Team 42

Dual inhibition of terminal oxidases eradicates antibiotic-tolerant Mycobacterium tuberculosis

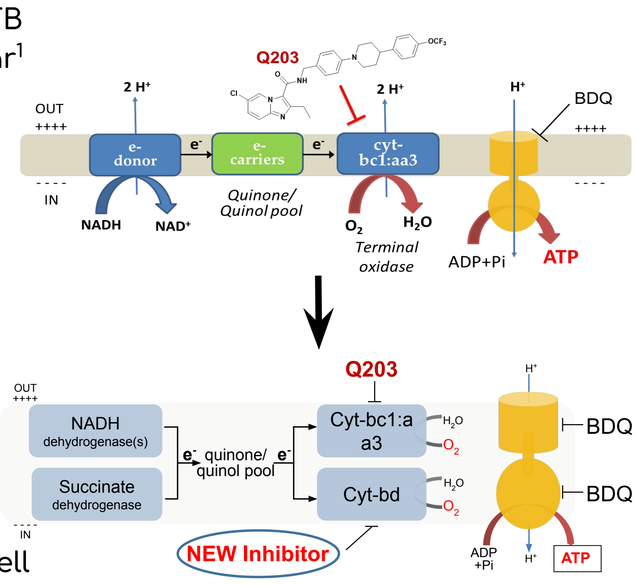
INTRODUCTION

Prevalence: >2 billion people around the globe infected with TB and causes 1.4 million death/year[1]

Urgency: Spread of MDR-TB has risen dramatically and its resistance to most potent drugs confer poor prognosis[2]

Focus 1: Discovery and characterization of a molecule targeting Cyt-bd

Focus 2: Determining its effectiveness when used with Q203 against M. TB strains, as well as hypoxic, and nutrient-starved antibiotic-tolerant subpopulations



METHODOLOGY

Wild type and Cyt-bd overexpressed M tuberculosis, M bovis, M smegmatis were used to test the anti-mycobacterial properties of ND-011992, individually and in combination with Bedaquiline, at various levels with the following methods:

1. Oxygen Consumption Assay
2. RNA Isolation and Sequencing
3. Drug Susceptibility Test on Solid Medium - Drug-resistant M. TB & M. Bovis

Following that, in vivo studies were also done on mouse models in order to determine the effectiveness of the drugs in vivo conditions in particular the bactericidal properties of the drugs which is the main limitation of ND-011992 monotherapy in previous studies [3].

These mice were infected with the mycobacterium species at 1.5×10^5 CFU per animal and subsequently treated. They were sacrificed 5 days after the end of drug treatment to determine the bacterial loads in the lungs and spleens by CFU enumeration.

RESULTS

At a CELLULAR level

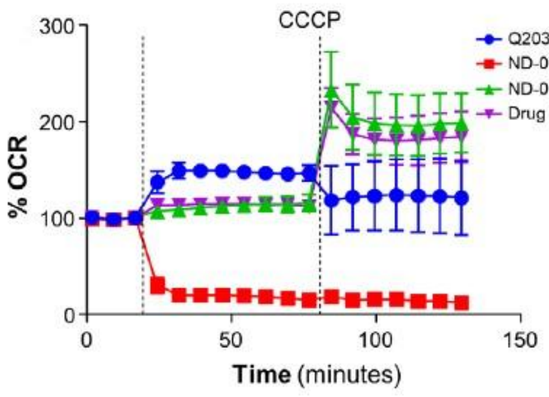


Fig 1
Graph of O_2 consumption rate against time for M.TB in different terminal oxidases

Immediate and complete inhibition of OCR on M.TB and M.bovis on Seahorse XFe96 platform

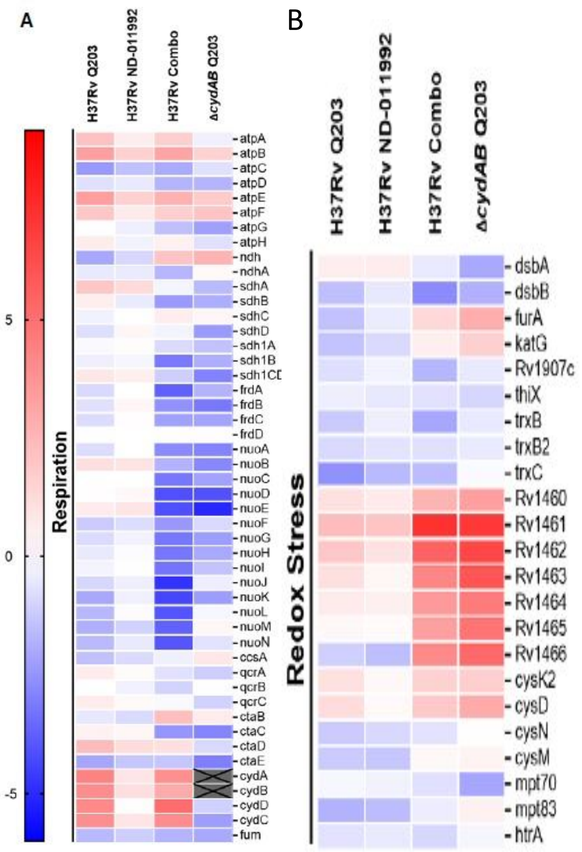
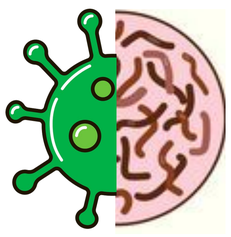


