIN	MIN 100 mg/d for 3 mo	Clinical and bacteriological and laboratory tests, BI and MI (7 d, 1, 2, 3 mo)	CI: 75% pts had complete resolution at 3 mo No reaction bac act: 100% wo viable ML at 3 mo	[75]
Chan et al. (1994)	Open clinical trial	9 (89)	wo RR or ENL	SPR	400 mg single loading dose + 200 mg/d × 12 wk	Clinical, BI, MI, PGL-I, oxi, mouse infectivity (2, 4, 6, 8, 10, 12 wk)	CI after 2 wk, 95% by 12 wk RR in two pts slight BI decrease MI = 0% at 4 wk PGL-I stable bac act > 99.9% at 4 wk	[129]
Chan et al. (1994)	Open clinical trial	9 (100)	LL (6) BL (3) BI > 4+ MI > 1%	CLR	3 g/d sdo + 1 g/d from d 8–21 + 0.5 g from d 22–96	Clinical, BI, MI, PGL-I, oxi, mouse infectivity (1, 3, 5, 8 wk)	Significant CI at 4 wk, BI reduction at 8 wk and MI reduction at 2 wk Reaction one case bac act > 99.9% at 3 wk	[92]
Franzblau et al. (1994)	Open clinical trial	9 (78)	BL (2) LL (7)	Fusidic acid	500 mg/d × 8 wk (n = 5) vs 750 mg/d × 4 wk (n = 4) + 500 mg/d × 4 wk (n = 9)	Idem	Active, slowly bactericidal, 500 mg = 750 mg regarding PGL-I, PCR	[93]
Fajardo et al. (2004)	Open RCT	22 (NA)	LL (18) BL (4)	PFL OFL	sdo PFL 800 mg + PFL 800 mg/d from d 8–56 (n = 11) vs sdo OFL 800 mg + OFL 400 mg/d from day 8–56 (n = 11), fb 2 years WHO-MDT (all pts)	Clinical improvement, reactional states and adverse events, BI, MI, ML viability (d 7, 14, 28, 56)	CI: PFL > OFL, reactions: PFL 2 cases ENL, OFL one case N Mild side effects: PFL = OFL bac act: PFL = OFL	[89]
Pardillo et al. (2008)		8 (100)	LL (7) BL (1) BI > 3.5	MXF	MXF 400 mg sdo + MXF 400 mg/d from d 8–56 After d 56, all pts WHO-MDT	Clinical improvement, BI and MI in skin smears, mycobacteria viability (d 7, 14, 28, 56)	Significant killing (p < 0.006) with sdo, no viable ML at d 28–56 Fast definite CI, ENL one case self-cured	[94]

AR: Adverse reactions; bac act: Bactericidal activity; BI: Bacterial index; BL: Borderline lepromatous; CI: Clinical improvement; CLR: Clarithromycin; d: Day; ENL: Erythema nodosum leprosum; fb: Followed by; LL: Lepromatous; MDT: Multidrug therapy; MI: Morphological index; MIN: Minocycline; ML: Mycobacterium leprae; mo: Months; MXF: Moxifloxacin; N: Neuritis; NA: Not applicable; OFL: Ofloxacin; oxi: Oxidation by radiorespirometry; PGL-I: Phenol glycolipid I; PFL: Pefloxacin; pts: Patients; RCT: Random clinical trial; RR: Reversal reaction; sdo: Single dose; SPR: Sparfloxacin; WHO-MDT: Standard multidrug therapy; wk: Week; wo: Without.

in the tuberculoid forms of the disease, will eliminate any residual viable bacteria. However, not all single lesions are PB [83], and not all bacterial organisms in a lesion are metabolically active, so dormant mycobacterium in the tissues would not be affected by this drug combination [84]. Would it not be more profitable to look for a sterilizing activity in the drug combination, eliminating both active and dormant organisms? Although initially believed to be promising given that ROM would dramatically reduce treatment duration and increase acceptability, it has not been shown to be superior to the current MDT [85,86].

