Drug repositioning in the treatment of malaria and TB

The emergence and spread of drug resistance in the malaria parasite *Plasmodium falciparum* as well as multi- and extremely drug-resistant forms of *Mycobacterium tuberculosis*, the causative agent of TB, could hamper the control of these diseases. For instance, there are indications that the malaria parasite is becoming resistant to artemisinin derivatives, drugs that form the backbone of antimalarial combination therapy. Likewise, *Mycobacterium tuberculosis* strains that are multidrug-resistant or extremely drug-resistant to first- and second-line drugs have been associated with increased mortality. Thus, more than ever, new antimalarials and anti-TB drugs are needed. One of the strategies to discover new drugs is to reposition or repurpose existing drugs, thus reducing the cost and time of drug development. In this review, we discuss how this concept has been used in the past to discover antimalarial and anti-TB drugs, and summarize strategies that can lead to the discovery and development of new drugs.

Malaria and **TB** are the two leading causes of mortally worldwide, killing approximately two million people annually [1]. Strategies to control and manage these two diseases rest primarily on the use of effective drugs.

In the case of malaria, the WHO has recommended the use of artemisinin combination therapies (ACTs) as first-line treatment for uncomplicated malaria with artemether–lumefantrine being the first ACT to be introduced into Africa [2]. Pyronaridine/artesunate and piperaquine/dihydroartemisinin combinations are at late development stages and have reached Phase III/IV clinical evaluation [201]. However, the emergence of *Plasmodium falciparum* parasites resistant to artemisinin derivatives has been reported in South East Asia, raising concern that this could spread to Africa, a scenario that would compromise the current ACT strategies [3].

Quinine (QN) remains the drug of choice for the treatment of severe malaria, and it has become the fall-back option for the treatment of uncomplicated malaria in many countries [202]. However, evidence indicates that resistance to this drug is also spreading in South Asia [4]. This observation led to the investigation of artesunate (an artemisinin derivative) as an alternative to QN for the treatment of severe malaria. This drug has proven superior to QN and, thus, has been recommended for the treatment of severe malaria [5]. However, the spread of artemisinin resistance could also compromise this option. To overcome this potential shortfall, non-artemisinin-based combinations have

been proposed as an alternative. However, the paucity of available **antimalarials** limits this strategy. More than ever, new antimalarials are urgently needed.

The combination of isoniazid, rifampicin, pyrazinamide and ethambutol has been the cornerstone of first-line TB treatment. However, this treatment requires at least a 6-month period, posing the challenge of compliance. In addition, the development and spread of multidrug-resistant strains of *Mycobacterium tuberculosis* (Mtb, MDR-TB), strains resistant to at least two of the most important first-line drugs isoniazid and rifampicin, have become a serious concern. An even more alarming development is the emergence of extensively drug-resistant strains of Mtb (XDR-TB), which are strains resistant to isoniazid and rifampicin, to any fluoroquinolone, and to one (or more) of the three injectable second-line drugs (i.e., amikacin, kanamycin or capreomycin) [6].

Multidrug-resistant TB and XDR-TB treatments are extremely difficult, requiring individualized therapy based on drug-sensitivity tests. Indeed, these treatments are lengthy (>18 months), complicated (require 5–7 drugs), poorly tolerated and extremely expensive, resulting in poor treatment outcomes, especially in patients co-infected with HIV [6–8]. In addition, the emergence and spread of XDR-TB have rendered possible the unacceptable scenario of the inability to clear TB infection using any available **anti-TB** drug regimen. Thus, new drugs with novel mechanisms of action are needed for the management of MDR- and XDR-TB.

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Key Terms

Malaria: Disease caused by a protozoa (apicomplexa) parasite *Plasmodium falciparum* or *Plasmodium vivax* in humans.

Tuberculosis (TB): Human disease caused by the mycobacterium agent *Mycobacterium tuberculosis*.

Antimalarial: Chemical entities that kill, block or prevent the multiplication of the malaria parasite.

Anti-TB: Chemical entities that kill, block or prevent the multiplication of *Mycobacterium tuberculosis*.

Drug repositioning: Finding new therapeutic uses of old and existing drugs.

Furthermore, effective treatment of TB in patients co-infected with HIV is often compromised due to drug–drug interactions. For both infections, drugs are taken for a long period (at least 6 months for TB, and on a chronic basis for HIV), and some drugs have severe drug–drug interactions. For instance, rifampicin, a potent cytochrome P450 3A4 enzyme inducer, increases the metabolism of protease inhibitors, thus diminishing the effectiveness of this important class of HIV drugs. Consequently, rifampicin should not be used when patients are on HIV treatment containing protease inhibitors. To overcome this limitation, analogs of rifampicin with a reduced effect on cytochrome P450, such as rifabutin, have been introduced in lieu of rifampicin. Unfortunately, safe and effective doses of this agent have yet to be established. Thus, new anti-TB drugs are needed that do not interact with anti-HIV drugs.

One of the strategies to discover new therapies against certain diseases is to reposition, repurpose or find new uses for drugs that are already used for other indications. This approach, which has the advantage of reducing the cost and shortening the time of drug development, has become an important area of research by the pharmaceutical industry [9,10]. For instance, in 2004, almost 40% of drugs registered by the US FDA found new uses in the treatment of various conditions in humans [9].

Drug repositioning has previously been exploited in the treatment of malaria and TB. Indeed, some drugs that are, or have been, central in malaria and TB treatment were initially developed for the treatment of non-malaria or TB diseases. In this review, we discuss work that has led to the discovery and development of such antimalarials and anti-TB drugs, and propose strategies to discover new uses for old drugs. We have limited our review to drugs that have reached advanced preclinical stages (animal models) or clinical development in human.

