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Toward a Single-Dose Cure for Buruli Ulcer

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ABSTRACT A single dose of Q203 (Telacebec), a phase 2 clinical candidate for tuberculosis, eradicates *Mycobacterium ulcerans* in a mouse model of Buruli ulcer infection without relapse up to 19 weeks posttreatment. Clinical use of Q203 may dramatically simplify the clinical management of Buruli ulcer, a neglected mycobacterial disease.

KEYWORDS *Mycobacterium ulcerans*, tuberculosis, Q203, Telacebec, oxidative phosphorylation, bioenergetics, cytochrome *bcc-aa3*, terminal oxidase, bedaquiline

Buruli ulcer is a chronic ulcerating disease of the skin and underlying tissues caused by *Mycobacterium ulcerans* isolates. The disease is regaining importance in West Africa and southeast Australia with increasing incidence and severity (1, 2). The current treatment strategy involves an 8-week regimen of rifampin administered with streptomycin or clarithromycin (3, 4). Disease management is complicated by underreporting, especially in rural Africa (5); social stigmata; and lack of awareness, which impede the deployment of medical treatment. Inadequate therapy may drive a substantial number of permanent disabilities, especially in children. Compliance with an 8-week therapy regimen is also a serious limitation.

Q203 (Telacebec) is an imidazopyridine amide drug targeting the mycobacterial cytochrome *bcc-aa₃* terminal oxidase. The drug candidate, currently in phase 2 clinical trial for tuberculosis (6), has excellent activity against *M. ulcerans* infection *in vitro* and *in vivo* (7–9). In *Mycobacterium tuberculosis* isolates, the bactericidal potency of Q203 is limited by the presence of the cytochrome *bd* oxidase, an alternate terminal oxidase. The exquisite sensitivity of *M. ulcerans* to Q203 is explained by the absence of a functional cytochrome *bd* oxidase in this species (10, 11).

Considering the distinct potency of Q203 coupled with a long half-life and favorable toxicological profile (7, 10), we evaluated the potency of a single dose or 4 weekly doses of Q203 to eradicate *M. ulcerans* in an established mouse model of Buruli ulcer infection. The oral standard of care rifampin plus clarithromycin, used as a positive control (4), was administered for 4 weeks (instead of the recommended 8 weeks) to match the longest period of Q203 dosing (12). The diarylquinoline bedaquiline (Sirturo) was selected as another comparator to Q203 because the drug acts on the oxidative phosphorylation pathway as well (13), shares a very long half-life with Q203 (13), and has demonstrated potency against *M. ulcerans* isolates (14). BALB/c mice were infected in the left hind footpad with 1.1×10^5 CFU of *M. ulcerans* strain S1013 as described by Fenner (15). Disease progression was monitored by weekly measurements of footpad thickness (Fig. 1A). Treatment was initiated 5 weeks postinfection when the mean footpad swelling reached 3.7 mm, reflecting the establishment and progression of infection. Fourteen animals were randomly assigned to the following treatment cate-

