Dual inhibition of the terminal oxidases eradicates antibiotic-tolerant *Mycobacterium tuberculosis*[1]

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Introduction

Mycobacterium tuberculosis (Mtb) employs two terminal oxidases, cytochrome *bcc:aa*$_3$ (Cyt-*bcc:aa*$_3$) and cytochrome *bd* (Cyt-*bd*) oxidase, to maintain respiration and survive under stress. Antibiotic-tolerant Mtb often relies on these oxidases, making them critical targets for therapeutic intervention.

Current treatments struggle to eradicate antibiotic-tolerant Mtb, contributing to treatment failure and prolonged therapy.

Aim

With Telacebec (Q203) found to be efficacious in inhibiting Cyt-*bcc:aa*$_3$, an inhibitor of Cyt-*bd* oxidase also needed to be identified to improve treatment outcomes.

This study aims to evaluate the efficacy of Q203 and Cyt-*bd* inhibitor ND-011992 in dual inhibition of Mtb's terminal oxidases, and combination therapy as a bactericidal strategy in replicating, hypoxic and nutrient-starved subgroups of antibiotic-tolerant Mtb.

Results

ND-011992 inhibits M. Bovis O_2 consumption

The representative molecule ND-011992 depleted ATP levels only in the presence of Q203 at an inhibitory concentration 50% (IC50) of 0.5–1.6 IM in M. bovis BCG.

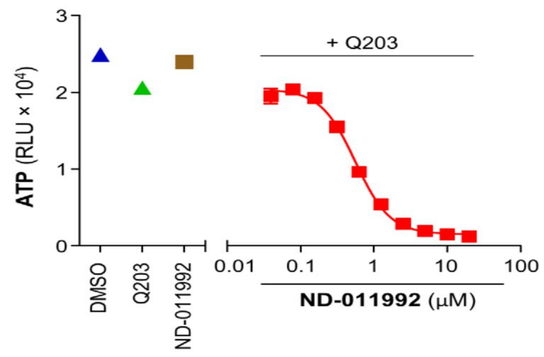


Figure 1: Effect of ND-011992 on the intracellular ATP level in M. bovis BCG with Q203

The combination of Q203 and ND-011992 resulted in an immediate and complete inhibition of the oxygen consumption rate (OCR) of M. bovis BCG on a Seahorse XFe96 platform.

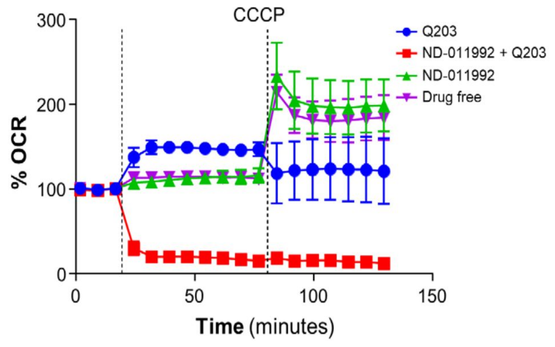
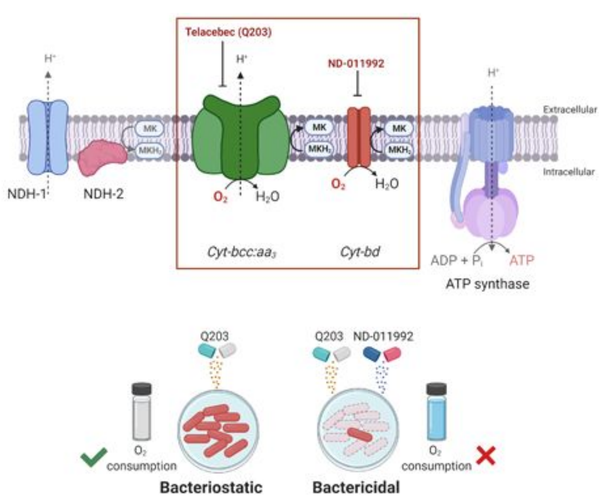


Figure 2: Oxygen consumption assay in M. bovis BCG using the Seahorse XFe96 Extracellular flux analyzer

Synopsis



Cytochrome bd oxidase inhibitor ND-011992 together with Q203 forms a bactericidal combination against M. tuberculosis.