Repositioning in malaria

■ Antibacterial sulfonamides & sulfones

The first drugs to be repositioned for the treatment of malaria were the sulfur-based antibacterial drugs. These drugs were developed in the early 1900s as industrial azo-dyes. The discovery that some of these compounds possessed antibacterial activity led to the development of Prontosil, the first drug ever discovered that could treat a wide range of bacterial infections [11]. Prontosil is a prodrug, which is converted *in vivo* to the active compound sulfanilamide, a sulfonamide derivative [11]. Its success in the treatment of bacterial infections led to the synthesis of several sulfonamide and sulfone derivatives, which were investigated for their potential to treat other infectious diseases, including malaria. These compounds are analogs of *para*-amino-benzoic acid, and therefore block the action of dihydropteroate synthase (DHPS), the enzyme that condenses *para*-amino-benzoic acid with pterin to generate dihydropteroate. The addition of glutamate to the latter gives rise to dihydrofolate, which is then reduced to tetrahydrofolate by dihydrofolate reductase (DHFR) [12]. This *de novo* folate synthesis pathway exists both in bacteria and the malaria parasite.

The sulfonamide drugs sulfanilamide and sulfadiazine, as well as the sulfone dapsone, were among the first sulfa drugs to be used to treat malaria infections [12]. Their use was abandoned because of their low efficacy and unacceptable toxicity. However, renewed interest in this class of antifolates was fostered when it was demonstrated that they synergized with inhibitors of DHFR, thus explaining their use as components in antifolate combinations.

The sulfonamide sulfadoxine has been combined with the DHFR inhibitor pyrimethamine (PM) under the name of Fansidar®. This drug had been extensively used as a first-line treatment for uncomplicated malaria replacing chloroquine (CQ). However, this combination is no longer used for mass treatment because of widespread resistance [12], although it is still of value in intermittent preventive treatment in pregnancy (IPTP) [13]. Another sulfonamide, sulfalene, and the sulfone dapsone have also been combined with PM, under the names Metakelfin® and Malorprim®, respectively. However, they have not been used as widely as Fansidar [12]. Recently, dapsone has been developed in combination with chlorproguanil, which is converted to the inhibitor of DHFR chlorcycloguanil *in vivo*, to treat Fansidar-resistant parasites. Unfortunately, this combination has been withdrawn because of toxicity associated with dapsone [14]. Thus, sulfa-based drugs, initially developed as antibacterial agents, have been central in the development of antifolate-based combinations against malaria. The chemical structures of sulfadoxine and dapsone, along with other repositioned drugs are given in **FIGURE 1**.

■ Antibacterial trimethoprim/sulfamethoxazole

In the past, attempts were made to use Co-trimoxazole® [15], the antibacterial combination of trimethoprim, a potent inhibitor of the bacterial DHFR, and sulfamethoxazole, inhibitor of DHPS, for the treatment of malaria infections. This drug has also been shown to treat malaria infection [16,17], and, in some studies, it was reported to be as efficacious as Fansidar [18]. Unfortunately, Fansidar-resistant parasites are also resistant to Co-trimoxazole, which, as a result, did not present any advantage over Fansidar [19]. Recently, this drug has been evaluated in combination with artemisinin derivatives [20].

Co-trimoxazole has been recommended by the WHO for the treatment of childhood febrile diseases and for prophylaxis against opportunistic infections in HIV-infected patients in Africa [21]. This use as a prophylactic agent has been associated with a reduction in malaria incidents in many parts of Africa [20] in areas of low to moderate Fansidar resistance. Thus, the use of Co-trimoxazole as an antibacterial prophylactic agent can also prevent the incidence of malaria.

The long safety history of Co-trimoxazole when used in pregnancy (to treat bacterial infections) and its antimalarial prophylactic properties have led to the evaluation of this combination in the prevention of malaria in pregnancy. This combination is now part of the Medicines for Malaria Venture portfolio, and clinical trials are currently under way to evaluate its prophylactic properties in pregnancy [203].

■ The anticancer antifolates: methotrexate

Cancer and malaria parasite cells are both rapidly dividing cells. Thus, some of the critical pathways that control cell division can be inhibited by the same compounds. The proof of this concept was provided in the 1970s, when methotrexate (MTX), an anticancer drug that disrupts folate metabolism, was shown to block malaria parasite growth *in vivo* (**FIGURE I**). A clinical trial of MTX at 2.5 mg/day for 5 days indicated that this drug was safe and efficacious to treat malaria infection [22,23]. However, these results have never been exploited because of concerns over MTX toxicity. Indeed, at the time these trials were carried out, MTX was only used in the treatment of cancer, where it was known to be toxic since it was used at high doses.

Methotrexate is used at high doses of up to 5000–12,000 mg/m²/week (130–300 mg/kg/week) for several weeks in the treatment of
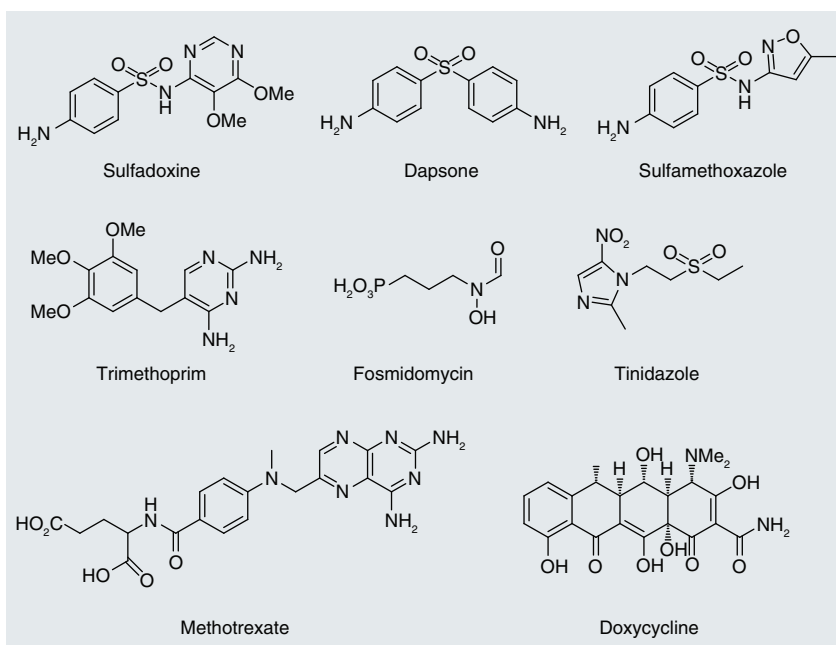