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FIG 1 A single dose of Q203 (Telacebec) is curative in a mouse model of Buruli ulcer. (A) Mice were infected with 1.1×10^5 CFU of *M. ulcerans* strain S1013 5 weeks before treatment initiation. Mice were randomly assigned to oral treatment with 1 dose of Q203 20 mg/kg (red diamonds), 4 doses of Q203 5 mg/kg (purple inverted triangles), 1 dose of bedaquiline 20 mg/kg (BDQ; green triangles), 20 doses of rifampin 10 mg/kg plus clarithromycin 100 mg/kg (brown squares), or dosing vehicle alone (Unt.; blue circles). Footpad thickness was measured with a caliper weekly over 24 weeks. Untreated mice ($n = 9$) and mice treated with BDQ ($n = 14$) were euthanized at week 8 postinfection because of unfavorable disease progression (cross). (B) Bacterial loads in the infected feet were enumerated by CFU count on agar plates at the indicated time points, except for the untreated group (weeks 5 and 8) and the BDQ-treated group (week 8). Six animals per time point were used; statistical analysis was performed using Student's *t* test. ***, *P* value, <0.0001 , when comparing CFU count of Q203 (1 and 4 doses) with that of rifampin-clarithromycin at corresponding 4 and 19 weeks. LOD, limit of detection. (C) Histopathological analysis of infected footpads after Ziehl-Neelsen/methylene blue staining. Analyses were performed at week 9 postinfection for the drug-treated groups (panels 1 to 3) and at week 8 for the untreated control group (panel 4). Note the faintly stained acid-fast debris and the absence of intact acid-fast bacillus (AFBs) in the animals treated with 1 dose (panel 1) and 4 doses (panel 2) of Q203. The structure of some of the bacilli was more preserved in animals treated for 4 weeks with rifampin-clarithromycin (panel 3), whereas numerous clusters of solid-stained AFBs were present in the untreated footpads at week 8 (panel 4).

gories: rifampin 10 mg/kg plus clarithromycin 100 mg/kg administered 5 times per week for 4 weeks (20 total doses), Q203 20 mg/kg administered only once (single dose), Q203 5 mg/kg administered weekly for 4 weeks (4 doses), and bedaquiline 20 mg/kg administered only once (single dose). All drugs were administered orally. The animal protocol used in this study was approved by the Institutional Animal Care and Use Committee of Nanyang Technological University (protocol A18022).

In the untreated group, the disease progressed steadily, as witnessed by an increase in foot inflammation and swelling. At 8 weeks postinfection, foot thickness reached 4.9 mm, and some of the lesions started to ulcerate (Fig. 1A). For these reasons, the untreated animals had to be euthanized. In comparison, the disease progression stopped rapidly in the animal treated with Q203; the feet reversed pathology as early as 1 week posttreatment (Fig. 1A). Complete reversal of swelling was not achieved in the animals treated with 20 doses of rifampin plus clarithromycin. The swelling of the feet remained consistently higher in this group than in the Q203 groups throughout the observation period (Fig. 1A). The high potency of single and weekly (4 doses) administration of Q203 was confirmed by CFU counts in the infected feet (Fig. 1B). The bacterial load diminished rapidly in the animals treated with Q203, reaching the limit of detection (1 CFU) at 4 weeks posttreatment. No relapse was observed up to 24 weeks postinfection (Fig. 1B). Conversely, under similar conditions, *M. ulcerans* bacilli were detected in all the animals treated with the rifampin-clarithromycin combination (average CFU, 2.3×10^4 ; range: 6×10^3 to 6×10^4) at 4 weeks posttreatment (Fig. 1B).

Histopathological analysis supported the curative potency of a single dose of Q203.

Only faintly stained acid-fast debris but no intact acid-fast bacilli (AFB) were observed at week 9 in the animals treated with a single dose (Fig. 1C1) or four doses (Fig. 1C2) of Q203. In animals treated for 4 weeks with 20 doses of rifampin-clarithromycin, the structure of some of the bacilli was more preserved, suggesting incomplete killing (Fig. 1C3), confirming the CFU count on agar plates. In contrast, numerous clusters of solid-stained AFB were present in the untreated footpads at week 8 (Fig. 1C4) (16).

The foot swelling of the animals treated for 4 weeks with the rifampin-clarithromycin combination diminished steadily until week 15 posttreatment but increased again thereafter, suggesting a relapse that was confirmed by an increase in CFU at week 19 posttreatment (average CFU, 4.3×10^5 ; range, 3.6×10^4 to 1×10^6) (Fig. 1A and B). This observation confirms that a 4-week regimen of this drug combination is insufficient to clear the infection (3). It is interesting to note that despite a favorable pharmacokinetic profile, a single dose of bedaquiline was ineffective at controlling disease progression (Fig. 1A and B). The lack of bedaquiline potency at a single dose is probably linked to its relatively modest potency compared with Q203 (10). Newer promising diarylquinolines with improved potency and favorable pharmacokinetic parameters should be explored for potency against *M. ulcerans* infection. Of note, Q203 treatment was well tolerated in mice without clinical signs of toxicity and without weight loss, as observed before (7, 10).