It is only recently that results have begun to be published regarding multicenter studies that had been recommended by the Steering Committee on the Chemotherapy of Mycobacterial Diseases and sponsored by the WHO/THELEP, to test these antibiotics in leprosy (Table 4). Different OFL regimens were compared against standard MB WHO-MDT in Brazil. A total of 23 relapses were diagnosed in 114 MB patients after a 7-year period of surveillance. Most of the relapses (83%) were registered among the 49 patients who received OFL plus RFM [42]. A well-designed and -reported double-blinded randomized trial evaluated the use of ROM in PB patients with two to five lesions. Relapse rates were significantly higher in patients treated with ROM (1.13 person/year) than in patients who received PB WHO-MDT (0.35 person/year) after a period of 3 years (p = 0.001; 95% CI: 1.6–7.2) [87].

Most of the reports listed in Tables 3 & 4 identify the measured variables, observational methods and criteria used to assess the therapeutic response. The myriad and sometimes profound differences in types of assessments, duration periods, sequential analyses of patient progress, length of time to end point, and measured outcomes render them incomparable. Although several studies randomly assigned the patients to the several treatment groups [42,79,88,89], very few explain the randomization process or provide a power statement [87,90]. Either no information on the comparable characteristics of the volunteer participants at intake was included, or the study groups were significantly different upon enrollment in some of the key end points evaluated. The study by Ji *et al.* is a case in point: the mean patient MI in the group receiving ROM was significantly smaller than the one receiving OFL–MIN (p = 0.001) [74].

Early bactericidal activity trials were developed to test various antibiotics [27,78,79,91–94]. Other studies were developed to test the drugs in small samples of patients. However, Phase I and II clinical trials require a larger sample size than those, in order to adequately determine the efficacy of intervention [95]. Thus, the observations of most of the studies are limited and their power of inference is low. If trials are not well designed from the beginning, the result is weak evidence for efficacy and the costly Phase III and IV trials are in vain.

Is it possible to translate the existing preclinical trials of potential antileprosy agents into viable treatments for patients?

Although there is little in terms of novel therapies being investigated for future treatments, preclinical studies have been developed for several new drugs such as dialkyldithiocarbamates, bipyridyl analogs, diarylquinolines and ansamycins, already tested for other mycobacteria. The macrolide derivatives roxithromycin and fosfomycin have anti-inflammatory, immunemodulatory in addition to anti-*M. leprae* activities [96], which is an advantage in antileprosy treatment.

The findings related to screening *in vitro* and in animal models are not always translated into clinical practice, as has been observed with regard to fusidic acid. *In vitro* experiments demonstrated that fusidic acid is highly bactericidal [97]; but in a Phase II trial, it was only weakly bactericidal [93]. Likewise, Ji *et al.* observed bactericidal activity in mice after 1 month of MDT (99.95%) were similar to that with a single dose of RFM, clarithromycin, OFL and MIN (99.4%) [74]. However, single-dose ROM treatment has been insufficient in humans [87].

Nonetheless, several promising drugs that have been screened in preclinical trials against *M. leprae* could offer interesting results for leprosy. Levofloxacin was found to have a twofold greater bactericidal activity than OFL and exhibited synergistic activity with rifabutin and other rifamycin analogs against *M. leprae* [98]. Other quinolones such as lomefloxacin, WIN 57273 and temafloxacin, are fully bactericidal [75]; but their effect on leprosy patients has yet to be studied. Similarly, moxifloxacin (MXF) and rifapentin (RFP) showed higher bactericidal effect than RFM [99], while the effect of gatifloxacin and linezolid was comparable to that of RFM and could be used in combination without antagonism between them [100]. Bedaquiline is a diarylquinoline with bactericidal activity against *M. leprae* comparable to that of MXF and RFP [101]. It has been found to have sterilizing activity in animal models of tuberculosis, being bactericidal against both actively metabolic and dormant mycobacteria [102].

Although many of these antibiotics are costly and only used in third-line treatments for other diseases, if an ultra-short or single-dose scheme is to be developed for leprosy, or a subgroup of patients with high bacterial loads is the focus, these drugs need to be considered.