Figure 1. Selected drugs that have been repositioned for the treatment of malaria.

cancer, and this dose can yield serum concentrations of >1000 μM, the range of concentrations that is associated with MTX's life threatening toxicity [24]. On the other hand, a 1000-fold lower dose of MTX (LD-MTX) (0.1–0.35 mg/kg [7.5–25 mg per adult]) has been used once weekly in the treatment of rheumatoid arthritis (RA) on a chronic basis for many years. At this dose, MTX is safe and remains the mainstay in the treatment of RA in the western world [25]. LD-MTX is also the drug of choice for the treatment of juvenile idiopathic arthritis in children (including infants of less than 1 year old), a common rheumatic disease in the western world [26].

The LD-MTX is now considered to be one of the safest drugs used in the treatment of RA and its safety profile has led to its new repositioning in the treatment of various conditions, including multiple sclerosis [27], inflammatory bowel disease [28], urticaria [29], chronic cholestatic disorder [30], Wegener's granulomatosis [31], primary biliary cirrhosis [32] and systemic lupus erythematosus [33], among others.

Taken together, this information has led us to revisit the potential of LD-MTX in the treatment of malaria. Our group and others have demonstrated that MTX is potent against both PM-sensitive and resistant laboratory strains and field isolates, including those carrying the Ileu-164-Leu *dhfr* codon (the most PM-resistant parasite) [34], with $IC_{90}/_{99}$ values (drug concentration

that kills 90 to 99% of parasitemia) of 250 to 450 nM, values within the range of concentrations achieved *in vivo* when LD-MTX is used [35,36]. We have carried out a Phase I evaluation of LD-MTX in 25 healthy male volunteers, as a step towards its development as an antimalarial (NCT00791531 [204]). The results from this trial demonstrate that 2.5 mg/day for 5 days is safe, although the *in vivo* achieved concentration is below 400 nM [37]. Thus, an evaluation of 5 mg/day for 3 days (instead of 5 days) would yield sufficient MTX concentration to clear malaria infection. Thus, the potential exists for this anticancer drug to become an antimalarial.

■ Other antibiotics
Agents with a long-acting effect (delayed-death effect)
Early studies using animal models showed the potential of the antibiotics chloramphenicol and tetracycline to treat murine malaria [38]. However, these drugs were slow-acting, requiring up to 1 week to clear malaria infection. This is now known as the 'delayed-death effect (DDE)', a hallmark of antibiotics such as tetracycline, doxycycline, clindamycin, erythromycin and azithromycin, among others. The mechanism of the DDE is now well understood. These antibiotics target the parasite apicoplast, a nonphotosynthetic plastid organelle unique to apicomplexa parasites. The apicoplast has its own genome and expresses a small number of genes (approximately 30 in total), but the vast majority of its proteome is encoded in the nuclear genome.

Antibiotics disrupt the apicoplast translation machinery during the first replication cycle, leading to a distribution of nonfunctional apicoplasts into the progeny. However, during this first cycle, these antibiotics do not affect the apicoplast metabolic functions, especially those catalyzed by nuclear-encoded proteins, since they are already present in the apicoplast at the time of the antibacterial exposure. This leads to normal parasite growth during the first cycle. The inhibition of translation machinery during the first cycle leads to disruptions in the import or export of new nuclear and apicoplast-encoded proteins during the second cycle, resulting in cell death, hence the DDE. We refer readers to three excellent reviews on this topic [39–41].

This DDE, which is translated *in vivo* by the delayed clearance of the malaria parasite, did not favor their development as antimalarials.

However, the spread of CQ resistance coupled with the need to develop new antimalarials led to a renewed interest in these antibiotics. The first use of these antibiotics was in the prevention of malaria. Tetracyclines (doxycycline and tetracycline) are commonly used as antimalarial prophylactic agents, mainly in Southeast Asia, an area where **multidrug resistance** is prevalent [42].

Antibiotics such as the lincosamide, clindamycin; the macrolide, azithromycin; and erythromycin have been evaluated in the treatment of malaria. Although in some studies radical cures were attained [43], their efficacy overall was limited, and in addition, as discussed earlier, they are all characterized by a delayed clearance time of parasite as a result of DDE [44,45], an effect also associated with delays in fever clearance, making these drugs unattractive for malaria treatment.

To counterbalance this DDE shortfall, these drugs have been combined with rapid-acting standard antimalarials. CQ or QN have been combined with azithromycin, erythromycin or clindamycin [46]. Apart from clindamycin combinations, these combinations have been proven synergistic *in vitro*, justifying their clinical evaluation [47]. Combinations with the antifolates PM/sulfadoxine (Fansidar) have also been investigated [18]. Although overall, the results have been encouraging, none of these combinations have reached Phase III/IV, probably due to concerns about resistance to the partner drugs CQ, QN or Fansidar.

Artemisinin-based combinations of artesunate with azithromycin or clindamycin are being evaluated [46]. However, this concept may be compromised by the emergence of artemisinin resistance [3].

