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Repurposing of Tuberculosis Drug Candidates for the Treatment of *Mycobacterium ulcerans* Disease

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Abstract: Buruli ulcer (BU) is a chronic necrotizing skin disease caused by *Mycobacterium ulcerans.* Historically, the disease was treated by surgical excision of the skin lesions, until an 8-week combination therapy of rifampicin and streptomycin was introduced in 2004. This treatment modality was effective and reduced recurrence rates. Rifampicin is the most efficacious antibiotic for the treatment of BU and, should rifampicin-resistant *M. ulcerans* strains emerge, there is currently no replacement for it. As for mycobacterial diseases in general, there is a pressing need for the development of novel, fast-acting drugs. Under market economy conditions, repurposing of new tuberculosis drug candidates is the most promising avenue for alternative BU treatments. Our drug repurposing activities have led to the identification of several actives against *M. ulcerans.* In particular, the cytochrome *bc*1 complex inhibitor telacebec (Q203) is a promising drug candidate for the treatment of BU in Africa and Australia. While an active cytochrome-*bd* oxidase bypass limits the potency of the cytochrome-*bc*1-specific inhibitor telacebec against *M. tuberculosis,* classical lineage *M. ulcerans* strains rely exclusively on cytochrome-*bc*1 to respire. Hence, telacebec is effective at nanomolar concentration against *M. ulcerans,* and a high treatment efficacy in an experimental mouse infection model indicates that treatment of BU could be substantially shortened and simplified by telacebec.

Keywords: Buruli ulcer · Drug repurposing · *Mycobacterium ulcerans* · Q203 · Telacebec

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Gerd Pluschke studied biochemistry and obtained his PhD at the University of Tübingen. After a post-doctoral stay at the Max-Planck-Institute for Molecular Genetics (Berlin, Germany) he worked at the Washington University (St. Louis, USA), the Basel Institute for Immunology, the University of Freiburg (Germany) and with Ciba Pharma Research. He joined the Swiss Tropical and Public Health Institute in 1995, where he is investigating host–pathogen interactions in malaria, meningococcal meningitis and *Mycobacterium ulcerans* disease. Translating research findings into the development of new drugs, vaccines and diagnostics is an important aim of his research.

1. Buruli Ulcer, a Neglected Tropical Disease

Mycobacterium ulcerans causes the chronic necrotizing skin disease Buruli ulcer (BU),[1] which has been reported from over 30 countries worldwide.[2a,b] Most BU cases occur in Central and West Africa, but some regions of Australia/Oceania, Asia and the Americas are also affected. *M. ulcerans* has developed from *M. marinum* by the acquisition of a plasmid that encodes the enzymes required for the production of a macrolide toxin designated mycolactone.[3a,b] At least three mycolactone-producing *M. ulcerans* sublineages have evolved through reductive evolution.[4a–c] Two of these sublineages are associated with BU disease in humans. Ancestral lineage strains have been isolated from BU patients from Asia and the Americas,[5] where they only sporadically cause disease in humans. In Africa and Australia, BU is caused by classical lineage strains and here the local incidence may be as high as >1/1,000 persons per year. Due to limitations in surveillance activities and access to biomedical treatment, there is considerable underreporting of BU in many endemic areas of Africa, as patients associated with traditional healers (*i.e.* outside of the formal health system) may not appear in the official prevalence statistics.

The mode of transmission of *M. ulcerans* is not clear, but direct human-to-human transmission seems to be rare.[6a,b] It has been suggested, however, that shedding of bacteria from the chronic ulcers may contribute to the contamination of the environment with *M. ulcerans.* Emergence of BU is associated with slow-flowing and stagnant freshwater bodies and it is assumed that patients are infected *via* skin trauma or insect bites from an environmental reservoir of the bacteria. Although the genomic diversity of African disease isolates is very limited, local clonal complexes have been identified by whole-genome sequencing in individual BU-endemic areas.[7a,b] Development of these local clonal complexes

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and the focal transmission of individual genomic *M. ulcerans* variants[8] speak against mobile reservoirs and an involvement of insect vectors that fly over large distances. In short-term visitors to BU-endemic areas of Australia, the incubation period for BU was estimated to be widely variable with a mean of about 4.5 months.[9] While BU patients in Africa and Papua New Guinea are primarily children, the average age of patients in Japan and in the south-eastern part of Australia is much higher.[10a,b] In African BU endemic settings, young teenagers and the elderly are at the highest risk of developing BU disease.[11] Children below the age of 4 years carry a low risk and serological studies indicate that their exposure to *M. ulcerans* is limited.[12] In endemic African communities, many inhabitants have developed immune responses against *M. ulcerans* without developing overt disease.[13] Several single nucleotide polymorphisms in genes implicated in the regulation of macrophage activation and apoptosis are associated with susceptibility to BU.[14] After inoculation of *M. ulcerans* into subcutaneous tissue, the efficacy of the innate immune defence mechanisms may thus determine the outcome of the infection.[15]