Especially important in leprosy therapy in which a drug combination is required, is the evaluation of interactions between the different drugs. Preclinical trials need to be developed with drugs combinations prior

Table 4. Reports of randomized clinical trials with drug combinations different from standard WHO-multidrug therapy.

Author (year)	Number of patients (% male)	Clinical form (n)	Drugs	Scheme	Improvement criteria (period)	Outcome	Ref.
Ji et al. (1993)	36	LL BL	CLR MIN	MIN 100 mg/d vs CLR 500 mg/d vs CLR 500 mg/d + MIN 100 mg/d × 56 d	Clinical and bacteriological and laboratory tests BI and MI, mouse infectivity (14, 28, 56 d)	CI and bac act: MIN = CLR = MIN/CLR AR were rare and mild	[130]
Ji et al. (1994)	24 (79)	LL (20) BL (4)	OFL	OFL 400 mg/d (n = 8) vs OFL 800 mg/d (n = 8) vs OFL 400 mg/d + DDS 100 mg/d + CLF 50 mg/d + CLF 300 mg/ mo (n = 8) × 56 d	Clinical and bacteriological and laboratory tests BI and MI (14, 28, 56 d)	CI, BI, MI and bac act: OFL 400 = OFL 800 = OFL/ DDS/CLF ENL: two cases in OFL/DDS/CLF Mild side effects: in 50% of cases	[79]
Ji et al. (1996)	50 (74)	LL (22) BL (28) high BI	CLR MIN OFL	MB WHO-MDT × 1 mo (n = 10) vs sdo RFM 600 mg (n = 10) vs DDS 100 mg/d + sdo CLF 100 mg + CLF 50 mg/× 1 mo (n = 10) vs sdo CLR 2 g + MIN 200 mg (n = 10) vs sdo CLR 2 g + MIN 200 mg + OFL 800 mg (n = 10)	Clinical, BI, MI, mouse infectivity, reactions	CI same all groups, BI reduction: MB WHO-MDT > RFM = DDS/CLF = CLR/MIN = CLR/MIN/OFL bac act: MB WHO-MDT = RFM >DDS/CLF = CLR/MIN = CLR/ MIN/OFL (p < 0.01) Reactions: CLR/MIN/OFL > MB WHO-MDT > RFM = DDS/ CLF mild side effects: CLR/MIN = CLR/MIN/OFL > MB WHO-MDT = RFM = DDS/CLF	[131]
Single-lesion Multicentre Trial Group (1997)	1381/1483 (NA)	PB single skin lesion	OFL MIN RFM	sdo ROM vs PB WHO-MDT × 6 mo	Clinical improvement, histopathology	12 treatment failures Mild side effects in both groups	[132]
Ji et al. (1998)	20 (89)	LL (14) BL (6)	RFM OFL MIN	sdo OFL 100 mg + MIN 400 mg (n = 10) vs ROM (n = 10)	CI, BI and MI in skin smears, viability (7 d)	CI, BI: OFL/MIN = ROM bac act: ROM > OFL/MIN (p < 0.01) ENL: two cases in ROM	[74]
Ura et al. (2007)	26 (NA)	BL LL	RFM OFL MIN	2 yr MDT (n = 14) vs ROM × 2 yrs (n = 12)	Clinical, BI, histopathology	No difference in CI, BI, histological parameters, ENL frequency	[133]
Girdhar et al. (2011)	300 (40) 15% children	PB single lesion	CLR	sdo ROM (n = 151) vs sdo ROM + CLR 500 mg (n = 149)	Complete healing of lesion Relapse (5 yr)	Healing: >90% at 24 mo ROM = CLR/ROM Relapse: ROM = CLR/ROM	[90]

AR: Adverse reactions; bac act: Bacterial activity; BB: Borderline borderline; BI: Bacterial index; BL: Borderline lepromatous; BT: Borderline tuberculoid; CI: Clinical improvement; CLF: Clofazimine; CLR: Clarithromycin; d: Day; DDS: Diethyl diaminosulfone; ENL: Erythema nodosum leprosum; LL: Lepromatous; MB: Multibacillary; MI: Morphological index; MIN: Minocycline; mo: Months; OFL: Ofloxacin; PB: Paucibacillary; RFM: Rifampin; ROM: RFM 600 mg/mo + OFL 400 mg/mo + MIN 100 mg/mo; sdo: Single dose; spv: Supervised; WHO-MDT: Standard multidrug therapy; yr/s: Year/s.

