Mechanisms of action and therapeutic efficacies of the lipophilic antimycobacterial agents, clofazimine and bedaquiline

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Summary

Drug-resistant (DR)-tuberculosis (TB) is the major challenge confronting the global tuberculosis (TB) control programme, necessitating treatment with second-line anti-TB drugs, often with limited therapeutic efficacy. This scenario has resulted in the inclusion of Group 5 antibiotics in various therapeutic regimens, two of which promise to impact significantly on the outcome of the therapy of DR-TB. These are the “re-purposed” riminophenazine, clofazimine, and the recently approved diarylquinoline, bedaquiline. Although they differ structurally, both of these lipophilic agents share cationic amphiphilic properties, which enable them to target and inactivate essential ion transporters in the outer membrane of *Mycobacterium tuberculosis.* In the case of bedaquiline, the primary target is the key respiratory chain enzyme, F_1/F_0-ATPase, while clofazimine is less selective, apparently inhibiting several targets, which may underpin the extremely low level of resistance to this agent. This review is focused on similarities and differences between clofazimine and bedaquiline, specifically in respect of molecular mechanisms of antimycobacterial action, targeting of quiescent and metabolically-active organisms, therapeutic efficacy in the clinical setting of DR-TB, resistance mechanisms, pharmacodynamics, pharmacokinetics, and adverse events.

Key words: Anti-inflammatory activity; bacterial sub-populations; diarylquinolines; early bactericidal activity; F_1/F_0-ATPase; potassium transporters; multidrug-resistance; *Mycobacterium tuberculosis*; resistance mechanisms; riminophenazines.

Introduction

Tuberculosis (TB) remains a major public health problem globally, killing more people than any other infectious disease.[1] In 2013, approximately nine million active TB patients and 1.5 million TB-related deaths were reported.[2-4] The two major factors underpinning this global public health crisis include the ongoing TB pandemic driven by human immunodeficiency virus (HIV) coinfection and the associated alarming increase in drug-resistant (DR)-TB, increasing the transmission of *Mycobacterium tuberculosis (Mtb)* and mortality from the disease.[2,5-7] In 2013, approximately 480 000 new multidrug-resistant (MDR)-TB cases and 210 000 deaths were reported worldwide with a very large proportion of these (60%) originating from Brazil, Russia, India, China and South Africa (BRICS).[2,8]

Unlike drug-susceptible (DS)-TB, the treatment of DR-TB is complicated, often resulting in poor treatment outcomes, which vary according to the resistance profiles of the infecting strains to the constituent drugs in the various regimens. For instance, treatment of MDR-TB has been successful in less than 44% of patients,[9,10] in comparison to the 95% success rate attained in the case of DS-TB cases.[2,11] In the setting of complicated MDR-TB, such as patients infected with MDR-plus-, pre-extensively drug-resistant (pre-XDR)-, XDR-, or totally drug-resistant (TDR)-TB strains of *Mtb*, the recommended regimens yield even poorer results, achieving treatment success rates of around 11%, with associated high mortality rates of about 73%.[6,11,12] Treatment outcome of TB/HIV coinfection is also poor, with an associated mortality rate of 70%.[8,13] Notwithstanding, the drug resistance profiles of the infecting *Mtb* strains, the outcome of chemotherapy in this setting is dependent on additional factors including the severity of the two diseases, as well as drug-drug interactions

(DDIs) between the anti-infective agents used in the treatment of the two diseases.[2,12]

The WHO regimen recommended for the treatment of DR-TB consists predominantly of several, under-researched and highly toxic, second-line antibiotics and is administered for a minimum of 18 months.[14] The progression of treatment involves a 6 – 8-month intensive phase of administration of at least four second-line drugs, which include newer fluoroquinolones (levofloxacin and moxifloxacin), an injectable agent (kanamycin), prothionamide, and cycloserine or para-aminosalicylic acid, in addition to pyrazinamide, followed by a 12 month continuation phase with at least four oral drugs.[7,11,15,16] Depending on the clinical and bacteriological responses, treatment duration can be extended if necessary.[17] However, the duration of therapy and/or the composition of the drug regimen may have to be revised due to development of drug toxicity.

Questionable efficacy and/or unacceptably high toxicity of the recommended DR-TB drug regimen prompted the WHO to formulate an alternative strategy in an attempt to overcome treatment failure. This was based on the inclusion of Group 5 antibiotics in the regimens of those DR-TB patients who experience treatment failure. Group 5 antibiotics consist of “re-purposed” older agents such as clofazimine, linezolid, amoxicillin plus clavulanate, imipenem plus cilastatin, and clarithromycin, as well as the new drugs, bedaquiline and delamanid.[9,10,16,18-20] However, as with the drugs which comprise the recommended WHO DR-TB regimen, Group 5 antimicrobial agents also have limitations as most have incomplete information in respect of their antimicrobial efficacies against mycobacterial subpopulations, DDIs,

safety, and mechanism(s) of action.[19] The availability of such data is essential in formulating new, more effective treatment regimens, which should be less amenable to development of resistance by acting on multiple targets, including those located in the cell membrane.[21]

This review is focused on two Group 5 anti-TB agents, clofazimine and bedaquiline, that promise to impact significantly on improving the efficacy and shortening the duration of therapy of DR-TB. Both clofazimine and bedaquiline are the prototypes of different classes of lipophilic, antimycobacterial agents, having predicted logP (logarithm of partition (P) coefficient in Poctanol/Pwater) lipophilicity values of 7.39 and 6.37, respectively.[22,23] Both drugs are operative at the level of the cell membrane *Mtb*, targeting the proton motive force (PMF).[24] In addition to comparing and contrasting the mechanisms of antimycobacterial action of clofazimine and bedaquiline, their antimicrobial spectrums,and activities against mycobacterial subpopulations, other topics covered include: i) overviews of the efficacy of recently developed DR-TB regimens containing either bedaquiline or clofazimine; ii) mechanisms of development of drug resistance; iii) effects on eukaryotic cells; and iv) the pharmacokinetic, pharmacodynamic and adverse event (AE) profiles of each agent.

Clofazimine

Background

Clofazimine is a riminophenazine antibiotic, originally developed for the treatment of TB.[25] Despite its impressive antimicrobial activity against *Mtb* isolates *in vitro,* clofazimine monotherapy was unsuccessful in earlier studies undertaken in higher primates and humans, while skin discolouration with associated mental disturbances, including depression, was also a deterrent to clinical application.[26] Poor treatment outcomes with clofazimine also coincided with the discovery of the first-line anti-TB agents, pyrazinamide and ethambutol in 1952 and 1961, respectively, both of which showed better therapeutic efficacy and fewer side-effects, surpassing clofazimine as preferred agents for the treatment of TB. Subsequently, rifampicin (1968) was discovered, which contributed significantly to shortened duration of treatment. These three agents, together with streptomycin and isoniazid, which were discovered prior to clofazimine in 1943 and 1945, respectively, were combined to form the earlier and current regimens used in the treatment of TB for the past 50 years. The efficacy of these regimens resulted in loss of interest in clofazimine as an anti-TB agent. However in 1981, clofazimine was recommended by the WHO for inclusion as a component of the multi-drug treatment of leprosy due to its beneficial combination of antimicrobial and anti-inflammatory properties.[18]

In the light of the growing XDR-TB epidemic, there has been a re-emergence of interest in clofazimine, which has become an important component of newer treatment regimens.[27,28] One of these, referred to as the 9-month short-course