BU is a chronic necrotizing skin disease which primarily affects subcutaneous adipose tissue. Mycolactone plays a key role in the necrotizing pathology of the disease. The macrolide toxin drives host cells into apoptosis and downregulates systemic immune responses by the suppression of cytokine production.[16] *M. ulcerans* grows best at temperatures below the core body temperature, favouring infection of the skin. Most BU lesions are located on the lower or upper limbs, but the skin of all body parts may be affected. BU disease starts with the development of a single painless subcutaneous nodule or papule. As the disease progresses, the dermis and epidermis overlying the initial infection focus degenerates and sloughs off. Tissue destruction spreads laterally and ulcers with undermined edges and a necrotic slough at the base develop. Contiguous coagulation necrosis leads to the destruction of local blood vessels and the development of interstitial oedema. Epidermal hyperplasia, fat cell ghosts, and extracellular clusters of acid-fast bacilli in an amorphous coagulum without living inflammatory cells are histopathological hallmarks of the disease.[17] The necrotic process may eventually extend to deeper structures like muscle or bone. Extracellular clusters of *M. ulcerans* are primarily found in the deep layers of the necrotic adipose tissue,[18] but tissue destruction extends far beyond through the diffusion of mycolactone.

2. Treatment of BU

Historically, chemotherapy of BU was considered ineffective, and wide surgical excision of lesions was the only recommended treatment. Clinicians who anecdotally tried chemotherapy of BU, most likely interpreted paradoxical reactions, which include progressive ulceration of primary lesions and the occasional development of new lesions, as treatment failure. In fact, >30% of patients enrolled in a clinical trial of the combination chemotherapy with streptomycin and rifampicin showed an increase in lesion size at week 8, as compared to week 6, 83% of non-ulcerative lesions ulcerated, and 7% of the patients developed new lesions.[19]

It had already been shown in 1975 that rifampicin has *in vitro* activity against *M. ulcerans*.[20a,b] However, only results of a clinical trial conducted nearly 30 years later, which demonstrated killing of *M. ulcerans* in early BU lesions,[21] changed the thinking about the potential of antimicrobial treatment for BU. In 2004, the World Health Organization (WHO) published provisional guidance recommending an 8-week combination therapy of oral rifampicin and intramuscular streptomycin.[22] This treatment modality was effective and reduced recurrence rates. Due to the ototoxicity and nephrotoxicity of streptomycin,[19] replacement of streptomycin by oral clarithromycin is now recommended for treatment of BU in Africa.[23] Rifampicin is the most efficacious antibiotic for the treatment of BU and, should rifampicin-resistant *M. ulcerans* strains emerge, there is currently no replacement for it.[24] This is a distinct possibility, as rifampicin-resistant strains have been isolated after monotherapy in experimentally treated mice.[25] Consequently, methods for the determination of resistance in clinical samples have been established,[26] although they are not routinely used. It would also be highly desirable to have alternative treatment options for patients experiencing rifampicin-related adverse events, such as hepatotoxicity or hypersensitivity reactions. Furthermore, there is an urgent need for the development of new fast-acting drugs for mycobacterial diseases in general.

3. Laboratory Methods for Drug Screening against *M. ulcerans* and the Repurposing of Drugs

Laboratory work with *M. ulcerans* is challenging as *M. ulcerans* is an extremely slow-growing mycobacterium having a generation time of several days. Furthermore, the bacteria form biofilms, generating problems with respect to the quantification of cultures. Determination of minimum inhibitory concentrations (MICs) is used as the 'gold' standard for the assessment of the susceptibility of a microorganism to antimicrobial agents. This typically involves the exposure of a microorganism to decreasing concentrations of an antimicrobial of interest and determining the lowest concentration required to inhibit a defined proportion (typically 50%) of the microorganisms. Due to the slow growth of *M. ulcerans*, it is necessary to incubate culture plates at a permissive temperature (28–33°C) for several months before colony-forming units (CFUs) can be counted. Hence, measurements of the metabolic activity of the bacteria using the redox-sensitive reagent resazurin are frequently used.[27]