Table 4. Reports of randomized clinical trials with drug combinations different from standard WHO-multidrug therapy (cont.).						
Author (year)	Number of patients (% male)	Clinical form (n)	Drugs	Scheme	Improvement criteria (period)	Outcome according to authors	Ref.
Manickam et al. (2012)	1359/1526 (48)	PB 2–5 lesions	RFM OFL MIN	PB WHO-MDT (n = 764) vs sdo ROM + DDS-placebo/d + RFM-placebo/mo × 6 mo (n = 762)	Clearance of skin lesions Relapse rate AR	Lesion clearance: ROM = PB >40% at 6 mo and 72% at 36 mo Relapse: ROM>PB WHO-MDT (p = 0.001)	[87]
Cunha et al. (2012)	114/198	BT (7), BB (45) BL (78) LL (42)	OFL	1 (n = 53) vs 2 yr MDT (n = 27) vs 1 yr MDT + OFL 400 mg/d/1 mo spv (n = 55) vs RFM 600 mg/d + OFL 400 mg/d × 1 mo spv (n = 63)	Relapse rate up to 7 yr follow up	RFM + OFL (39%) > 1 yr MDT (4%) = 1 yr MDT + OFL (p < 0.001)	[134]

AR: Adverse reactions; bac act: Bacterial activity; BB: Borderline lepromatous; BI: Bacterial index; BL: Borderline lepromatous; BT: Borderline tuberculoid; CI: Clinical improvement; CLF: Clofazimine; CLR: Clarithromycin; d: Day; DDS: Diethyl diaminosulfone; ENL: Erythema nodosum leprosum; LL: Lepromatous; MB: Multibacillary; MI: Morphological index; MIN: Minocycline; mo: Months; OFL: Ofloxacin; PB: Paucibacillary; RFM: Rifampin; ROM: RFM 600 mg/mo + OFL 400 mg/mo + MIN 100 mg/mo; sdo: Single dose; spv: Supervised; WHO-MDT: Standard multidrug therapy; yr/s: Year/s.

to the development of clinical trials. For instance, in the case of telithromycin, a ketolide used for community acquired pneumonia and upper respiratory tract infections, combination with RFM reduces its plasmatic concentration [202]. Drug interactions bring added complexities and challenges to the trials not encountered by those of monotherapy; for example, they can be associated with more adverse effects. The design and interpretation of combination therapy studies need to take these factors into consideration.

Conclusion

Various issues related to the design of clinical trials for antibacterial drugs against leprosy were observed in the revised literature. The methods adopted to assess patient responses to treatment as well as end point measures need to be well defined, reliable, and facilitate comparative studies.

Unmet needs

Antibacterial treatment does not exhaust the therapeutic need to treat leprosy complications such as reactions and disability. Accessibility to therapy remains a major issue in many endemic countries, especially in those with low prevalence rates. The lack of an efficient surveillance system capable of detecting relapse, drug resistance and defaulters is a matter of concern in view of the public health risk posed by the likelihood of continued transmission of infection by these cases. The development of effective drug schemes to be used for chemoprophylaxis also needs to be addressed.

The availability of second-line drugs is not uniformly and regularly distributed in endemic countries as most treatments are supported by national governments or nongovernmental organizations. As a consequence, many MB leprosy patients may have received insufficient MDT treatment after presenting adverse side effects from one of the three drugs used in the MDT standard regimen. This is a worrisome scenario as several reports have documented relapses and identified drug-resistant strains of *M. leprae*. No new regimen trials are taking place for these cases. Therefore, after introducing untested short-term treatment regimes, it is imperative to record the number of relapse cases and identify the *M. leprae* strains involved.