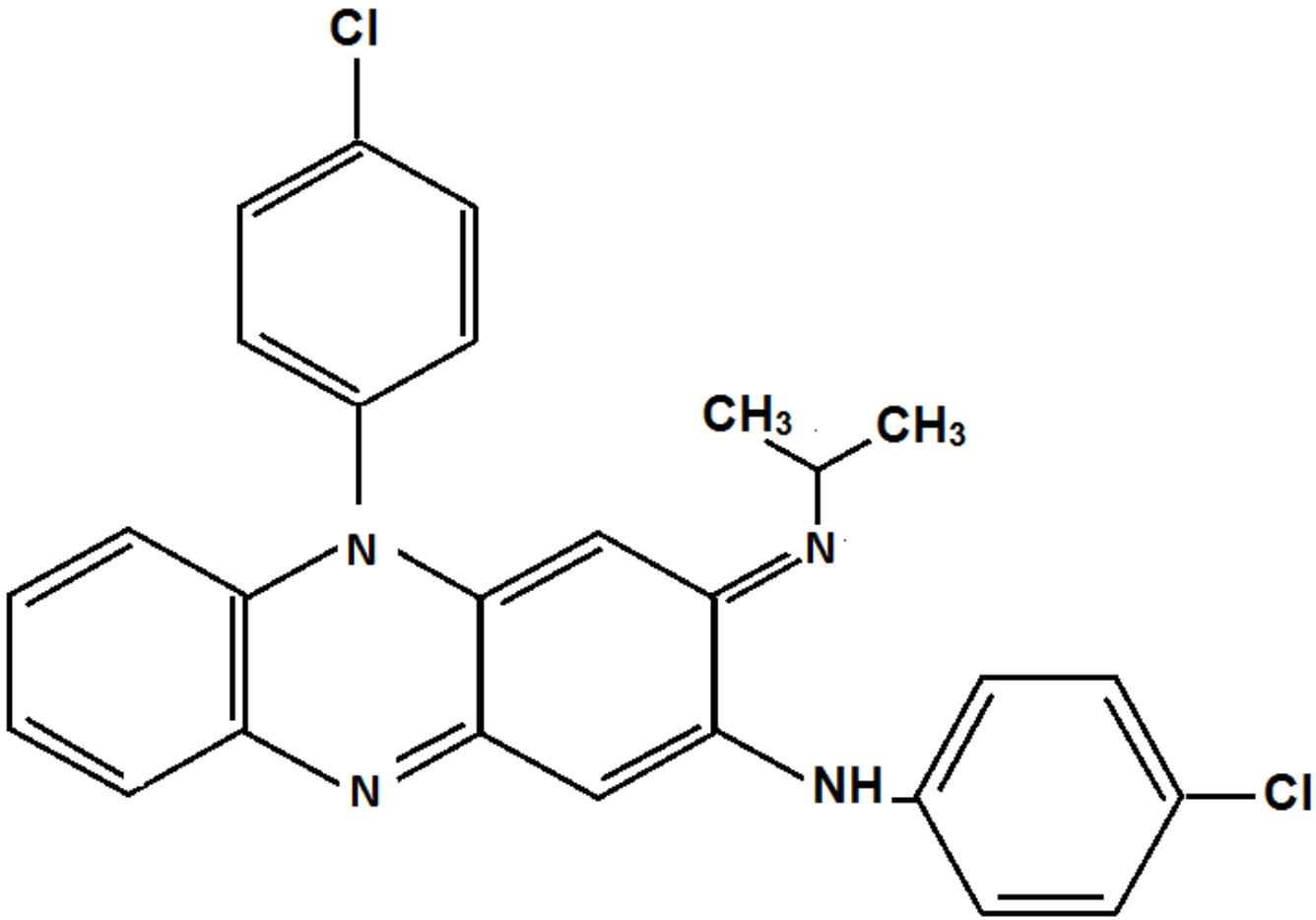
regimen, based on the outcome of a clinical trial conducted in Bangladesh, was found to be efficacious and is currently being evaluated for its possible application as a future standard regimen for the treatment of DR-TB patients.[27,29,30]

Chemical structure of clofazimine

Clofazimine [3-(p-chloroanilino)-10-(p-chlorophenyl)-2,10-dihydro-2-(isopropylimino) phenazine] has a molecular formula of $C_{27}H_{22}C_{12}N_4$ and a molecular weight of 473.14 Daltons (Figure 1a).[26,31] The key structural features of the riminophenazines are the phenazine nucleus, with an alkylimino (R-imino) group at the C-2 and phenyl substituents at the C-3 and N-10 positions of the phenazine nucleus.[32] The basic nitrogen atom of the isopropylimino group at position C-2 of clofazimine contributes to the cationic amphiphilic properties of the molecule. Cationic amphiphilic drugs contain both a hydrophobic domain in the aromatic ring system and a hydrophilic domain in the ionisable amine functional group. [33,34,35]

Modifications of the substituents at positions C-2, C-3 and N-10 of the clofazimine molecule have resulted in analogues that have demonstrated alterations in antimicrobial activity and pharmacological properties. The most promising of these are the tetramethylpiperidyl-substituted phenazines in which the isopropyl group at position C-2 of the phenazine nucleus is replaced by the tetramethylpiperidyl group,[26,36-38] or by a methoxypyridylamino group at C-3.[39,40] However, none of these, to our knowledge, are currently in clinical development.

(a)



(b)

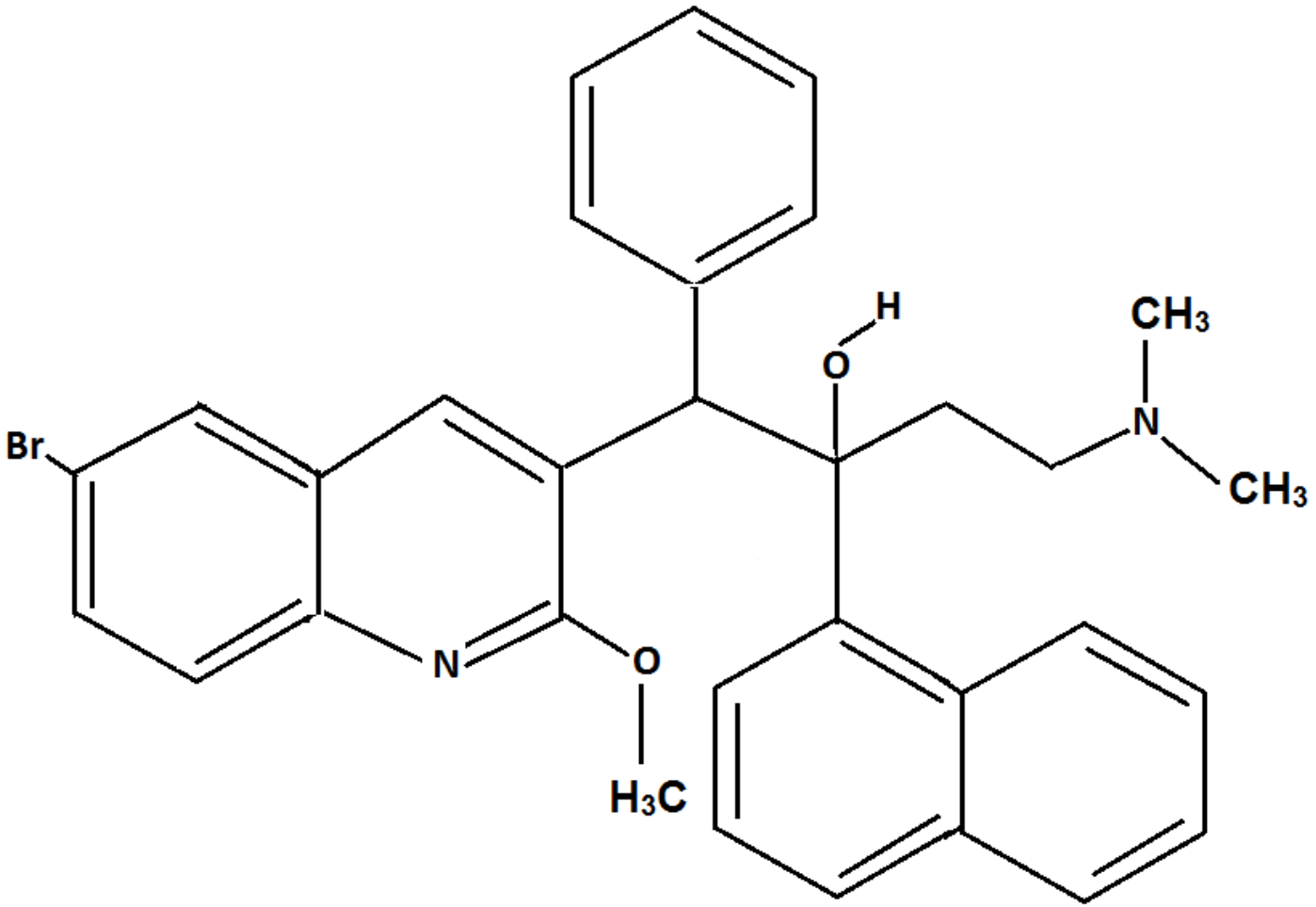


Figure 1. Molecular structures and systematic names of (a) clofazimine ([3-(p-chloroanilino)-10-(p-chlorophenyl)-2,10-dihydro-2-(isopropylimino) phenazine]),[32] and (b) bedaquiline ([1-(6-bromo-2-methoxy-quinolin-3-yl)-4-dimethylamino-2-naphthalen-1-yl-1-phenyl-butan-2-ol]).[130]