Although a broad range of animal models have been described for experimental infection studies with *M. ulcerans,*[28] a mouse footpad infection model is most commonly used for *in vivo* drug efficacy testing.[29] Progression of the infection and of treatment effectiveness are assessed by measuring footpad thickness. Bacterial multiplication in footpads of sacrificed animals is determined by quantitative polymerase chain reaction (qPCR) and by CFU plating of tissue lysates. Histopathological analyses can give deeper insights into pathogenesis and treatment efficacy.[30]

The slow-growing *M. ulcerans* and the evolutionarily related *M. tuberculosis* complex may share certain drug targets. However, *M. ulcerans* has only limited susceptibility to a range of anti-tubercular drugs. When comparing a chemically diverse set of 83 anti-mycobacterial agents in different stages of development for activity against both *M. tuberculosis* and *M. ulcerans,*[31] we found that most *M. tuberculosis* active compounds were inactive or only weakly active against *M. ulcerans* (Fig. 1). Marked resistance of *M. ulcerans* to a variety of scaffolds with anti-tubercular activity may in part be related to loss through genome reduction of certain drug targets or of enzymes required for prodrug bio-activation.[4a] Furthermore, the production of a highly hydrophobic extracellular matrix by *M. ulcerans* may also play an important role.[32] Compounds with high activity against *M. tuberculosis* may thus not necessarily be suitable leads for *M. ulcerans* drug development.

Nevertheless, in view of market economy conditions and the massive costs involved in drug discovery and development, repurposing of new scaffolds under development for tuberculosis treatment, represents the most viable strategy for the search for new antibiotics against *M. ulcerans*. Such drug repurposing strategies can build on pre-existing pharmacology, formulation and safety data and can potentially lead rapidly to clinical testing for BU treatment. Our drug repurposing activities for BU initially led to the identification of several moderately active compounds,[33a,b] as well as some highly active compounds. For instance, we found that an acid-oxidizing solution developed for the treatment of

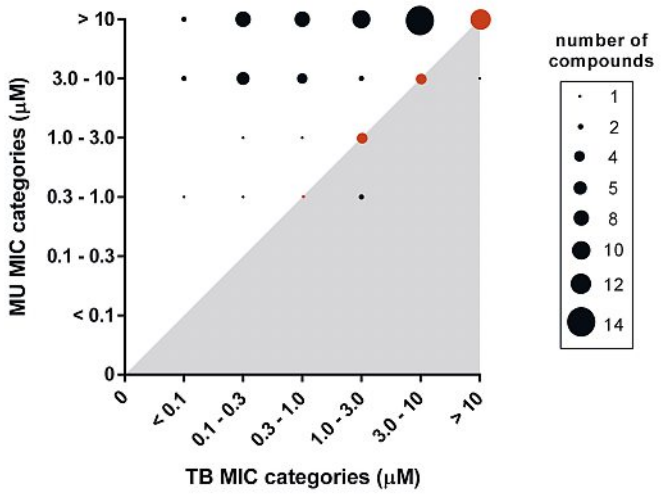
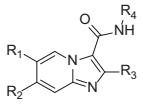


Fig. 1. Comparison of MIC values of *M. tuberculosis*-active compounds of different development stages against *M. ulcerans* and *M. tuberculosis*. The number of compounds at a given coordinate are reflected by the size of the dots. Dots in the grey triangle represent the few compounds that are more active against *M. ulcerans* than against *M. tuberculosis*. Dots shown in red represent compounds which have the same activity against both mycobacteria. Figure taken from ref. [31] with permission.



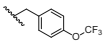
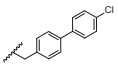
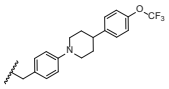
#	R¹	R²	R³	Mol. Wt	cLogP*	R⁴	MIC (nM)
80	H	H	Me	215.2	1.90		>100,000
31	H	H	Cl	328.8	3.2		950
13	Cl	H	Et	397.8	5.4		98
5	Cl	H	Et	424.3	7.0		15
telacebec	Cl	H	Et	557.0	7.6		0.9
ND-10885	H	Me	Me	321.5	3.65		54
ND-11176	Me	H	Me	347.3	4.5		2.3

Fig. 2. Activity of IPA derivatives against the classical *M. ulcerans* strain S1013. Compounds **5**, **13**, **31** and **80** are representatives of different IPA activity groups. *cLogP was calculated with PerkinElmer ChemDraw Professional 16.0.1.4. Figure taken from ref. [38] with permission.

chronic wounds, is highly active against *M. ulcerans*.[34] In view of the broad-spectrum microbicidal activity of the acid-oxidizing solution and that it does not disturb granulation tissue formation, it may be a suitable adjunct to systemic antibiotic treatment preventing secondary infections[35] and the spread of *M. ulcerans* into the environment. Activities in the nanomolar range were eventually observed, when we screened imidazopyridine carboxamide (IPA) compounds for activity against *M. ulcerans*.