The safety of some of these antibiotics in pregnancy, mainly azithromycin, has led to its use in combination with CQ in the treatment of pregnant women and, especially in IPTP, as an alternative to Fansidar [48]. This combination is now in Phase II/III clinical trials for IPTP (NCT01103063 [205]) [203].

Antibiotics with rapid-acting effects
In addition to the aforementioned antibiotics (those associated with DDE), other antibiotics, such as fosmidomycin, rifampicin or ciprofloxacin, also possess antimalarial activities and eliminate parasites more rapidly than short-acting antibiotics. Fosmidomycin is the most potent rapid-acting antibiotic against malaria, and its discovery has been the most informative. Indeed,

the fatty acid precursor isoprene is synthesized through the 'mevalonic pathway' in mammalian and many other cells. However, bacteria cells have an alternative pathway, known as the 'non-mevalonic pathway'. Fosmidomycin, an inhibitor of one of the critical enzymes of this pathway, deoxy-xylulose 5-phosphate reductoisomerase, is a potent antibacterial agent. Using the malaria genome information, Jomaa and colleagues discovered that malaria parasites use a 'non-mevalonic pathway' to synthesize isoprene, as bacteria cells do, leading to the discovery of fosmidomycin as a potent antimalarial both *in vitro* and *in vivo* [39].

Because it is rapid-acting, fosmidomycin has become an ideal partner to be used in combination with short-acting antibiotics to treat uncomplicated malaria. Fosmidomycin and clindamycin have been evaluated and have reached Phase II/III clinical trials; the combination has proven efficacious [49], although in one study, reduced efficacy was observed in younger children (younger than 2 years) [50]. Fosmidomycin has also been combined with artemisinin derivatives [51]. Clearly, more studies are still needed to establish the efficacy and effectiveness of these fosmidomycin combinations.

The antiprotozoa & antibacterial agent tinidazole

Tinidazole is a nitroimidazole compound, a derivative of metronidazole. Like metronidazole, tinidazole has been used for the treatment of anaerobic protozoa, including amoeba and bacteria. In 1985, James treated a patient with amoeba infection using emetine; this patient also had a co-infection of malaria *Plasmodium vivax*, which was cleared in the same treatment, leading to the investigation of emetine as an antimalarial. Unfortunately, because of its toxicity, the anti-amoebic emetine was replaced by metronidazole, and studies indicate that metronidazole could treat *P. vivax* and *P. falciparum* infections [52]. However, metronidazole toxicity prevented its further investigation as an antimalarial. Tinidazole, a metronidazole derivative, which has a lower toxicity profile than metronidazole, has been developed, and one trial indicated that this drug could clear *P. vivax* infection [53]. Currently, a Phase II investigation of tinidazole efficacy for radical cure (including against the hypnozoites or the dormant forms) of *P. vivax* is being conducted by the Walter Reed Army Institute of Research (NCT00811096 [206]).

Drug repositioning in TB

Over the last five decades, much effort has been dedicated to the discovery and development of new antibacterial agents. The discovery of one agent active against one bacterium species, in most cases, leads to the testing of the same agent against other species. This concept is known as 'spectrum expansion', and drugs that suppress the growth of several different bacteria species have a 'broad-spectrum activity'. As TB is a bacterium species, many broad-spectrum agents have also been tested against this species. Thus, the repositioned drugs in TB are mainly derived from two approaches: 'spectrum expansion' of antibacterials and the new use of non-antibacterial agents. The chemical structures of some repositioned drugs are given in FIGURE 2.

■ Spectrum expansion of antibacterial agents
Oxazolidinones: linezolid
In the 1980s and 1990s, the emergence and spread of resistant Gram-positive bacteria, mainly cocci of the group of methicillin-resistant *Staphylococcus*, vancomycin-resistant enterococci and penicillin-resistant *Streptococcus pneumoniae*
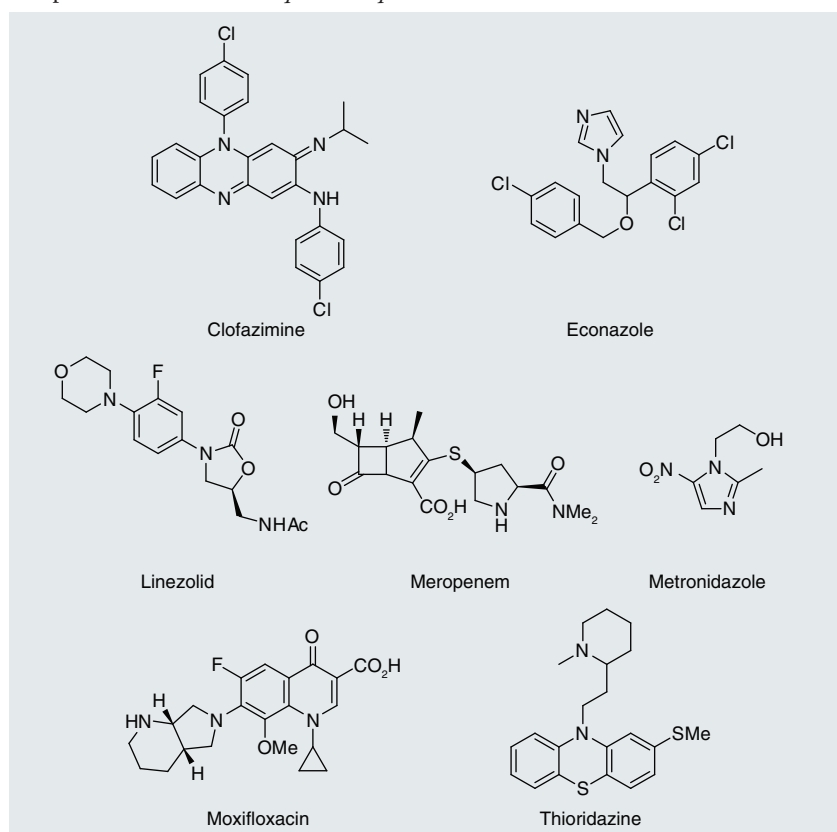

Figure 2. Selected drugs that have been repositioned for the treatment of TB.

prompted extensive work to discover new antimicrobials active against drug-resistant bacteria. This effort led to the discovery of linezolid, an oxazolidinone derivative with broad-spectrum activity. The mode of action of this drug stems from the inhibition of protein synthesis, and unlike most known protein synthesis inhibitors, this drug targets the early stage of protein synthesis [54]. Linezolid was introduced in the 1990s for the treatment of several types of infections caused by methicillin, vancomycin and penicillin-resistant strains [54].