Various formulations of clofazimine, oral, intravenous and inhaled, have also been evaluated in the experimental setting, but none has yet undergone clinical evaluation.[32,41,42]

Antimicrobial activity of clofazimine

Clofazimine has a broad spectrum of antimicrobial activity, acting against many types of microorganisms, including bacteria, parasites and fungi. Among the bacteria, clofazimine is active against Gram-positive organisms, while Gram-negative organisms are uniformly resistant.[32,43-46] Parasites that are susceptible to clofazimine include *Plasmodium falciparum*,[47] *Leishmania donovani,*[48,49] *Trypanosoma cruzi*,[50] *Babesia, Theileria*[51] and *Schistosoma* species[52,53] while the yeast, *Candida albicans,* is also susceptible.[53]

Clofazimine has demonstrated impressive activity against various mycobacterial species,[54,55] including rapidly- (*M. abscessus*, *M. fortuitum* and *M. smegmatis*) and slow-growing bacilli [*Mtb*, *M. avium intracellulare* complex (MAC) and *M. leprae*].[54,56-59] In addition, clofazimine acts synergistically with other antimicrobial agents, such as amikacin and clarithromycin, against several mycobacterial species, including *M. avium* and *M. abscessus in vitro.*[57] In the case of *Mtb*, clofazimine, at low concentrations, is active against both DS- and DR-TB strains *in vitro* and *in vivo*, exhibiting differential activities against *Mtb* populations according to the stage of growth.[32,37,60] Clofazimine has demonstrated impressive bacteriostatic activity, but poor bactericidal activity, against actively-replicating bacilli, *in vitro* and *in vivo*, the former achievable at minimum inhibitory concentration (MIC) values ranging

from 0.06 - 2 mg/L.[32,61,62] Clofazimine also acts synergistically in combination with the primary anti-TB agents, ethambutol and pyrazinamide,[63,64] as well as with the second-line agents, linezolid,[65] bedaquiline and moxifloxacin, against *Mtb* isolates.[66] Clofazimine monotherapy has been reported to reduce the bacterial load in BALB/c mice infected with the H37Rv strain of *Mtb*, reducing it from $6\log_{10}$ to $4\log_{10}$ within 8 weeks.[67] In another murine model of experimental chemotherapy, combining clofazimine with the primary anti-TB drugs, rifampicin, isoniazid and pyrazinamide, shortened the treatment time to achieve cure from 6 months to 4 months in comparison with mice treated with a primary anti-TB drug regimen alone (rifampicin, isoniazid and pyrazinamide).[68] In humans, however clofazimine fails to kill this actively-growing microbial population during the first 14 days of therapy, which is attributable to its lack of early bactericidal activity (EBA). *In vitro*, clofazimine also failed to demonstrate EBA during the first 10 days of treatment when used at concentrations of 0.3 - 2.5 mg/L. However, an EBA was achievable when higher concentrations of clofazimine (5 - 20 mg/L) were used.[62]

Among the different *Mtb* subpopulations, clofazimine has demonstrated highest activity against slow-replicating bacilli. The MIC and minimum bactericidal concentration (MBC) values against this bacterial subpopulation *in vitro* were reported to be 0.06 and 0.3 mg/L, respectively,[62] while reducing the slow-replicating *Mtb* bacterial load in C3HeB/FeJ infected mice by $5.8\log_{10}$ cfu/mL.[67] Slow-replicating bacilli are responsible for the formation of biofilm *in vitro* and granuloma *in vivo,* both of which are attenuated by clofazimine, possibly facilitating exposure of the bacteria to other antimicrobial agents.[62,67]

In the case of non-replicating bacilli, only those cultured in an aerated, streptomycin-starved (SS18b) model of dormancy *in vitro* were found to be susceptible to the lethal activity of clofazimine,[69] while those residing in non-aerobic, enclosed environments, such as preformed mycobacterial biofilm cultures were not affected.[62] Likewise, in a C3HeB/FeJ murine model of experimental TB, organisms contained in the matured granuloma lesions in the lungs were only slightly reduced by 1.6$\log_{10}$ following treatment with clofazimine.[67] These observations seemingly support the requirements for the availability of oxygen and/or accessibility of the bacteria to the antibiotic to achieve a mycobactericidal effect on dormant bacilli.[62,67,70,71]The preferential microbicidal action of clofazimine on non-replicating bacilli, may explain the lack of EBA, while contributing to shortening of the duration of chemotherapy via late bactericidal activity (LBA).

Mechanisms of action of clofazimine

Effect of clofazimine on microbial cells

The Irish group which discovered clofazimine suggested that the antimycobacterial activity of this agent was attributable to two unusual properties, these being its high lipophilicity, enabling efficient transmembrane penetration, together with a redox potential of –0.18V at pH7,[25] favouring intracellular redox cycling.[43] Intracellular oxidation of reduced clofazimine was proposed to result in the generation of antimicrobial reactive oxygen species (ROS).[25] However, convincing evidence for the existence of such a mechanism was provided only 50 years later by Yano *et al*.[70] These authors, using isolated membrane fractions from *M. smegmatis*,

demonstrated that clofazimine appears to compete for electrons with menaquinone, the substrate for type 2 nicotine adenine dinucleotide hydrogen (NADH):quinone oxidoreductase, which is the initial event in the mycobacterial respiratory chain.[70] Reduced clofazimine generated by this mechanism was proposed to undergo spontaneous oxidation, resulting in the generation of antimicrobial ROS such as superoxide and hydrogen peroxide.[70,72] This putative mechanism of antimicrobial activity is supported by a more recent study which reported that supplementation of the bacterial growth medium with high concentrations of menaquinone antagonized the antimycobacterial activity of clofazimine.[73] In addition, and seemingly consistent with an inhibitory effect on bacterial respiration, selective inactivation of the cytochrome *bd*-type quinol oxidase of the branched respiratory chain operative in mycobacteria was found to increase the susceptibility of *M. smegmatis* to clofazimine.[74] The authors speculated that the protective action of cytochrome *bd* is achieved via neutralization or inhibition of clofazimine-generated ROS.[74]

Although redox cycling as described by Yano *et al.*[70] appears to contribute to the antimycobacterial activity of clofazimine, others believe that this is unlikely to be the only mechanism, favouring the existence of a multifaceted mechanism of antimicrobial activity. If correct, this may explain the remarkably low level of resistance to clofazimine in both the clinical and experimental settings. Evidence in support of this contention originates from several sources. Firstly, although clofazimine is assumed to compete with menaquinone for electrons generated via the activity of type 2 NADH:quinone oxidoreductase, the existence of such a mechanism remains to be conclusively established.[73] In addition, menaquinone possesses secondary membrane–stabilizing properties,[75] which may counteract the

disruptive effect of clofazimine on the mycobacterial membrane. Secondly, in an earlier study, Van Rensburg *et al*.[43] reported that exposure of a single strain each of *Staphylococcus aureus* and *Streptococcus pyogenes* to clofazimine under anaerobic conditions actually increased the susceptibility of these microorganisms to clofazimine.[43] More recently, Lu *et al*.[76] using a low oxygen recovery assay (LORA), reported that exposure of *M. tuberculosis* to clofazimine at very low oxygen concentrations (<0.16%) resulted in only moderate loss of antimycobacterial activity of the antibiotic.[76] These authors proposed that different clofazimine-mediated antimycobacterial mechanisms may be operative under different environmental conditions.[76] Thirdly, the susceptibility of Gram-negative bacteria to the antimicrobial actions of ROS is not entirely consistent with their relative lack of susceptibility to clofazimine.[32,43]