4. Identification of Telacebec as Promising Drug Candidate for the Treatment of BU

Mycobacteria require oxidative phosphorylation for energy generation and this process is carried out *via* their electron transport chain (ETC).[36] Energy generation *via* the ETC involves several enzymes, including the cytochrome oxidases that generate a proton gradient and the ATP synthase that uses this proton gradient to generate ATP. The major cytochrome oxidase is the cytochrome-bc_1:aa_3 (cyt-bc_1:aa_3), although some mycobacteria also have a secondary *bd*-type cytochrome oxidase (cyt-*bd*). Only one type of ATP synthase is present in the mycobacterial electron transport chain.[37]

Screening of IPA compounds targeting the cyt-bc_1:aa_3 revealed that they have exquisite activity against *M. ulcerans*.[38] In particular, Q203 (now designated telacebec), a phase 2 drug candidate for tuberculosis, showed activity at nanomolar concentration. While the presence of an alternate *bd*-type terminal oxidase limits the potency of telacebec against *M. tuberculosis*, cyt-bc_1:aa_3, is the only terminal electron acceptor of *M. ulcerans* strains belonging to the classical lineage, which cause BU in Africa and Australia.[38] In these strains, the cyt-*bd* oxidase gene *cydAB* harbours a nonsense mutation converting the tryptophan encoding codon at amino acid position 231 into a stop codon. Reductive evolution can thus eliminate metabolic redundancy and drive hyper-susceptibility to certain classes of drugs. Such reductive evolution has also made *M. leprae* exquisitely sensitive to telacebec.[39]

Profiling of 87 IPA compounds revealed a comparable structure–activity relationship for *M. ulcerans* and *M. tuberculosis,* with telacebec being the most active derivative (Fig. 2).

While for classical *M. ulcerans* strains the MIC_{50} was <1 nM, the MIC_{50} for ancestral lineage isolates from Japan possessing a functional cyt-*bd* were 4- to 8-fold higher (Table 1). In contrast, classical and ancestral lineage isolates were equally sensitive to the ATP synthase inhibitor bedaquiline (Table 1).

While telacebec is only bacteriostatic for *M. tuberculosis,*[40] oxygen respiration of classical lineage *M. ulcerans* strains is completely inhibited (Fig. 3), leading to ATP depletion and rapid cell death.[38]

Table 1. Growth inhibitory activity (MIC_{50}) of telacebec and bedaquiline against classical *M. ulcerans* strains from Africa and Australia and against ancestral strains from Japan. Average MIC_{50} values for three strains per geographical origin are given.

	Classical lineage		Ancestral lineage
	Africa	Australia	Japan
telacebec	0.86 nM	0.57 nM	4.04 nM
bedaquiline	71.1 nM	70.7 nM	47.4 nM

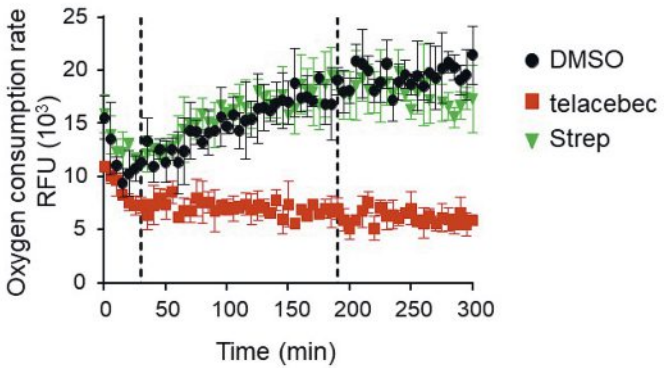


Fig. 3. Arrest of oxidative phosphorylation in classical lineage *M. ulcerans* strains by inhibition of cyt-*bc1:aa3*. Oxygen consumption rates in classical lineage *M. ulcerans* bacteria treated with telacebec (5 nM), streptomycin (5,000 nM) or DMSO (1%). Rates were measured using the MitoXpress® Xtra-oxygen probe. Figure taken from ref. [38] with permission.