Fig 2a
Scale of respiration measured in M.TB H37Rv strain in different terminal oxidases

Lowest transcription of respiratory chain with Q203-ND011992 combination

Fig 2b
Scale of redox stress measured in M.TB H37Rv in different terminal oxidases

Highest redox stress measured with drug combination



At a COLONY level

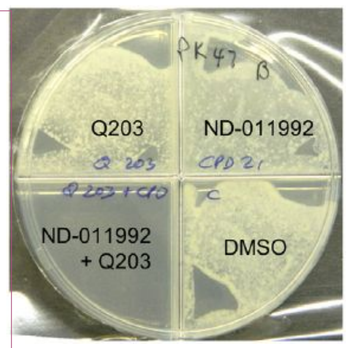


Fig 3
Culture of drug-resistant M.TB 123-20-0047 strain in different terminal oxidase(s) mediums

Dual inhibition by combination drug had greatest potency

in vitro

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

Fig 4
Susceptibility testing of multiple TB lineages, including multi- and extreme drug resistant strains, to Q203-ND011992 drug combination

Drug-susceptible, MDR and XDR TB strains were susceptible to combination drug

in vivo

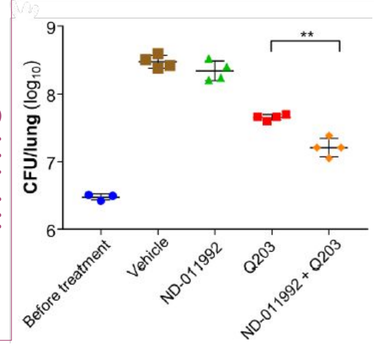


Fig 5
Graph of Colony Forming Units (CFU) measured in mouse treated for acute TB with different terminal oxidase(s) drugs

DISCUSSION

- Q203 potent against TB but may not shorten treatment unless combined with other drugs[4]
- Greatest bactericidal effect by Q203-ND-011992 combination drug (Fig 1, 2a, 2b, 3)
- Combination drug has wide range of effectiveness - potent against different TB lineages, regardless of antibiotic profile (Fig 4). Slight variations in susceptibility to combination drug possibly due to different expression levels of the 2 terminal oxidases
- Frequency of resistance not increased with dual inhibition compared to Q203 alone[5]. Main mechanism of resistance to combination drug is mutations against Q203, not ND-011992, possibly due to strict essentiality of ND-011992-interacting residues of cyt-bd function
- Enhanced potency of dual inhibition limited by ND-011992's pharmacokinetics (required lead to optimise in in vivo study)

CONCLUSION

- Potency of Q203 greatly limited by the presence of the cyt-bd
- Addition of ND-011992 enhanced the potency of Q203
- Synergism is limited by ND-011992's less-than-optimal pharmacokinetic properties
- Further research in multidrug combination therapies and optimising ND-011992 pharmacokinetic properties

References

(1) Houben, R. M., & Dodd, P. J. (2016). The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS medicine, 13(10), e1002152.
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(3) Foo CS, Lupien A, Kienle M, Vocat A, Benjak A, Sommer R, Lamprecht DA, Steyn AJC, Pethe K, Piton J et al (2018) Arylvinylpiperazine amides, a new class of potent inhibitors targeting QcrB of Mycobacterium tuberculosis. mBio 9: e01276–18
(4) Arora K, Ochoa-Monta~no B, Tsang PS, Blundell TL, Dawes SS, Mizrahi V, Bayliss T, Mackenzie CJ, Cleghorn LAT, Ray PC et al (2014) Respiratory flexibility in response to inhibition of cytochrome C oxidase in Mycobacterium tuberculosis. Antimicrob Agents Chemother 58: 6962 – 6965
(5) Pethe K, Bifani P, Jang J, Kang S, Park S, Ahn S, Jiricek J, Jung J, Jeon HK, Cechetto J et al (2013) Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. Nat Med 19: 1157 – 1160