The broad-spectrum activity of linezolid led to the investigation of its potency against TB bacteria. *In vitro* and *in vivo* investigations in the mouse model indicated that this drug was active or moderately active against TB [55]. The use of this drug in a small number of patients yielded encouraging results [56], although low efficacy was also reported [57]. However, its long-term use in the treatment of TB was associated with toxicities, mainly severe anemia, peripheral and optic neuropathy [6,8]. Nevertheless, this drug is currently being evaluated in a Phase I/II trial to treat MDR-TB and XDR-TB in South Korea, Brazil and South Africa (NCT00727844 [207], NCT00396084 [208], NCT00691392 [209] and NCT00664313 [210]).

PNU-100480, a linezolid analog, is being evaluated for both drug-sensitive and drug-resistant TB. This compound is slightly more active than linezolid *in vitro* but significantly more efficacious *in vivo* in the mouse model [58,59], and has pronounced activity in combination with moxifloxacin and pyrazinamide. Currently, two Phase I clinical trials have been completed to evaluate its safety and pharmacokinetics in healthy volunteers (NCT00990990 [211] and NCT01225640 [212]) [6,8]. Another oxazolidinone compound, AZD5847, is also in clinical development for TB (NCT01037725 [213] and NCT01116258 [214]).

Fluoroquinolone

The discovery of quinolone (which eventually gave rise to fluoroquinolone) resulted from research aimed at synthesizing analogs of CQ as antimalarial agents [60]. Chloroquinolone derivatives proved active against bacteria, and further investigations led to the discovery and development of nalidixic acid, the first agent from the quinolone class, which was used for the treatment of urinary tract infections in the 1960s [60]. The insertion of a fluorine atom to the 6-position of quinolone resulted in increased

activity against the enzyme target, the DNA gyrase, and improved transport through the bacteria cell membrane. Norfloxacin (the first fluoroquinolone) was developed in the 1980s, and since then, many fluoroquinolones have been developed and marketed [60]. Several fluoroquinolones have proved to be potent against Mtb, among them, ofloxacin (the first to be tested against TB in humans), ciprofloxacin, levofloxacin, sparfloxacin, gatifloxacin and moxifloxacin. Of these, gatifloxacin and moxifloxacin are the most active [7,8,61]. Fluoroquinolones have been widely used as second-line agents for the treatment of MDR-TB.

One of the limitations associated with current TB treatment is the lengthy time required for a full treatment course, usually varying from 6 to 24 months. *In vivo* studies in mice have demonstrated that gatifloxacin and moxifloxacin have the potential to shorten the treatment of drug-sensitive TB from 6 to 4 months [62], and preliminary clinical studies confirmed similar observations in human when gatifloxacin or moxifloxacin was substituted for ethambutol or isoniazid [63,64]. Further investigations at the Phase III stage are under way to establish the full potential of these two new fluoroquinolone-containing regimens to shorten the treatment duration to 4 months. To date, more than 14 clinical trials have been conducted for the treatment of TB with these two drugs [215]. It is worth noting that gatifloxacin has recently been withdrawn from the market due to safety concerns.

Riminophenazine derivatives: the case of clofazimine

The repositioning of clofazimine is one of the most interesting. Indeed, clofazimine was previously synthesized and tested against TB *in vitro* [65]. This encouraging result led to its investigation *in vivo*, in guinea pig and simian (monkey) models of TB. Unfortunately, its *in vitro* activity did not translate to *in vivo* efficacy and as a result, the drug was abandoned [65].

Although the development of clofazimine for TB was abandoned because of its low efficacy in guinea pig and monkey models, the two models that were then used for TB. However, careful examination of the data indicated that this low efficacy was the result of poor oral bioavailability of this compound in these two animal species, indeed good efficacy was reported in mice and hamster models [65]. In spite of these findings, there was no interest in clofazimine, until its potential against *Mycobacterium leprae*, the

pathogen responsible for leprosy was shown by Browne and Hergerhe [66]. This drug was developed as Lamprene® and marketed for the treatment of leprosy [67]. This drug has also been found to be central in the treatment of infections caused by bacteria species of *Mycobacterium avium* complex, responsible for a disseminated form of infection (not lung infection), and the disease is commonly found in immune-compromised conditions, such as HIV infections [68].

In addition to its bactericidal effect, this compound also has anti-inflammatory properties, leading to its use in non-mycobacterial chronic inflammatory diseases of the skin, such as lupus erythematosus and pyoderma gangrenosum, among others [67]. In addition, it has been reported to possess interesting anti-tumor properties [69].

One of the issues associated with clofazimine is its extremely long half-life (~70 days in humans), which leads to drug accumulation in tissues and skin pigmentation [70]. The emergence of MDR-TB and XDR-TB has led to a renewed interest in clofazimine. This drug has been included in the anti-TB armamentarium for MDR-TB and XDR-TB treatment, albeit its efficacy in humans has yet to be fully established [6,7]. New and improved analogs, with a shorter half-life and than clofazimine without its skin pigmentation liability, are needed to fully capitalize on the potential of this interesting compound class.