Additional mechanisms of antimicrobial activity, unrelated to redox cycling, are likely to result from the cationic amphiphilic, membrane disruptive properties of clofazimine alluded to above. Mycobacteria and Gram-positive bacteria are particularly susceptible to the membrane disruptive actions of cationic amphiphiles and other types of membrane disruptive agent.[32,43,46,77-79] In this context, it is noteworthy that ion-transporting adenosine triphosphatases (ATPases), are particularly vulnerable to inhibition by cationic amphiphiles, which appear to induce conformational changes in protein molecular structure and loss of function.[80-83] We have previously reported that interference with cation uptake, specifically potassium (K^+), is one of the earliest occurring changes during exposure of *Mtb* to clofazimine at MIC concentrations, and is followed by depletion of ATP and inhibition of growth.[32] Although we have previously proposed that selective targeting of mycobacterial K^+

active transporters may underpin the antimycobacterial activity of clofazimine, a non-specific membrane disruptive mechanism, resulting in loss of activity of several different ion transporters, appears more likely.[24,32,37,61]

Taken together, the currently available evidence is consistent with the existence of at least two mechanisms of clofazimine-mediated antimycobacterial activity *viz.* intracellular redox cycling and membrane disruption. As mentioned above, the relative contributions of these mechanisms may vary according to environmental conditions and may also explain the very low level of resistance to clofazimine encountered in the therapeutic setting. It is, however, noteworthy that the membrane disruptive antimycobaterial mechanism related to the cationic amphiphilic properties of clofazimine may not be effective in regions of the granuloma which are slightly alkaline, thereby neutralising the positive charge on the molecule.[34,35]

Effect of clofazimine on eukaryotic cells

The effects of clofazimine on eukaryotic cells have been reviewed by us previously and are considered only briefly here.[32] Not surprisingly, these include inhibition of the plasma membrane K^+ transporters, sodium (Na^+), K^+-ATPase,[84] and the Kv1.3 potassium channel,[85,86] both of which are electrogenic and essential for the activation and proliferation of T-lymphocytes. Clofazimine-mediated interference with T-cell activation via inhibition of transmembrane K^+ fluxes is likely to underpin the reported benefit of this agent in the treatment of autoimmune and other chronic inflammatory disorders, as well as in controlling immune-mediated tissue damage during mycobacterial infection.[32,84-86] Additional mechanisms of clofazimine-mediated

immunosuppressive activity include increased production of both prostaglandin E_2, and the interleukin (IL)-1 receptor antagonist by immune, inflammatory and other cell types.[32,87-89]

As proposed previously, the immunosuppressive properties of clofazimine may be either beneficial or detrimental depending on the timing of administration of this agent.[32] If administered at the outset of therapy, immunosuppressive activity may compromise the antimycobacterial efficacy of clofazimine, possibly contributing to the lack of EBA, as well as that of other agents in the drug regimen. Administration later in the course of therapy may contribute to the eradication of slow-growing persisters in the setting of controlled recovery of *Mtb*-specific immune reactivity.

Clinical efficacy of clofazimine

As with other anti-TB therapeutic agents, clofazimine is used in multidrug regimens to prevent the emergence of drug resistance. However, due to lack of a standardised regimen for clofazimine, several different regimens have been evaluated for efficacy in the treatment of DR-TB, most frequently at daily dosages ranging from 50-100 mg, with few reaching to 300 mg.[17,18,90]

One of the most effective clofazimine-containing regimens evaluated to date has been the 9-month short-course regimen, based on a clinical trial conducted in Bangladesh from 1997 - 2007.[29] This regimen consisted of gatifloxacin, clofazimine, ethambutol and pyrazinamide throughout the treatment period of nine months, supplemented with prothionamide, kanamycin and high-dose isoniazid during the 4-

month intensive phase, followed by a continuation phase of five months with gatifloxacin, ethambutol, pyrazinamide and clofazimine. This regimen administered to treatment-naive MDR-TB patients resulted in a reduction in the duration of treatment from the 20-month period when using the WHO-recommended regimen to nine months. It was also associated with impressive relapse-free cure rates in patients who were followed for 24 months.[29] The efficacy of this regimen was confirmed in a follow-up clinical trial undertaken in Bangladesh[91] and has since been adopted by many low-income countries, including Cameroon[92] and Niger.[93] These studies, summarised in Table 1, have also demonstrated impressive treatment outcomes, with success rates ranging from 84 - 89%.[91] A number of patients recruited to some of these studies harboured isolates resistant to at least six anti-TB agents, and also included those who were coinfected with HIV. The outcomes of therapy reported from these trials appear to confirm the efficacy of the 9-month short-course regimen, irrespective of disease severity and socio-economic status of the patients. This regimen is currently being evaluated in several other West African countries and preliminary data has demonstrated sputum-culture conversion within four months of the intensive phase of therapy in 96% of patients.[30]

The shortening of treatment associated with the Bangladesh-based regimen may be related to targeting of slow/non-replicating bacterial populations by clofazimine as described in experimental settings both *in vitro* and *in vivo*.[60,62,67] The sustained relapse-free cure rates have been attributed to the prolonged half-life (70 days), high tissue accumulation,[60,94] and/ or the long post-antibiotic effect (PAE) of clofazimine. The latter effect was recently demonstrated in the experimental setting when *Mtb*-infected BALB/c mice treated with clofazimine alone and as a constituent

Table 1. 9-month clofazimine-based short-course treatment regimen for DR-TB patients in different countries

Study name[a]	**1**	**2**	**3**	**4**	**5**
Study site	Bangladesh	Bangladesh	Niger	Cameroon	STREAM stage 1[b]
Period (years)	1997-2007 (10)	2005-2011 (6)	2008-2010 (2)	2008-2011 (3)	2012-2015 (3)
No of patients	206	515	65	150	424
Treatment regimen **Months (drugs)**	4 (KCGEHZP) 5 (GEZC)	4 (KCGEHZP) 5 (GEZC)	4 (KCGEHZP) 5 (GEZC)	4 (KCGEHZP) 8 (GEZCP)	4 (KCMEHZP) 5 (MEZC)
Treatment duration (months)	9-12	9-12	12	12-13.6	9-11
Follow-up period (months)	24	24	24	24	27
Successful treatment outcome	182/206 (87.9%)	435/515 (84.4%)	58/65 (89%)	134/150 (89%)	NYA
Relapse-free cure rate	90%	82.3%	49/49 (100%)	100%	NYA
Treatment failure	1/206 (0.5%)	7 (1.4%)	1/65 (1.5%)	1/150 (0.7%)	NYA
Default	12 (5.8%)	40/515 (7.8%)	1/65 (1.5%)	5/150 (3.3%)	NYA
Death	11 (5.3%)	29/515 (5.6%)	6 (9.2%)	10/150 (5%)	NYA
FQ/KM resistance	20/206 (10%)	51/515 (10%)	1/65 (1.5%)	0	NYA

HIV-positive	0	0	1/58 (1.7%)	30/150 (20%)	NYA
Side effects	Vomiting (21.4%) Hearing impairment (6.3%) Ataxia (3.9%) Dysglycemia (3.9%)	Vomiting (21.6%)	Vomiting (26%) Hearing impairment (20%) Hyperglycaemia (9.2%) Gastritis (7.7%) Arthralgia (6.2%) Peripheral neuropathy (4.6%) Skin pigmentation (3.1%)	Hearing loss (43%) Vomiting Retrobulbar neuritis Transaminases (0.7%)	NYA

K, kanamycin; C, clofazimine; G, gatifloxacin; E, ethambutol; H, isoniazid; Z, pyrazinamide; P, prothionamide; M, moxifloxacin; NYA, not yet available.