The high activity of telacebec against classical *M. ulcerans* strains translated into high treatment efficacy in the mouse footpad infection model.[38,41a–e] Oral treatment with 0.5 mg/kg body weight three times per week for 4 weeks led to a complete regression of footpad swelling as early as 10 days after treatment initiation and no relapse was observed over a 6-month post-treatment observation period.[38] When the drug was administered to the infected mice once (20 mg/kg) or weekly for 4 weeks (5 mg/kg), the bacterial load diminished rapidly, reaching the limit of detection at 4 weeks post-treatment (Fig. 4) and no relapse was observed up to 24 weeks.[41b] An addition of a second drug (rifampicin or clarithromycin) does not significantly increase efficacy of telacebec in the mouse model.[41d,e] The natural loss of cyt-*bd* function in *M. ulcerans* indicates that telacebec is likely to have a higher efficacy and safety margin for BU treatment in Africa and Australia than for tuberculosis treatment, and even a single-dose cure of BU may become possible.

Clinical development of telacebec for treatment of BU can build on results of first clinical trials. A dose-escalation study to evaluate safety, tolerability and pharmacokinetics of single doses of telacebec in normal, healthy volunteers (NCT02530710) showed that single oral doses of 10 mg and up to 800 mg are well tolerated.[42] Subsequently, a dose-escalation study to evaluate safety, tolerability and pharmacokinetics of multiple doses of Q203 in normal healthy volunteers (NCT02858973) showed that daily doses of 20 mg up to 320 mg given for 14 days are well tolerated.[42] Peak serum concentrations found in this clinical trial ranged from 76 to 1,502 ng/ml. In a phase 2 study to evaluate early bactericidal activity and safety of multiple oral doses of telacebec in tuberculosis patients (NCT03563599), increasing doses were associated with greater reductions of the viable mycobacterial sputum load.[43] While no serious adverse drug reactions were observed in the phase 2 study in adults, the safety profile in children has yet to be determined. In view of these promising clinical data, in spring 2023 the TB Alliance entered into a license agreement with Qurient Co. Ltd. (Gyeonggi-do, Korea), the developer of telacebec. The goal is to develop and commercialize telacebec for the treatment of tuberculosis and other non-tuberculosis mycobacterium infections. For the treatment of BU, telacebec holds exceptional promise, opening the possibility of developing a drastically simplified and safe treatment regimen.

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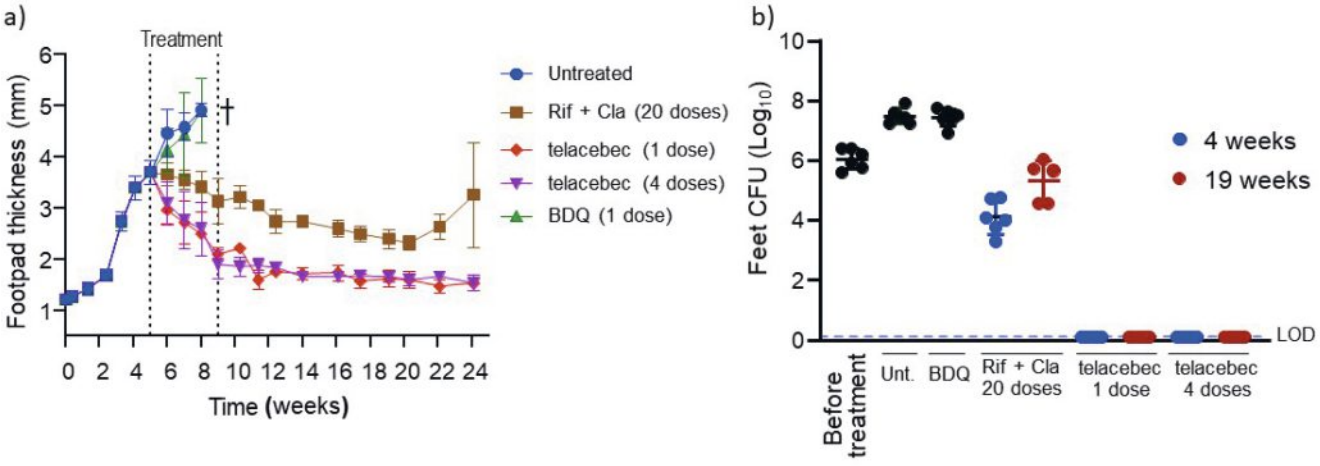

Fig. 4. Treatment of *M. ulcerans*-infected mice with telacebec. a) Mice infected with *M. ulcerans* 5 weeks before treatment initiation were randomly assigned to oral treatment with 1 dose of telacebec (20 mg/kg), 4 doses of telacebec (5 mg/kg), 1 dose of bedaquiline (BDQ; 20 mg/kg), 20 doses of rifampin (10 mg/kg) plus clarithromycin (100 mg/kg) or dosing vehicle alone. Footpad thickness was measured with a calliper weekly over 24 weeks. b) CFU counts in the infected feet were enumerated 4 and 9 weeks after start of treatment. Figure taken from ref. [41b] with permission.

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