The nerve function deterioration that may occur after completion of WHO-MDT [103] has generated distrust regarding the efficacy of MDT. It is difficult for local health workers and patients to accept being pronounced cured after release from treatment if nerve impairment continues to progress and successive reactions require further medical care. Some authors have argued that 'cure rates' should be replaced by 'treatment completion rates' [60]. Considering the current

WHO target for reduction of deformities by the year 2020, there is an urgent need to intensify research to develop an effective treatment and address nerve function impairment head on.

Recommendations

Cure involves much more than the killing of bacteria and the disappearance of skin lesions and peripheral nerve involvement. A person affected by leprosy has other medical needs that may or may not be the result of *M. leprae* infection, but are a product of disability, social or cultural background. For future trials, it would be desirable to have a well-defined and reliable clinician-reported outcome instrument in addition to biomarkers for PB patients.

Known treatments have been proven to effectively kill circulating bacteria but not persistent ones. To achieve sterilizing activity in patients remains one of the biggest challenges in developing useful regimens for leprosy. Persister organisms are a fraction of an antibiotic-treated bacterial population that are refractory to killing without becoming genetically resistant [104]. Recent advances in mycobacterial pathogenesis demonstrate the ability of some species to induce modifications in the host cell to improve the niche and ensure dissemination [105], as well as to modify their adaptative response to the pressures exerted by prolonged drug exposure [106]. These epigenetic effects may also be present in *M. leprae*.

Trials with an adequate sample size that adhere to the principles of good clinical practice [13] and include the well-established PB and MB WHO-MDT regimens as controls are required to have good evidence of the effect of new drug combinations. Noninferiority trial designs could be a resourceful option for testing short schemes in leprosy and they may be more feasible than superiority trials. Efficacy decisions need to be based on consistent clinical and bacteriological improvement and take relapses into account. The intent-to-treat correction could be used to protect against bias and strengthen study conclusions. The use of placebo in addition to the standard MDT to test against other combination therapies would also strengthen the observations. It is recommended that protocol designs follow the Standard Protocol Items: Recommendations for Interventional Trials 2013 statement to ensure the designs contain the requisite information for critical appraisal and interpretation [107].

Better end points should also be designed for clinical trials in leprosy. New molecular tests that could determine the viability of relatively small bacterial numbers would be of particular interest. Comparing the amounts of *M. leprae* RNA and DNA in tissue samples, such as the 16S rRNA/RLEP ratio, has already been proven effective in determining bacterial viability [30]. These molecular tests are less time-consuming and expensive than traditional *in vitro* bacterial metabolism analyses. Other promising surrogate end points could be the serological biomarkers currently under investigation. Several *M. leprae* recombinant proteins (LID-1, ML2028, ML0286 and ML2038) elicit an antibody response in both PB and MB cases while the response rapidly declines after completion of MDT even in patients with a high initial BI [23].

Treatment of mycobacterial infection in the nerves is still far from satisfactory. Understanding the pathogenesis of *M. leprae*-induced nerve injury may pave the way toward new pathways in leprosy therapeutics. For example, trastuzumab, a humanized monoclonal ErbB2 antibody, has been proposed as a potential agent as *in vitro* studies demonstrated it was effectively able to block the binding of *M. leprae* to Schwann cells [108].

Any considerations regarding new antibiotics also need to address their ability to cross the blood–nerve barrier and their interaction with antireactional drugs. It is well established that drug concentrations in the tissues vary according to the presence or absence of inflammation. In the course of a CNS infection, drug concentrations found during early infection and its resolution are different from those found when the meninges are inflamed. In addition, penetration and concentration may be affected by other drugs. Molecular size, lipophilicity, plasma protein binding and active transport affect the penetration of anti-infectives into the cerebrospinal fluid and brain tissue [19]. Thus, it is highly desirable to obtain effective antibiotic concentrations in the nerve compartments, not only of inflamed, but also of normal nerve tissue. Carefully designed experiments are needed to assess the pharmacokinetics of antibiotics at the blood–nerve barrier and their pharmacodynamic properties in the nerve tissue. This knowledge may help to improve the treatment of *M. leprae* nerve infection.