[a]Studies numbered according to authors' names; 1, 2, 3, 4 and 5 represent studies conducted by van Deun *et al*., 2010[29]; Aung *et al*., 2014[91]; Piubello *et al*., 2014[93]; Kuaban *et al*., 2015[92] and Nunn *et al*., 2015[27], respectively.

[b]Different sites included in Stream stage 1 trial were Ethiopia, South Africa, Vietnam and Mongolia.

of a multi-drug regimen showed a delay in bacterial regrowth six weeks after treatment cessation, while those treated with an alternative clofazimine-free regimen showed regrowth immediately after treatment cessation.[95]

Despite its success, several caveats exist in relation to widespread implementation of the 9-month short-course regimen. Most importantly, the efficacy of this regimen may be affected by the drug resistance profiles of the isolates. In Bangladesh, patients with *Mtb* strains with high-level fluoroquinolone (gatifloxacin) and pyrazinamide resistance had a poor treatment outcome.[28,91] In this setting, cessation of the 9-month short-course regimen and replacement with a bedaquiline-containing regimen is recommended.[96]

In addition to the ongoing West Africa study, the efficacy of the Bangladesh regimen is currently being evaluated in the STREAM trial, encompassing several countries including South Africa, Ethiopia, Mongolia and Vietnam, comparing its efficacy and safety with that of the WHO regimen as a necessary prerequisite prior to possible recommendation as a standard regimen for the treatment of MDR-TB patients. In this study, 424 patients, including those who are HIV-positive, have been enrolled during the period 2012 - 2015, while patients infected with fluorquinolone- and injectable agent-resistant DR-TB isolates have been excluded. Modifications to the 9-month treatment regimen include replacement of gatifloxacin with moxifloxacin due to the association of the former with dysglycaemia.[28] Patients are being monitored for 27 months post-treatment. The trial is ongoing and the expected completion date is early 2018.[27,28]

Based on the successful implementation of the STREAM trial, also referred to as STREAM stage 1, a stage 2 trial was planned and will be initiated during 2016 continuing for three years thereafter, with the results expected in 2021.[28] In this trial, the two major objectives are the formulation of: i) a less toxic fully oral 9-month regimen; and ii) a shortened 6-month regimen. With respect to the first objective, a fully oral 9-month regimen consisiting of isoniazid, prothionamide, bedaquiline, levofloxacin, ethambutol, clofazimine and pyrazinamide during a 4-month intensive phase, and bedaquiline, levofloxacin, ethambutol, clofazimine and pyrazinamide during a 5-month continuation phase is under investigation. In this fully oral 9-month regimen, bedaquiline replaces the injectable kanamycin, while in both regimens levofloxacin is used instead of moxifloxacin to reduce the risk of QT prolongation that occurs during coadministration of bedaquiline and moxifloxacin. In the case of the second objective, a 6-month regimen consisting of bedaquiline, clofazimine, pyrazinamide, levofloxacin, isoniazid and kanamycin is administered during a 2-month intensive phase, followed by bedaquiline, levofloxacin, clofazimine and pyrazinamide during a 4-month continuation phase. On a cautionary note, however, several investigators have expressed concern about coadministration of clofazimine and bedaquiline, due to shared efflux pump-based mechanisms of drug resistance,[6,97] high risk for increased QT prolongation[98,99] and a possible increased occurrence of DDIs due to metabolism of both antibiotics by CYP3A4,[2,100,101] necessitating high-level vigilance.

Other clofazimine-containing regimens, although resulting in improved treatment outcomes, have been less successful than the 9-month short-course regimen.[29,102,103] These trials were undertaken in Benin, South Africa, Ukraine, Brazil

and Sri Lanka with reported cure rates ranging from 60 - 66%. Interestingly and importantly, comparable successful treatment outcomes of MDR- (65%) and XDR-TB (66%) cases were reported,[90,104] illustrating that the efficacy of clofazimine is independent of the resistance of *Mtb* to the other antimicrobial agents in the regimens, especially the primary anti-TB agents. In most of these trials treatment duration ranged from 12 - 18 months.[17,104]

Inclusion of clofazimine in the DR-TB drug regimens of TB/HIV-coinfected patients has also been reported to result in improved treatment success rates, increasing from 28.6% to 50%.[13,17,18] However, treatment success in these patients is affected by the DDI effect of the anti-TB and antiretroviral (ARV) agents.[100]

Mechanisms of resistance to clofazimine

Currently, no primary clofazimine-resistant *Mtb* clinical isolate, has been described, probably due to an extremely low mutation rate, necessitating exposure at a high bacterial density in a clinical lesion or culture ($1/10^{26}$ cfu/mL) for selection of resistance traits.[6,105-108] Alternatively, the existence of multiple targets may underpin the low level of development of resistance to clofazimine.[24,32]

In the absence of resistant clinical isolates, the mechanism of clofazimine resistance has been investigated using clofazimine-resistant mutant strains developed *in vitro*. Ninety-seven percent of these laboratory mutant strains had mutations at the *rv0678* gene, encoding the Rv0678 protein drug efflux pump.[6,107,109] The Rv0678 protein is the transcriptional regulator, which represses expression of

mmpS5-mmpL5, the gene encoding the multi-substrate MmpS5-MmpL5 efflux pump.[99,107] Interestingly, the *rv0678-mmpS5-mmpL5* locus is absent in *M. leprae*, which is also highly susceptible to clofazimine, and for which no clofazimine-resistant mutant has been isolated to date.[107] Mutations at the *rv0678* gene also lead to cross-resistance to bedaquiline in *Mtb* isolates.[6,97] Although other genes associated with clofazimine resistance in *Mtb* (*rv1979c* and *rv2535c*) have been identified, their mechanisms of resistance have not been determined.[109]

Pharmacokinetic and pharmacodynamic properties of clofazimine

Clofazimine is highly lipophilic leading to high accumulation in fat-tissues and relatively low serum concentrations (0.7 - 1 mg/L).[32,39,94,104] The fat-tissues include macrophage-rich organs such as the lungs, livers, spleen, brain[110] and the bone marrow.[111-113] As mentioned above, the drug has a long half-life of approximately 70 days, which contributes to skin discolouration, its most frequent AE.[75]

During its long-term tissue accumulation, clofazimine undergoes xenobiotic sequestration resulting in the formation of crystal-like drug inclusions (CLDI) in the cytoplasm of tissue macrophages.[114-117] These bodies are formed through an intracellular chloride transport mechanism within the cells and are composed of several layers 5-15 nanometers in thickness.[115-,118] Intracellularly, they do not destabilise mitochondria, neither do they induce oxidative damage as shown *in vitro* cultures.[111,117]

In spite of its high lipophilicity, clofazimine is unable to penetrate and accumulate in caseous granulomas. Examination of granuloma lesions in patients, using matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry has shown a strong accumulation of the drug in the highly cellular peripheral zone of the granuloma, consisting of macrophages, epitheloid macrophages, and lymphocytes, to levels considerably higher than in the enclosed necrotic core. Due to its high lipophilicity, it is internalised in the macrophages through endocytosis and lysosomal trapping, but fails to diffuse passively through the acqeous necrotic region.[94] The poor accumulation of clofazimine in this region of the granuloma may explain its failure to act on the non-replicating bacilli in the granuloma core of C3HeB/FeJ mice as described above.[32,94]

Administration of clofazimine to DR-TB patients has been associated with occasional, usually manageable, AEs, with frequencies comparable to those of the standard first-line anti-TB agents.[90] The most common are transient discolouration of the skin and mucous membranes, and gastrointestinal tract (GIT) discomfort,[32,90] such as vomiting and nausea.[18,55,93,102] While skin discolouration can lead to mental disturbance, the GIT AEs may be, albeit infrequently, severe and occasionally fatal.[30] In a meta-analysis of cohort studies involving clofazimine therapy, the pooled proportion of all of these AEs attributable to clofazimine has been shown to be 21.9%, although those that required withdrawal or discontinuation was 0.1% and the frequency of all AEs was 5.1%.[90] In the study by Xu *et al.*[102] 39 of 44 patients (87%) were reported to have experienced AEs after starting clofazimine, predominantly skin discolouration.[94] It has, however, been noted that administration of clofazimine in the 9-month short-course regimen, was associated with a relatively low frequency (3%)

of skin pigmentation (Table 1). Clofazimine therapy has also been reported to be associated with an increased QT interval.[99]

Mortality associated with clofazimine therapy is uncommon.[29,55,68] In several studies, mortality rates ranged from 1 - 63.4%, although none of the deaths was apparently attributable to clofazimine use,[104] but rather to respiratory failure complications, haemoptysis and cerebral malaria.