β-lactam antibiotics

β-lactam antibiotics are derivatives of penicillin, cephalosporin and carbapenem and form one of the most important classes of antibacterial agents. Indeed, β-lactam antibiotics have broad spectrum activity, killing both Gram-negative and -positive bacteria.

However, this drug class has not been used against TB. An early report in the late 1940s indicated that TB expresses a β-lactamase (penicillinase), an enzyme that degrades the lactam ring of the drug, rendering the drug inactive. Subsequent studies confirmed the low activity of penicillin against TB [71]; as a result, β-lactam antibiotics were not tested further against TB.

The expression of β-lactamase in bacteria has been associated with resistance to β-lactam antibiotics. To overcome this resistance, clavulanic acid, a potent inhibitor of β-lactamases, has been developed. This compound restores β-lactam activity [72], and has been combined with amoxicillin under the trade name of Augmentin™. This combination is now the drug of choice in the treatment of respiratory tract infections [73].

Several reports have shown the inhibitory potency of clavulanic acid against the Mtb β-lactamase, raising a renewed interest in β-lactam antibiotics [74]. Further studies indicated that β-lactam antibiotics of the carbapenem family (especially meropenem and imipenem) and of the cephalosporin family were more active against Mtb *in vitro* than penicillin derivatives [74,75]. The combination of meropenem–clavulanic acid has been proposed as a potential anti-TB agent [76,77]. However, clinical evaluation of this combination has yet to be conducted.

■ Drugs with new indications
Phenothiazines: the case of thioridazine

Phenothiazines are drugs with antihistaminic or antipsychotic properties. Early studies showed the antibacterial activity of chlorpromazine, the first commercially available phenothiazine, which could suppress bacteria growth [78]. However, the serious side effects associated with the use of this agent for psychoses reduced interest in its exploration as an antimicrobial agent.

The search for new drugs active against MDR-TB has led to a renewed interest in this family of drugs. Indeed, new phenothiazines with improved toxicity profiles have been developed, and used in the treatment of psychoses. One of them, thioridazine, has shown promising activity against MDR-TB and XDR-TB *in vitro* and *in vivo* in a mouse model [79]. Evidence indicates that phenothiazines inhibit NADH:menaquinone oxidoreductase activity, an essential enzyme in the energy metabolism pathway and, therefore, represent a novel mechanism of action [80].

Thioridazine has demonstrated unique activity against the nonreplicating Mtb cultures grown under hypoxic conditions [81]. The first-line TB drugs rifampicin and pyrazinamide have demonstrated some activity against the nonreplicating Mtb cultures and are largely responsible for shortening TB therapy from 18 months to 6 months. However, the safety and efficacy of thioridazine against TB has yet to be established in clinical trials [82].

Nitroimidazole derivatives: metronidazole

These drugs belong to the imidazole family, and are used for the treatment of anaerobic protozoa and bacterial infections. One of these drugs, tinidazole, has been repositioned for the treatment of malaria.

Mtb responsible for human TB may adopt various metabolic states. The fast-replicating populations are metabolically active and more susceptible to drug treatment. The slow-growing or nonreplicating populations are metabolically inactive or dormant and much harder to kill. Most available drugs are poorly active against the dormant forms, and these forms are likely responsible for long-term treatment and post-therapy relapse. Thus, drugs active against these dormant forms are needed in the armamentarium against TB.

Most actively replicating tubercle bacilli die rapidly when they are abruptly deprived of oxygen, and few of them shift into a state of dormancy where they adapt to a gradually decreasing supply of oxygen. This adaptation to an hypoxia environment is one of the main characteristics of the dormancy state [83]. Other parameters, such as the lack of nutrients and the production of nitric oxide, can contribute to dormancy [84]. Available information on metronidazole as being selective and effective against anaerobes led Wayne and Sramek to test it against TB [83]. The results revealed the bactericidal effect of this drug against the dormant form of TB, providing a rationale for the use of nitroimidazole derivatives as potential anti-TB agents. Although limited, clinical evaluations of metronidazole in combination with streptomycin, rifampicin and isoniazid have been conducted in human, with encouraging results [85]. Another study evaluating the potency of a metronidazole-containing regimen to clear MDR-TB has recently been completed in South Korea (NCT00425113 [216]).

Two members of a new generation of the nitro-imidazole class are currently under development for the treatment of TB: OPC-67683 and PA-824 [86]. It is noteworthy that these compounds are prodrugs that require activation by mycobacteria, through nitro-reduction. This process produces multiple, highly reactive species that have been shown to inhibit lipid (and therefore cell wall) and protein biosynthesis [87]. These drugs have now reached Phase II clinical trials for the treatment of both drug-sensitive and -resistant TB (NCT00685360 [217] and NCT00567840 [218]), and PA-824 is part of the TB Alliance portfolio [219].

Azole antifungal drugs: econazole
The rationale behind the repositioning of this drug family is based on exploiting the Mtb genome. The mechanism of action of azoles involves inhibition of the fungus enzyme 'lanosterol 14 α-demethylase' (also known as CYP51A1 and P45014DM). This enzyme leads to the synthesis of ergosterol from lanosterol, an important constituent of the fungus cell membrane [88]. Using genome information, a homologous gene that encoded for a sterol 14 α-demethylase was identified, raising the hypothesis that azole compounds could also inhibit TB growth. This hypothesis was later proven by Ahmad *et al.* who showed that the azole antifungals econazole and clotrimazole have anti-TB effects both *in vivo* and *ex vivo*, including against MDR-TB [89–91]. However, azoles, in general, have poor oral bioavailability, limiting their use as oral drugs. To overcome this limitation, nanoparticles encapsulated with an econazole-containing regimen are being evaluated, and have reached the animal evaluation stage [92], a step towards their development for potential oral use against MDR-TB and XDR-TB.