Instead of only targeting the infectious agent, a different potential therapeutic approach might involve the induction of a strong adaptive immune response in the patient to limit the infection and promote healing, for example, by prescribing vitamin D [109].

All of the above-cited elements need to be considered in the implementation of new treatment regimens. Furthermore, since the general trend is to unify and shorten the present regimens, the probability of increasing complications post-MDT should be measured. Many studies with promising positive results have not yet been implemented while others have not been published. The cost and operational factors involved in new treatment regimens must be considered, keeping

in mind the priority of early case detection, especially if zero transmission is to be achieved.

In agreement with other authors that suggest the use of highly bactericidal agents to replace bacteriostatic DDS or CLF [52], a more bactericidal MDT definitely needs to be used in the treatment of MB cases with a high bacterial index. Several potential candidate drugs are already in the market. The combination of RFP, MXF and MIN has been the most potent anti-*M. leprae* drug scheme found so far. MXF presents the advantage of having good penetration in skin macrophages, especially in infected ones [110], and has already been trialled for combination therapy in tuberculosis [111]. Finally, a recently approved drug for the treatment of resistant tuberculosis, bedaquiline [203], or the long known telithromycin could also be tried in combination regimens. The use of highly bactericidal antibiotics could also allow for a considerable reduction of treatment duration for the other forms of leprosy.

Nevertheless, among the drugs currently in use, CLF, in spite of only being bacteriostatic against *M. leprae,* possesses anti-inflammatory and immunosuppressive properties that render it still of interest [112]. It is used for chronic inflammatory diseases such as pyoderma gangrenosum [113] and systemic lupus erythematosus [114]. However, it reduces the macrophage half-life, which might lead to reduction of bacterial clearance, and produces minor adverse effects. Still, several analogs have shown improved properties and reduced adverse effects that could be used in the drugs combination [115].

The Nippon Foundation and Novartis currently provide financial support for WHO-MDT supply and will continue to do so until 2020 with additional donations and logistics support from the Novartis Foundation for Sustainable Development [204]. Until that time, alternative drug combinations need to be sufficiently screened and evaluated. To be of advantage to replace the standard WHO-MDT, the new combination must be the shortest possible, as well as a simple and, consequently, affordable treatment.

Future perspective
The use of new, highly bactericidal antibiotics in combination with drugs that induce a strong adaptive immune response may prove effective to finally reach sterilizing effect against *M. leprae*. Furthermore, the inclusion of novel serological biomarkers and molecular testing will be helpful in proving the absence of both circulating and persister organisms in patients treated with these new treatment regimens, thereby reducing the need for long follow-up periods for confirming their effect.

Executive summary

Principles of leprosy treatment
- Leprosy is caused by *Mycobacterium leprae* infection and the particular immunological response elicited, producing a spectrum of clinical forms and immune reactions.
- The current established cure parameter for leprosy imposes many difficulties for defining comparable, clear-cut end points for clinical trials.

Unmet needs
- Validated surrogate end points to evaluate the effect of new regimens in clinical trials such as serological and molecular biomarkers are needed.
- Defining objective and comparable parameters to evaluate the therapeutic effect in all patients is a cornerstone of clinical trials in leprosy.
- To date, no standardized, reproducible and consistent end point has been used to evaluate the meaning of a cure in leprosy.

Recommendations
- Although little is being investigated in terms of novel therapies for the treatment of leprosy, there are few drug combinations that could be explored in well-designed clinical trials that follow the Standard Protocol Items: Recommendations for Interventional Trials 2013.
- Short new schemes need to include a combination of two or more highly bactericidal drugs that also have an immune/anti-inflammatory effect, such as rifapentin and moxifloxacin, in combination with drugs that induce a strong adaptive immune response.
- Long surveillance periods after trials of uniform and standard multidrug therapy must be ensured in order to allow firm conclusions regarding relapse rates.
- New regimens are required for patients, especially children, who are resistant to or do not tolerate any of the drugs in the current multidrug therapy.

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