Bedaquiline

Background

Bedaquiline belongs to a new class of antimicrobial agents known as the diarylquinolines.[105,119,120] It is the first drug that has been approved for the treatment of TB in over 40 years by the US FDA. Approval was based on the outcome of two phase IIb studies demonstrating improved efficacy in the clinical setting of DR-TB with cure rates of 62% and 44% in patients who received the bedaquiline/background regimen (BR) and placebo/BR, respectively, with corresponding reductions in the duration of therapy (83 vs 125 days). The preferred BR consisted of five antibiotics, ethionamide, pyrazinamide, ofloxacin, kanamycin and cycloserine.[121]

Despite the improved therapeutic efficacy of bedaquiline-containing regimens, safety issues remain a concern due to incomplete safety data based only on phase II clinical trials in the absence of phase III and IV trial data.[12,119,122,123] Of concern, the

phase II studies revealed more deaths and AEs in the bedaquiline/BR-treated group than in the placebo/BR group. These safety issues led the WHO to recommend only conditional use of bedaquiline, limited to patients with pulmonary MDR-TB for whom no suitable alternatives are available.[10,20,124] The WHO has also emphasized the necessity for timely completion of phase III and phase IV clinical trials.[125]

Nevertheless, despite limited availability of safety data, usage of bedaquiline in MDR-TB chemotherapy through compassionate use programmes has been beneficial, resulting in the survival of many MDR-TB patients with more than 1258 patients worldwide having experienced clinical benefit as of October 2015.[123,126-129] In addition, the US Agency for International Development has decided to make bedaquiline available free-of-charge to >100 global fund-eligible countries for a 4-year period.[12,99,126]

Chemical structure of bedaquiline

Bedaquiline [1-(6-bromo-2-methoxy-quinolin-3-yl)-4-dimethylamino-2-naphthalen-1-yl-1-phenyl-butan-2-ol] has a molecular formula of $C_{32}H_{31}BrN_2O_2$ and a molecular weight of 555.51 Daltons (Figure 1b).[130] Its alternative names are R207910 and TMC207.[130] Bedaquiline contains planar hydrophobic moieties and hydrogen-bonding acceptor or donor groups.[131] The hydrophobic moieties, which include hydroxyl and N,N dimethyl ($-N(CH_3)_2$) groups, play an important role in binding to the mycobacterial target F_1/F_0-ATP synthase, interacting with amino acid residues Arg-186 and Glu-61 at the A and C subunits, respectively, while the hydrogen bonding provides stability.[5,132] The diarylquinoline ring has also been shown to play a role in

the anti-TB activity of bedaquiline, although it is not indispensable for this activity. Other structural moieties that play a role in anti-TB activity include the hydroxyl group, the side chain with the N,N-dimethyl amino terminus and/or the naphthalene moiety.[5,133]

Several bedaquiline analogues have been synthesised with the aim of improving the antimicrobial activity and spectrum of bedaquiline, as well as the pharmacological properties of the antibiotic, however, none of these has progressed to clinical evaluation.[134,135]

Antimicrobial activity of bedaquiline

Unlike clofazimine, which has a broader spectrum of antimicrobial activity, bedaquiline has demonstrated weak activity against Gram-positive and Gram-negative bacteria, generally exhibiting MIC values >32 mg/L.[2,120,130] Activity against other types of microorganisms, such as parasites and fungi, has not yet been reported. Importantly, bedaquiline has demonstrated selective activity against a wide variety of pathogenic mycobacteria, such as *Mtb*, *M. leprae*[136] and *M. avium*,[137] as well as non-pathogenic organisms including *M. smegmatis,* with MICs ranging from 0.003 - 0.5 mg/L.[119,120,138] *Mtb,* and *M. smegmatis*, are the most susceptible mycobacterial species to bedaquiline with equivalent MIC values of 0.003 mg/L.[2,119,120,138] On the other hand, several mycobacterial species are naturally resistant to bedaquiline with high MIC values (>8 mg/L) reported for *M. novocastrense, M. shimoidei* and *M. xenopi in vitro.* Resistance of these organisms to bedaquiline has been associated with phenotypic variations in the F_1/F_0-ATP

synthase enzyme, which, as described below, is the target for bedaquiline in susceptible mycobacterial species.[2,139]

Like clofazimine, bedaquiline has showed varying activity against the different bacterial subpopulations within DS and DR strains of *Mtb*. It is highly bacteriostatic against actively-replicating organisms,[140] achieving MIC values ranging from 0.003 - 0.12 mg/L *in vitro*.[2,119,120,138] It also acts synergistically when used in combination with other anti-TB agents, including the primary anti-TB drugs rifampicin, ethambutol and pyrazinamide, as well as second-line drugs such as AZD5847, tedizolid, oxazolidinone, rifapentine, linezolid, clofazimine,[141-143] BTZ043 and PBTZ169.[144] Other agents such as sutezolid and SQ109 have demonstrated additive interactions,[145] while others like pretonamid, interact antagonistically with bedaquiline.[146]

Bactericidal activity of bedaquiline against actively-replicating *Mtb* organisms has also been demonstrated *in vivo*, resulting in accelerated sterilizing activity in a murine model of experimental chemotherapy as shown by earlier culture conversion.[145] During two months of chemotherapy, approximately 20% of mice treated with bedaquiline monotherapy demonstrated culture conversion to negativity, which was not observed in those treated with individual primary anti-TB drugs. The sterilizing activity of bedaquiline improved when this agent was added to the primary anti-TB drug regimen, resulting in culture conversion in 70 - 100% of infected mice.[145,147] In humans, bedaquiline, like clofazimine, has demonstrated poor EBA during the first 14 days of chemotherapy. Like clofazimine, earlier onset of

bactericidal activity of bedaquiline can, however, be achieved by increasing the dosage of the drug.[148,149]

Importantly, and similarly to clofazimine, bedaquiline possesses bactericidal activity against dormant/non-replicating *Mtb* bacilli at low, therapeutically attainable MBCs.[2] This highly beneficial property of bedaquiline on the dormant bacilli, has been attributed to its LBA,[150,151] which is also consistent with shortening of chemotherapy. However, like clofazimine, it fails to act on mycobacterial populations residing in caseous granuloma lesions of C3HeB/FeJ mice due to its poor penetration of the caseous core.[152]

Mechanisms of antimycobacterial action of bedaquiline

Bedaquiline, unlike clofazimine, is highly selective against *Mtb*, including MDR strains of the pathogen, as well as against other types of mycobacteria as mentioned above.[130] The drug selectively targets and inactivates, the F_1/F_0-ATP synthase of the pathogen,[130] but importantly, has no inhibitory effect on mammalian F_1/F_0-ATP synthase.[142] F_1/F_0 -ATP synthase is a highly conserved and key enzyme in the process of oxidative phosphorylation, which utilises the kinetic mechanical energy of PMF to drive ATP production.[153,154] Protons generated by the bacterial, membrane-associated electron-transport chains coupled to oxidative phosphorylation are captured by, and funnelled into the membrane-embedded, F_0 proton channel of the enzyme and transported to the catalytic F_1 component, which undergoes a conformational change resulting in synthesis of ATP.[2,154,155]

Computer-based molecular modelling/docking, mutational analyses and other approaches have identified the lipophilic F_0 component of F_1/F_0 ATP synthase, specifically the transmembrane, oligomeric subunit C (AtpE) as being the primary target of bedaquiline.[130,139,156,157] Binding of bedaquiline is located to a "cleft between two adjacent C subunits in the C-ring," a region which contains the proton-binding acidic residue, Glu-61.[139,156,157] The consequence is interference with proton movement and synthesis of ATP.[158]