Q203 holds exceptional promise as a drug candidate for treatment of Buruli ulcer. The role of the drug candidate in abbreviating therapy to 2 weeks has been recognized in the mouse model (9). The proof of concept of a single-dose cure opens the possibility of developing a drastically simplified and safe regimen to treat the disease. Although no serious adverse drug reactions were observed in a phase 2 proof-of-concept study in adults (6), the safety profile in children has yet to be determined. Future combination studies between Q203 and optimized diarylquinolines should be performed to develop a single-dose-combination cure with minimum risks for emergence of resistant mutants (17).

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REFERENCES

1. Zingue D, Bouam A, Tian RBD, Drancourt M. 2018. Buruli ulcer, a prototype for ecosystem-related infection, caused by *Mycobacterium ulcerans*. *Clin Microbiol Rev* 31:e00045-17. <https://doi.org/10.1128/CMR.00045-17>.
2. Tai AYC, Athan E, Friedman ND, Hughes A, Walton A, O'Brien DP. 2018. Increased severity and spread of *Mycobacterium ulcerans*. *Emerg Infect Dis* 24:58–64. <https://doi.org/10.3201/eid2401.171070>.
3. World Health Organization. 2012. Treatment of *Mycobacterium ulcerans* disease (Buruli ulcer): guidance for health workers. World Health Organization, Geneva, Switzerland.
4. Phillips RO, Robert J, Abass KM, Thompson W, Sarfo FS, Wilson T, Sarpong G, Gateau T, Chauty A, Omollo R, Ochieng Otieno M, Egondi TW, Ampadu EO, Agossadou D, Marion E, Ganlonon L, Wansbrough-Jones M, Grosset J, Macdonald JM, Treadwell T, Saunderson P, Paintsil A, Lehman L, Frimpong M, Sarpong NF, Saizonou R, Tiendrebeogo A, Ohene SA, Stienstra Y, Asiedu KB, van der Werf TS. 2020. Rifampicin and clarithromycin (extended release) versus rifampicin and streptomycin for limited Buruli ulcer lesions: a randomised, open-label, non-inferiority phase 3 trial. *Lancet* 395:1259–1267. [https://doi.org/10.1016/s0140-6736\(20\)30047-7](https://doi.org/10.1016/s0140-6736(20)30047-7).
5. Mavinga Phanzu D, Suykerbuyk P, Saunderson P, Ngwala Lukanu P, Masamba Minuku JB, Imposo DB, Mbadu Diengidi B, Kayinua M, Tamfum Muyembe JJ, Tshindele Lutumba P, de Jong BC, Portaels F, Boelaert M. 2013. Burden of *Mycobacterium ulcerans* disease (Buruli ulcer) and the underreporting ratio in the territory of Songololo, Democratic Republic of Congo. *PLoS Negl Trop Dis* 7:e2563. <https://doi.org/10.1371/journal.pntd.0002563>.
6. de Jager VR, Dawson R, van Niekerk C, Hutchings J, Kim J, Vanker N, van der Merwe L, Choi J, Nam K, Diacon AH. 2020. Telacebec (Q203), a new antituberculosis agent. *N Engl J Med* 382:1280–1281. <https://doi.org/10.1056/NEJMc1913327>.
7. Pethe K, Bifani P, Jang J, Kang S, Park S, Ahn S, Jiricek J, Jung J, Jeon HK, Cechetto J, Christophe T, Lee H, Kempf M, Jackson M, Lenaerts AJ, Pham H, Jones V, Seo MJ, Kim YM, Seo M, Seo JJ, Park D, Ko Y, Choi I, Kim R, Kim SY, Lim S, Yim S-A, Nam J, Kang H, Kwon H, Oh C-T, Cho Y, Jang Y, Kim J, Chua A, Tan BH, Nanjundappa MB, Rao SPS, Barnes WS, Wintjens