ND-011992/Q203 combination enhanced inhibition of oxygen respiration, intracellular ATP depletion, and bactericidal activity. The ND-011992/Q203 combination did not significantly increase the frequency of acquired resistance, whilst showing enhanced killing effect *in vivo*.

Quantifying ND-011992's mechanistic effect *in vitro* and *in vivo*

In vitro studies

Consistently, ND-011992 inhibited the growth of an H37Rv strain deficient in Cyt-*bcc:aa*$_3$ expression at a concentration 33- to 110-fold lower compared with the concentration required to inhibit the growth of the parental strain.

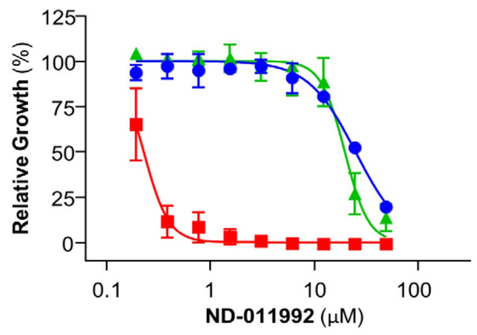


Figure 3: Growth inhibition of the Cyt-bcc:aa3 deficient H37Rv by ND-011992.

The transcription of respiratory chain and redox stress response genes was particularly affected by the addition of ND-011992, indicating a more drastic poisoning of the electron transport chain.

The difference spectrum of the M. smegmatis DqcrCAB IMVs energized with NADH and treated with ND-011992 showed the peaks corresponding to the Cyt-*bd* hemes strongly reduced or disappeared. This demonstrates that the compound selectively binds electron transfer in the mycobacterial Cyt-*bd*, indicating that ND-011992 is a quinone binding site inhibitor.

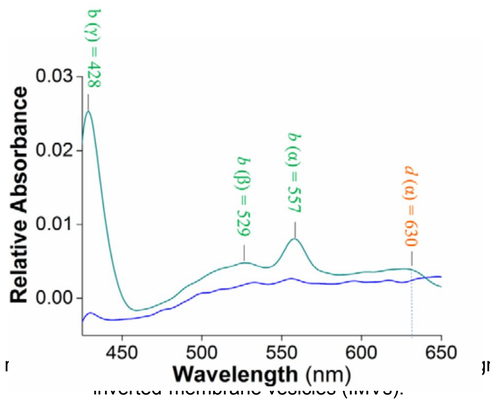
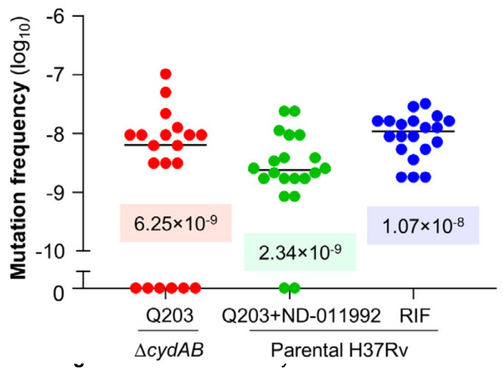


Figure 4: The r ɯmatis DqcrCAB inverted membrane vesicles (IMVs).

Fluctuation analysis confirmed that addition of ND-011992 to Q203 did not raise the pathogen's spontaneous frequency of resistance to the treatment.



When the ΔcydAB mutant strain was complemented with the cydABDC operon from M. tuberculosis, OCR sensitivity to ND-011992 was restored, supporting that the cytochrome bd oxidase is the primary target of ND-011992.

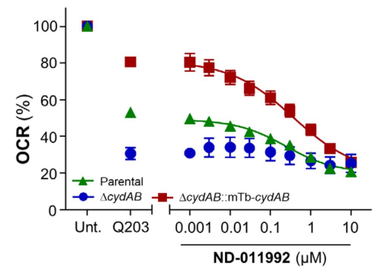


Figure 6: Effect of ND-011992 on the OCR of M. smegmatis IMVs using the Oroboros O2k fluorespirometer