Although rapid depletion of ATP occurs following exposure of *Mtb* to bedaquiline, bactericidal action, as mentioned above, is delayed for several days, apparently as a result of induction of dormancy and utilisation of alternative energy sources and pathways such as glycolysis.[43,142,159] It has even been proposed that the bactericidal mode of action of bedaquiline, at least in the case of *M. smegmatis*, results from the collapse of the transmembrane pH gradient and dissipation of the PMF which is lethal to mycobacteria.[160] This contention is consistent with observations that exposure of *M. smegmatis* to bedaquiline is associated with upregulated expression and utilisation of cytochrome *bd* oxidase, the non-proton-pumping terminal oxidase, which improves bacterial survival.[74,160] In addition, and supportive of these findings, others have reported that selective knockout of cytochrome *bd* oxidase in *Mtb* results in increased susceptibility of the pathogen to bedaquiline,[161] which,as mentioned earlier, is also evident following exposure of *M. smegmatis* to clofazimine.[74]

Very recently Lamprecht and colleagues have reported that a combination of clofazimine, bedaquiline and the novel imidazopyridine amide antimycobacterial

agent, Q203, is extremely effective in accelerating the rate of extracellular and intra-macrophage killing of *Mtb in vitro,* being superior to the individual agents and the various two drug combinations.[162] Mechanistically, bedaquiline and Q203, via their inhibitory effects on the bacterial electron-transport chain were found to increase the intracellular reductive potential via elevated NADH/NAD^+ ratios, which, in turn, augmented clofazimine-mediated generation of bactericidal ROS.[162] Although interesting, the therapeutic potential of this drug combination remains to be addressed, as do concerns in relation to cross-resistance to these agents.[162,163]

Alternative mechanisms of antimycobacterial action of bedaquiline

Like clofazimine, bedaquiline is a cationic amphiphilic drug, a property, which, albeit speculatively, may be related to its primary F_1/F_0-ATP synthase-directed mode of antimycobacterial action. Surprisingly, however, the existence of alternative, secondary mechanisms of bedaquiline -mediated antimycobacterial activity possibly related to the cationic amphiphilic properties, especially effects on membrane ion-transporting ATPases, appear to be largely unexplored.

Effects of bedaquiline on eukaryotic cells

This important aspect of bedaquiline research is, to our knowledge, also largely unexplored. The observed prolongation of the QT interval and potentially fatal cardiac arrhythmias, may, or may not, be related to interference with membrane-associated cation transporters, specifically K^+ transport in cardiomyocytes secondary to cationic amphiphilic properties, an issue which requires investigation.[24] Very

recently, bedaquiline, like clofazimine,[164] has been reported to possess anti-tumour properties *in vitro*, which are related to inhibition of mitochondrial respiration and intracellular generation of ROS.[165,166]

Clinical efficacy of bedaquiline

As mentioned above, bedaquiline may be added to a relevant BR for the treatment of DR-TB patients who do not respond to the current WHO-recommended treatment regimen. The dosing regimen consists of 400 mg orally once daily for two weeks, followed by 200 mg orally three times weekly with a total treatment duration of 24 weeks.[121,155] Inclusion of bedaquiline in the treatment of DR-TB has led to improvements in treatment outcomes, resulting in shorter duration of treatment and low relapse rates. The shorter duration of therapy is associated with faster sputum and culture conversion.[105,107,128,150] The improved sputum conversion demonstrated in two studies during the first two months of treatment was achieved in 48% and 84% of patients treated with bedaquiline/BR as opposed to 9% and 65% in the placebo/BR-treated groups, respectively.[99,167,168] With respect to overall duration of chemotherapy, this ranged from 78 - 83 days in the bedaquiline/BR-treated group in comparison with 125 - 129 days in the placebo/BR-treated group.[121]

The rates of culture conversion were also significantly faster in patients treated with the bedaquiline/BR than those treated with placebo/BR. During the six months of therapy 79.5% and 81% of patients who received bedaquiline achieved culture conversion to negativity, while approximately 65% of those who received placebo/BR converted.[99,124] Although not statistically significant, data reported by

Pym *et al.*[99] showed that the culture conversion rate is affected by the drug resistance profiles of *Mtb* isolates, decreasing as the degree of drug resistance of the isolates increases. In their study, the rates of culture conversion were 73.1%, 70.5% and 62% for MDR-, pre-XDR- and XDR-TB patients, respectively.[99] The use of bedaquiline has also been beneficial in preventing the emergence of resistance to the companion drugs in the regimens.[2,99] Inclusion of bedaquiline in the drug regimen also resulted in lower relapse rates, with the majority of patients who achieved culture conversion to negativity maintaining this status for long periods, recording a median of 5.4 months of treatment-free follow-up.[99]

Bedaquiline can also be used effectively for the treatment of patients infected with HIV.[99,169] However, as with clofazimine, many ARV agents, such as efavirenz and lopinavir, have demonstrated DDIs with bedaquiline, necessitating replacement therapy with alternative agents such as nevirapine.[170,171]

Mechanisms of resistance to bedaquiline

Since its introduction, an increase in the number of bedaquiline-resistant *Mtb* isolates has been reported.[172] Based on this concern, the WHO has advised that bedaquiline resistance development be carefully monitored. To date, however, standardised assays for the detection of drug resistance in bedaquiline have not been developed. Implementation of surveillance to monitor the emergence of resistance via serial MIC determinations of isolates from patients on bedaquiline therapy, especially when there is a history of prior exposure to clofazimine, has been proposed. In this context, approximately 97% of clinical isolates at baseline drug susceptibility testing

(DST) have shown bedaquiline MIC values ranging from 0.0075 - 0.24 mg/L.[99,125,173,174] Isolates with MIC values >0.24 mg/L, as well as those exhibiting a 4-fold increase in MIC from baseline occurring during treatment, are contenders for possible development of bedaquiline resistance and should be closely monitored. Used in conjunction with early detection of clinical signs of non-response to treatment, monitoring of drug resistance development involves sequential determination of the MIC values of *Mtb* present in sputum samples taken at baseline, and at weeks 8 and 24, following initiation of chemotherapy.[99,175]

In the case of most DR-TB regimens, inclusion of bedaquiline together with a minimum of three other anti-TB agents has been recommended.[2,176] These combination regimens, consisting of fewer anti-TB drugs, do, however, pose an increased risk of development of drug resistance.[12] In this respect, the WHO has recently issued an interim guideline[14] for more discerning use of bedaquiline in DR-TB regimens. In this context, an effective treatment regimen containing four second-line drugs, including pyrazinamide, a fluoroquinolone and a second-line injectable agent, is recommended, with bedaquiline held in reserve for those clinical settings in which such regimens are deemed to be ineffective.[4]

Several molecular mechanisms underpinning bedaquiline resistance have been identified. Most prominent amongst these are mutations occurring at two separate genes. The first gene is the *atpE*, which encodes for F_1/F_0-ATP synthase. Currently, the occurrence of resistance due to mutations at this gene has been reported in approximately 30% of bedaquiline-resistant clinical isolates.[107,177] To

date, this type of resistance has been exclusively associated with mutations at the C subunit of the enzyme.[158]

The second gene associated with bedaquiline resistance is the *rv0678*, which encodes the Rv0678 protein. The majority of bedaquiline-resistant mutants, reported in several studies, have mutations at the *rv0678* gene.[105,107,177] In South Africa, all of the bedaquiline-resistant isolates, as well as some with the potential to develop bedaquiline resistance (>4-fold increase in MIC), have had mutations at the *rv0678* gene.[99] A case of bedaquiline resistance reported from Switzerland, also involved mutation of this gene.[97] Importantly, as mentioned above, *rv0678* gene mutations also lead to cross-resistance with clofazimine, potentially restricting treatment options.[2,6,107]

The impact of *rv0678*-based resistance on the outcome of DR-TB chemotherapy appears variable. In the study by Pym *et al*.[99] 5 of 12 patients who harboured *Mtb* strains which demonstrated a >4-fold increase in their bedaquiline MICs during therapy, nevertheless had a successful treatment outcome.[99] On the contrary, Pule *et al.*[98] have emphasized the significance of efflux-mediated bedaquiline resistance as a contributor to treatment failure.