How to identify new antimalarial & anti-TB agents from old drugs
The aforementioned repositioned drugs were discovered using different strategies, which we have summarized below.

■ Similarity in cell biology & biological processes
As discussed earlier, several strategies have been used to identify drugs for repositioning. The first is cell biology-based. In this regard, it is noteworthy that bacteria, malaria parasites and cancer cells are rapidly dividing cells, thus drugs that target one of these cells could also potentially block the division of the other cell. The previously mentioned sulfa-drugs as antimalarial agents are excellent examples of this approach. Further investigation demonstrated that sulfa-based drugs target the same enzyme in both bacteria and malaria, the DHPS enzyme. Likewise, MTX, an inhibitor of the mammalian folate pathway, also blocks the synthesis of folate in the malaria parasite, highlighting its potential as an antimalarial. *In vitro* studies have demonstrated that many other anticancer drugs are endowed with antimalarial activity, thus could potentially be repositioned to become antimalarial drugs if they demonstrate an acceptable safety profile at a dose that can yield sufficient drug concentration to clear malaria infections.

Another example where similarities in cell biology and/or biological processes in different organisms could be targeted for drug

repositioning in malaria is antiretroviral protease inhibitors (APIs). Aspartic proteases are also important in malaria parasites (as plasmepsins), and APIs have been reported to have inhibitory effects on the malaria parasite *P. falciparum*. The APIs saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir and atazanavir directly inhibit erythrocytic stages of *P. falciparum* grown *in vitro* at concentrations achieved *in vivo* [93,94]. Some APIs also seem to exert an effect on the pre-erythrocytic stages of the malaria parasite. The APIs saquinavir and lopinavir inhibited the development of extra-erythrocytic liver stages *in vitro* using *Plasmodium berghei*, a rodent strain of malaria. *In vivo* animal (mouse) studies using the rodent strain *Plasmodium yoelii* showed a reduction in liver parasite burden when lopinavir/ritonavir was administered [95]. Thus, APIs could be repositioned for use in malaria chemotherapy, with the advantage of being used in malaria–HIV co-infections.

TB and other bacteria species share more than 99% of biochemical pathways, thus antibiotics with broad-spectrum activity are also likely to be active against Mtb, so long as they are able to efficiently cross the mycobacterial cell wall. The use of this approach has led to the discovery of important anti-TB drugs, such as moxifloxacin and gatifloxacin (fluoroquinolone), linezolid (oxazolidinone), clofazimine (riminophenazine) and meropenem-clavulanic acid (lactam/lactamase inhibitor). Other antibiotics, such as the macrolide clarithromycin, although not active against TB, can synergize with standard anti-TB drugs, such as rifampicin and isoniazid *in vitro,* raising the possibility of combining macrolides with anti-TB drugs [96]. However, *in vivo* studies are still needed to confirm this concept.

The existence of similar biological processes in cells, even phylogenetically far from each other, can also be exploited. For instance, metronidazole is known to be active against anaerobic organisms, and it is reasonable to postulate that the process of dormancy or latency in TB is similar to hypoxia conditions. As already mentioned, this observation led to the testing of metronidazole against TB.

■ Exploitation of genome information
Another approach is the use of cell genome information to identify potential drug targets that have been validated in another organism. For instance, using the malaria genome information, Jomaa and colleagues discovered that bacterial and malaria parasites utilize the same non-mevalonic pathway to synthesize isoprene. Since fosmidomycin, a drug targeting this pathway, had already been developed against bacteria, the testing of the same drug led to its discovery as an antimalarial. In TB treatment, this approach has been exploited with the discovery that mycobacteria have a gene that encodes for a sterol 14 α-demethylase, an enzyme that is the target of azole compounds in fungi. Thus, this family of drugs could also kill Mtb. One of the azoles, econazole, has proven potent against TB.

Another strategy could explore interactions of proteins with existing drugs, on a proteome-wide scale, using an integrated chemical and biological computational strategies. The binding site of an existing drug can be predicted from a 3D structure or model of the target protein, and using this information, off-targets with similar ligand-binding sites can be identified across the proteome using functional site search algorithms. Drugs that give rise to favorable protein–drug complexes would be good potential candidates for drug repositioning. This approach has been used recently, and has led to the discovery of entacapone and tolcapone, two drugs used for the treatment of Parkinson's disease, as potential anti-TB drugs [97].

■ Revisiting or reconsidering data from the failed repositioning of drugs
Attempts have previously been made to reposition many drugs. So far this has met with little success due to failures at various stages of the value chain. The revisiting or reconsideration of data generated during these studies could lead to their 'rediscovery'. For instance, since the 1950s, β-lactam antibiotics have been abandoned as potential treatments for TB because the cell expressed β-lactamase (penicillinase), the enzyme that degrades these drugs. However, since then, inhibitors of β-lactamase (such as clavulanic acid) have been developed, and combined with β-lactam antibiotics for clinical use. Thus, the same combinations could also be tested against TB; one of these combinations, meropenem-clavulanic acid has proven active in preclinical stages [76,77].

Clofazimine was initially discovered as an anti-TB agent but it was abandoned for this purpose. Instead, it was developed as an antileprosy agent. As discussed earlier, it was abandoned because it was found not to be efficacious in the TB animal models that were in use at the time. Subsequent studies indicated that the two animal species used are associated with poor bioavailability

of clofazimine, explaining the observed lack of efficacy. Clearly, these previous models were not appropriate for clofazimine. The potential of this drug class as anti-TB agents has been revisited, and it could become part of the anti-TB armamentarium [6,7].

Drugs used in one disease can present high risks of toxicity in another disease, and some attempts at drug repositioning failed because of the risk of toxicity. However, the development of analogs with better safety profiles could lead to successful drug repositioning.