- R, Walker JR, Alonso S, Lee S, Kim J, Oh S, Oh T, Nehrbass U, Han S-J, No Z, Lee J, Brodin P, Cho S-N, Nam K, Kim J. 2013. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. *Nat Med* 19:1157–1160. <https://doi.org/10.1038/nm.3262>.
8. Converse PJ, Almeida DV, Tyagi S, Xu J, Nuermberger EL. 2019. Shortening Buruli ulcer treatment with combination therapy targeting the respiratory chain and exploiting *Mycobacterium ulcerans* gene decay. *Antimicrob Agents Chemother* 63:e00426-19. <https://doi.org/10.1128/AAC.00426-19>.
9. Chauffour A, Robert J, Veziris N, Aubry A, Pethe K, Jarlier V. 2019. Q203 containing fully intermittent oral regimens exhibited high sterilizing activity against *Mycobacterium ulcerans* in mice. *bioRxiv* <https://doi.org/10.1101/813253>.
10. Scherr N, Bieri R, Thomas SS, Chauffour A, Kalia NP, Schneide P, Ruf M-T, Lamelas A, Manimekalai MSS, Grüber G, Ishii N, Suzuki K, Tanner M, Moraski GC, Miller MJ, Witschel M, Jarlier V, Pluschke G, Pethe K. 2018. Targeting the *Mycobacterium ulcerans* cytochrome bc1:aa3 for the treatment of Buruli ulcer. *Nat Commun* 9:5370. <https://doi.org/10.1038/s41467-018-07804-8>.
11. Stinear TP, Seemann T, Pidot S, Frigui W, Reysset G, Garnier T, Meurice G, Simon D, Bouchier C, Ma L, Tichit M, Porter JL, Ryan J, Johnson PD, Davies JK, Jenkin GA, Small PL, Jones LM, Tekaia F, Laval F, Daffé M, Parkhill J, Cole ST. 2007. Reductive evolution and niche adaptation inferred from the genome of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer. *Genome Res* 17:192–200. <https://doi.org/10.1101/gr.5942807>.
12. Chauffour A, Robert J, Veziris N, Aubry A, Jarlier V. 2016. Sterilizing activity of fully oral intermittent regimens against *Mycobacterium ulcerans* infection in mice. *PLoS Negl Trop Dis* 10:e0005066. <https://doi.org/10.1371/journal.pntd.0005066>.
13. Andries K, Verhasselt P, Guillemont J, Göhlmann HWH, Neefs J-M, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P, de Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V. 2005. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 307:223–227. <https://doi.org/10.1126/science.1106753>.
14. Ji B, Lefrançois S, Robert J, Chauffour A, Truffot C, Jarlier V. 2006. *In vitro* and *in vivo* activities of rifampin, streptomycin, amikacin, moxifloxacin, R207910, linezolid, and PA-824 against *Mycobacterium ulcerans*. *Antimicrob Agents Chemother* 50:1921–1926. <https://doi.org/10.1128/AAC.00052-06>.
15. Fenner F. 1956. The pathogenic behavior of *Mycobacterium ulcerans* and *Mycobacterium balnei* in the mouse and the developing chick embryo. *Am Rev Tuberc* 73:650–673.
16. Ruf M-T, Schütte D, Chauffour A, Jarlier V, Ji B, Pluschke G. 2012. Chemotherapy-associated changes of histopathological features of *Mycobacterium ulcerans* lesions in a Buruli ulcer mouse model. *Antimicrob Agents Chemother* 56:687–696. <https://doi.org/10.1128/AAC.05543-11>.
17. Working Group for New TB Drugs. 2020. Clinical pipeline. <https://www.newtbdrugs.org/pipeline/clinical>. Accessed 20 March 2020.