Rv0678-associated bedaquiline and clofazimine resistance can be attenuated *in vitro* via the use of efflux pump inhibitors, such as verapamil and the protonophores,[106,178] timcodar, reserpine and valinomycin, all of which inhibit the pump by reducing the transmembrane potential.[105,178] Verapamil is particularly effective, decreasing the bedaquiline and clofazimine MICs by at least 8-fold *in*

vitro.[98,106,179] However, the clinical utility of this approach is dubious due to the high levels of verapamil required to achieve these effects, as well as metabolism of this agent by CYP3A4, indicative of a probable DDI when coadministered with bedaquiline and clofazimine.

The second factor contributing to the development of bedaquiline resistance in *Mtb* is the mutation rate, which is dependent on the dynamics of the bacterial population in the clinical lesion or culture. For bedaquiline, the rate of drug resistance development for *Mtb* in culture has been found to be $1/10^8$ cfu/mL. This rate of resistance is relatively low, being comparable to that of rifampicin.[130,180] This bacterial density may be achievable in granuloma lesions of chronic TB patients.[181,182] However, which of the two resistance genes has the highest mutation rate remains unknown, with *rv0678* seemingly the most likely contender.[6,97,99]

An additional factor associated with bedaquiline resistance development is its half-life. Bedaquiline has a longer half-life (4 - 5.5 months) than those of other anti-TB agents in the current DR-TB regimens.[2,142,170] After termination of therapy, its long half-life may favour selection of resistant populations.[4,150]

Pharmacokinetic and pharmacodynamic properties of bedaquiline

Like clofazimine, bedaquiline has poor pharmacokinetic and pharmacodynamic properties. It is highly lipophilic, being distributed mainly in macrophage-rich tissues such as the lungs, while it is found in low concentrations in the blood, increasing with duration of chemotherapy.[183] In other bodily fluids, such as CSF, bedaquiline is

undetectable due to failure of this antibiotic to cross the blood-brain barrier.[184] During TB meningitis, when the blood-brain barrier is inflamed and disrupted, bedaquiline is detectable in the CSF especially in the early phase of treatment, albeit at very low concentrations, diminishing as treatment continues and the integrity of the blood-brain barrier is restored.[184,185]

Like clofazimine, bedaquiline is metabolised by CYP3A4[2,119,150] to a less active metabolite, M2, which is altered during coadministration with anti-TB and ARV agents.[101] In the case of anti-TB drugs, two rifamycins, viz. rifampicin and rifapentine, which are potent CYP3A4 inducers, have been shown to increase bedaquiline clearance (4.78-fold and 3.96-fold for RIF and rifapentine, respectively), resulting in substantial reductions in bedaquiline tissue concentrations (79% and 75% for rifampicin and rifapentine, respectively), necessitating dosage adjustment to achieve the required chemotherapeutic levels.[3,101,155,186] These adjustments may, in turn, contribute to the severity of AEs.[101] In the case of the ARV drugs, lopinavir/ ritonavir, these agents have been shown to increase bedaquiline retention time and serum concentrations.[2,171] On the other hand, bedaquiline can be safely administered with nevirapine without dosage adjustment.[2]

Various AEs associated with the use of bedaquiline have been reported. The most common events are hepatic and cardiac in nature.[187] The former include increased liver enzyme levels. Despite the hepatic complications, patients with mild to moderate hepatic impairment such as those with hepatitis B and C and heavy alcohol use, can still be treated with bedaquiline.[124] In the case of the latter, corrected QT interval prolongation[155] and disturbances of the heart's electrical

rhythm are most prominent.[8,119,172,176] The QT elongation is exacerbated when bedaquiline is used in combination with other antibiotics such as clofazimine, moxifloxacin and ketoconazole in DR-TB therapy.[4,150,188] Accordingly, the use of bedaquiline in regimens containing any of these antimicrobial agents should be closely monitored.[4] Because both clofazimine and bedaquiline carry risk of prolongation of the QT interval and cardiac arrhythmia, which is additive in patients treated with these agents, WHO guidelines recommend weekly electro-cardiograms (ECGs) during the first month, and thereafter monthly in patients treated with both agents.[14] When bedaquiline is used without clofazimine, monthly ECGs are sufficient.

Other AEs, which are nonspecific, include nausea, dizziness, arthralgia, headache, hyperuricemia and vomiting.[8,119,150,155] However, these events were reported to be mild and tolerable in most studies.[99,186]

Worryingly, an increased mortality rate has been associated with bedaquiline therapy. In the pivotal phase II licensing study, a fatality rate of 12.7% was recorded in patients receiving a bedaquiline/BR, compared with 2.5% in the standard comparator group.[121,124] In a subsequent study, a fatality rate of 7% was recorded in the group of patients receiving the bedaquiline/BR relative to placebo/BR.[99] The increased mortality rates reported in these studies were attributed mainly to respiratory disorders of infective and non-infective origin as opposed to bedaquiline toxicity.[121,124]

Table 2. Similarities and differences between clofazimine and bedaquiline

Property	Similarities	Differences	
		Clofazimine	**Bedaquiline**
Structural properties	• are strongly lipophilic.	• is a riminophenazine.	is a diaryquinoline.
Antimicrobial activity	• have a slow onset of bactericidal action. • active against both DS- and DR-*Mtb* strains. • have limited activity against Gram-negative bacteria.	• has dose-independent antimicrobial activity. • has broad spectrum antimicrobial activity against Gram-positive bacteria, parasites and fungi.	• has dose-dependent antimicrobial activity. • has limited antimicrobial activity against mycobacterial species.
Microbial subpopulations	• active against planktonic, slow/non-replicating bacteria. • show high activity against slow/non-replicating bacteria.	• unknown.	• unknown.
Cellular target	• .target the mycobacterial membrane, collapsing the pH gradient and membrane potential. • interfere with mycobacterial energy metabolism. • are antagonised by cytochrome *bd* oxidase.	• probable multiplicity of microbial targets.	• primarily targets the mycobacterial F_1/F_0-ATPase.

Clinical trials	• contribute to shortening of therapy. • result in low relapse rate after treatment cessation. • lead to increased QT prolongation.	• is associated with lower mortality than bedaquiline therapy.	• is associated with higher mortality than clofazimine
Drug resistance mechanisms	• have common mechanism of acquired resistance due to mutations in the mycobacterial transcriptional regulator Rv0678, resulting in upregulation of the MmpS5-MmpL5 drug efflux pump.	• is also associated with mutations at *rv1979c* and *rv2535c* genes. • has a mutation rate of $1/10^{26}$ cfu/mL.	• is also associated with mutations at a*tpE* gene. • has a mutation rate of $1/10^{8}$ cfu/mL.
Pharmaco-properties	• accumulate in fatty tissues. • metabolised by CYP3A4 enzymes. • have DDI effect with several anti-TB and anti-retroviral agents.	• discolours skin and tissues.	• does not cause skin and tissue discolouration.

DDI, drug-drug interaction.

Summary

Similarities and differences between clofazimine and bedaquiline are summarised in Table 2.

Conclusion

Clofazimine and bedaquiline are lipophilic, prototype antimycobacterial agents of the riminophenazine and diarylquinoline classes, respectively. Both antibiotics target the outer membrane of *Mtb*, but differ with respect to target selectivity. The lipophilicity of both agents accounts for their unusual pharmacodynamic/pharmacokinetic properties, resulting in slow and prolonged tissue accumulation, which, in turn, appears to underpin their efficacy in countering the slow-growing, persistent pathogen. Given the differences in phospholipid composition between eukaryotic and prokaryotic cells, these two agents may act as templates for the design of novel membrane-active antimicrobial agents which interact selectively with the outer membrane of prokaryotic cells, enabling improved efficacy and reduced toxicity. With respect to therapeutic efficacy, the early promise of the STREAM trials in evaluating the utility and safety of short-course clofazimine- and/or bedaquiline-based DR-TB regimens is encouraging. However, compelling, definitive recommendations are awaited.

Transparency declarations

None to declare.

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