For instance, the antiprotozoa metronidazole was abandoned as an antimalarial agent because of its toxicity. However, the development of a safer analog, tinidazole, to treat protozoa, led to its testing against malaria. This drug is now undergoing clinical evaluation to treat *P. vivax* malaria [53]. Another interesting example is with the phenothiazine chlorpromazine, the antihistaminic and antipsychotic agent. Its potential to inhibit bacteria growth was proven in the 1950s but toxicity prevented its further development [78]. A new phenothiazine, thioridazine, an antipsychotic has been developed, and this drug has a better safety profile than chlorpromazine.

■ **Exploitation of co-infection drug efficacy**
Drugs can be repositioned by careful observation of co-infection treatment. Indeed, many diseases occur as co-infections with malaria and TB, or malaria and HIV, or HIV and TB, thus the treatment of one disease could also clear the concurrent disease. For instance, the discovery of metronidazole and, later, its analog tinidazole, a drug that is now undergoing clinical evaluations against *P. vivax*, has its origins in studies on the treatment of the protozoa amoeba in patients co-infected with malaria.

■ **Drug-screening studies**
Finally, new therapies could be discovered by screening of old and existing drugs against malaria parasites and Mtb. Recently, a screening of a library of 1000 known drugs against *P. falciparum in vitro* led to the discovery of the antimalarial activity of astemizole, an antihistaminic drug [98]. Pharmacokinetics data indicate that safe and tolerable doses of this drug could yield effective concentrations that could clear malaria infection *in vivo*, warranting its further evaluation in humans [98]. More recently, a medium throughput screening of a library of 1514 known drugs using the Alamar Blue assay resulted in the identification of 17 novel inhibitors of Mtb, and

among them the antimalarial primaquine, a drug used to treat the dormant forms (hypnozoites) of malaria [99]. The antimalarial mefloquine has also been discovered to be active against Mtb [100]. This drug is known to have serious neurological side effects; as a result, analogs are being synthesized and tested as potential anti-Mtb agents [101–103].

Conclusion
The emergence of artemisinin resistance is threatening the current concept of ACTs. The spread of MDR-TB and XDR-TB is reducing the effectiveness of current anti-TB drugs. Therefore, new drugs are urgently needed. The human pharmacopoeia is rich. It is estimated that 12,000 drugs have been used in humans. It is, therefore, not unreasonable to propose that several of these drugs have the necessary pharmacological properties to become new anti-TB and/or antimalarial drugs. The challenge remains to identify them. Strategies exist to identify such drugs, but the main limitation is human and financial resources. An excellent opportunity now exists to identify new drugs at a low cost and in a relatively short time period.

However, while new drugs can be identified by drug repositioning, the challenge remains to discover active drugs against dormant/persister tubercle bacilli in TB, and the dormant liver stage parasites (hypnozoites) in malaria. Thus, drug repositioning is only the first step in finding new uses of old drugs, subsequent 'drug evolution' (optimization of repositioning drugs in new applications) should be undertaken afterwards. These specific unique challenges are critical in controlling the spread of multidrug resistant TB and malaria.

Future perspective
The selection and spread of resistance to antimalarial and anti-TB agents require that new drugs be discovered urgently. Drug development is a long and costly undertaking. Given the lack of market incentives and adequate funding to support drug development in these areas, drug repositioning is going to become a core strategy in malaria and TB drug development. As we discussed, strategies exist to streamline this process.

Acknowledgments

Executive summary

Background: repositioning in malaria & in TB

- Emergence of resistance to artemisinin, the backbone of antimalarial therapy, and the development of multidrug-resistant strains and extensively drug-resistant strains are of great concern.

- New drugs are urgently needed to counterbalance this burgeoning drug-resistance problem.

- One of the strategies to discover new therapies is to reposition, repurpose or find new uses for drugs that are already used for other indications.

- Drug repositioning has already been exploited in the past in the treatment of malaria and TB. For instance, sulfa-based drugs for malaria, and fluoroquinoline for TB were initially developed for the treatment of non-malaria or TB diseases.

How to identify new antimalarial & anti-TB agents from old drugs

- Similarities in cell biology and biological processes: a drug that targets one pathway in one organism can also target the same pathway in a different organism. For instance, sulfa-based drugs were discovered as antibacterials but have been used as antimalarials. Likewise, some antibiotics discovered for non-TB infections have been repositioned in TB.

- Exploitation of genome information: the discovery of the non-mevalonic pathway to synthesize isoprene in malaria by genome analysis, led to the clinical evaluation of fosmidomycin, a drug developed as an inhibitor of the non-mevalonic pathway in bacteria. Likewise, the exploitation of TB genome indicated that this microorganism encodes for sterol 14 α-demethylase enzyme, the enzyme target of azole compounds in fungal organisms. One of the azoles, econazole, has proven potent against TB.

- Revisiting or reconsidering data from failed repositioning drugs: some drugs failed to be repositioned in TB or malaria because they were deemed to be toxic. However, since then, new analogs have been developed with reduced toxicity, and these analogs could be repositioned. One of the best examples is tinidazole replacing metronidazole (used in amoeba infection) in malaria treatment, or the antihistaminic and antipsychotic chlorpromazine being replaced by thioridazine in the treatment of TB.

- Exploitation of co-infection drug efficacy: drugs can be repositioned by careful observation of co-infection treatment. For instance, the discovery of metronidazole, and later its analog tinidazole, a drug that is now undergoing clinical evaluations against *Plasmodium vivax*, has its origins in studies on the treatment of the protozoa amoeba in patients co-infected with malaria.

- Drug-screening studies: new therapies could be discovered by *in vitro* screening of old and existing drugs using high-throughput methods. For instance, screening studies have indicated that the antihistaminic astemizole possesses antimalarial activity, whereas the antimalarial primaquine is a potential anti-TB drug.

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