In vivo studies

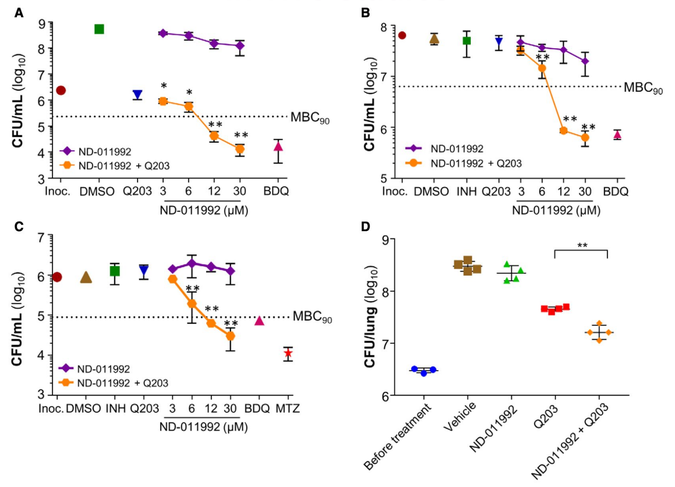
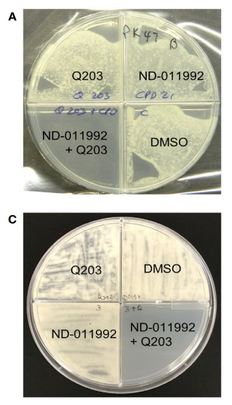


Figure 7A: Bactericidal activity of ND-011992 and ND-011992-Q203 combination in replicating Mtb H37Rv.
Figure 7B: Bactericidal activity in nutrient-starved Mtb H37Rv.
Figure 7C: Bactericidal activity in Mtb H37Rv under hypoxia.
Figure 7D: Efficacy of ND-011992-Q203 combination treatment in a mouse model of acute tuberculosis.

A combination of Q203 and ND-011992 was bactericidal against M. tuberculosis and M. bovis BCG, against both antibiotic-tolerant, nutrient-starved and hypoxic non-replicating mycobacteria.



Strain ID	Profile	Minimum inhibitory Concentration Q203 + ND-011992 (μM)
H37Rv	S	2.0
N0072	S	≤ 0.2
N0157	S	≤ 0.2
N0145	S	≤ 0.2
N0052	S	0.5
N0054	S	0.2
N1274	S	1.0
N1283	S	≤ 0.2
N0136	S	1.0
123-20-0015	XDR	≤ 1.0
123-20-0091	XDR	≤ 1.0
123-20-0041	MDR	≤ 1.0
123-20-0047	MDR	≤ 1.0

Figure 8A: Combined potency of ND-011992 + Q203 against Mtb 123-20-0047.
Figure 8B: Potency of the combination ND-011992 + Q203 against various clinical isolates in an agar plate MIC assay.
Figure 8C: Growth inhibition assay in M. bovis BCG.

ND-011992 was potent at an MIC value ranging from ≤ 0.2 to 1 IM against pan-susceptible clinical isolates, and MIC ≤ 1 IM against the clinical isolates of South African origin. showing that the drug is active against a diverse pool of clinical isolates, including those resistant to first- and second-line antituberculosis agents.

Discussion

Tuberculosis treatment faces two key challenges of the rise in rapidly spreading multi-drug resistant strains and the lack of effective drug combinations to fight infections. Oxidative phosphorylation remains a critical factor in maintaining energy production in Mtb. The new discovery of ND-011992 as a cytochrome *bd* oxidase inhibitor has enhanced the potency of cytochrome *bcc:aa*$_3$ inhibitor Q203. Together, the combined drug therapy exhibits synergistic bactericidal activity, including against replicating and non-replicating antibiotic-tolerant Mtb. However, while the combination Q203/ND-011992 shows significant potential against Mtb, optimization of ND-011992's pharmacokinetic properties is still needed before clinical application. The effectiveness of ND-011992 also relies heavily on its combination with Q203, limiting its standalone therapeutic potential.

References

1. Lee BS, Hards K, Engelhart CA, Hasenoehrl EJ, Kalia NP, Mackenzie JS, et al. Dual inhibition of the terminal oxidases eradicates antibiotic-tolerant Mycobacterium tuberculosis. EMBO Molecular Medicine. 2021 Jan 11;13(1):e13207.