II WORKSHOP DA REDE SUL DE MICOBACTÉRIAS
II MOSTRA ESTADUAL DA ATENÇÃO À SAÚDE PRISIONAL
IV ENCONTRO REGIONAL DE TUBERCULOSE

22 E 23 DE OUTUBRO DE 2018
UNISC- SANTA CRUZ DO SUL, RS

Drogas para o tratamento da tuberculose e resistência antimicrobiana: o que há de novo

NUPEMM

Universidade Federal do Rio Grande - FURG

Pedro Eduardo Almeida da Silva
Universidade Federal do Rio Grande – FURG – Brazil
pedrefurg@gmail.com

RESUMI
REDE SUL DE MICOBACTÉRIAS

REDE-TB

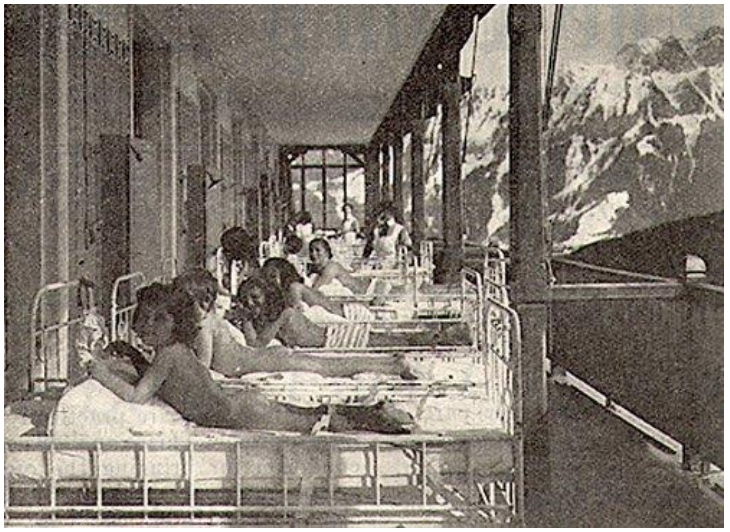
ROTEIRO

1) PASSADO, PRESENTE E FUTURO

2) RESISTÊNCIA AOS ATB COM UM FENÔMENO EVOLUTIVO

3) PARADIGMAS DA RESISTÊNCIA EM *M. tuberculosis*

4) NOVOS ATB, REGIMES E MECANISMOS DE RESISTÊNCIA




"Bottled
Sunlight"
Extra rich in "sunshine vitamin" D. Possesses a fine, wholesome flavour.
115
Ayerst
10-D
COD LIVER OIL

1944 STREPTOMYCIN

1948

STREPTOMYCIN RESISTANCE IN PULMONARY TUBERCULOSIS

A RESISTÊNCIA

Registr... Trials, Brompton Hospital; Lecturer in Medicine, Postgraduate Medical School of London

AND

D. A. MITCHISON,* M.B.

Lecturer in Bacteriology, Postgraduate Medical School of London; Formerly Assistant to the Pathologist, Brompton Hospital

WORLD TB DAY
March 24

in 2016

AROUND 600,000

people had drug-resitant TB

There is slow progress in tackling MDR-TB

ONLY 1 IN 4

MDR-TB cases is diagnosed

ONLY 130,000

patients were started on MDR-TB treatment last year

ONLY 1 IN 8

MDR-TB cases is successfully treated

Each dollar invested in TB yields **up to US $85 in return***

*The figure reflects the return of investment following the accelerated scenario as set forth in the Global Plan to End TB 2016-2020.

Stop TB Partnership

World Health Organization

•6 % do total de casos de TB são MDR-TB

•10 % dos MDR são XDR-TB (MDR, FQ e uma droga injetável)

International Journal of Infectious Diseases 32 (2015) 101–104

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Therapeutic drug monitoring: how to improve drug dosage and patient safety in tuberculosis treatment

Giovanni Sotgiu [a], Jan-Willem C. Alffenaar [b], Rosella Centis [c], Lia D'Ambrosio [c], Antonio Spanevello [d,e], Andrea Piana [a], Giovanni Battista Migliori [c,*]

DESFECHOS

ESTUDOS COM GRANDES COHORT COM MDR-TB/XDR

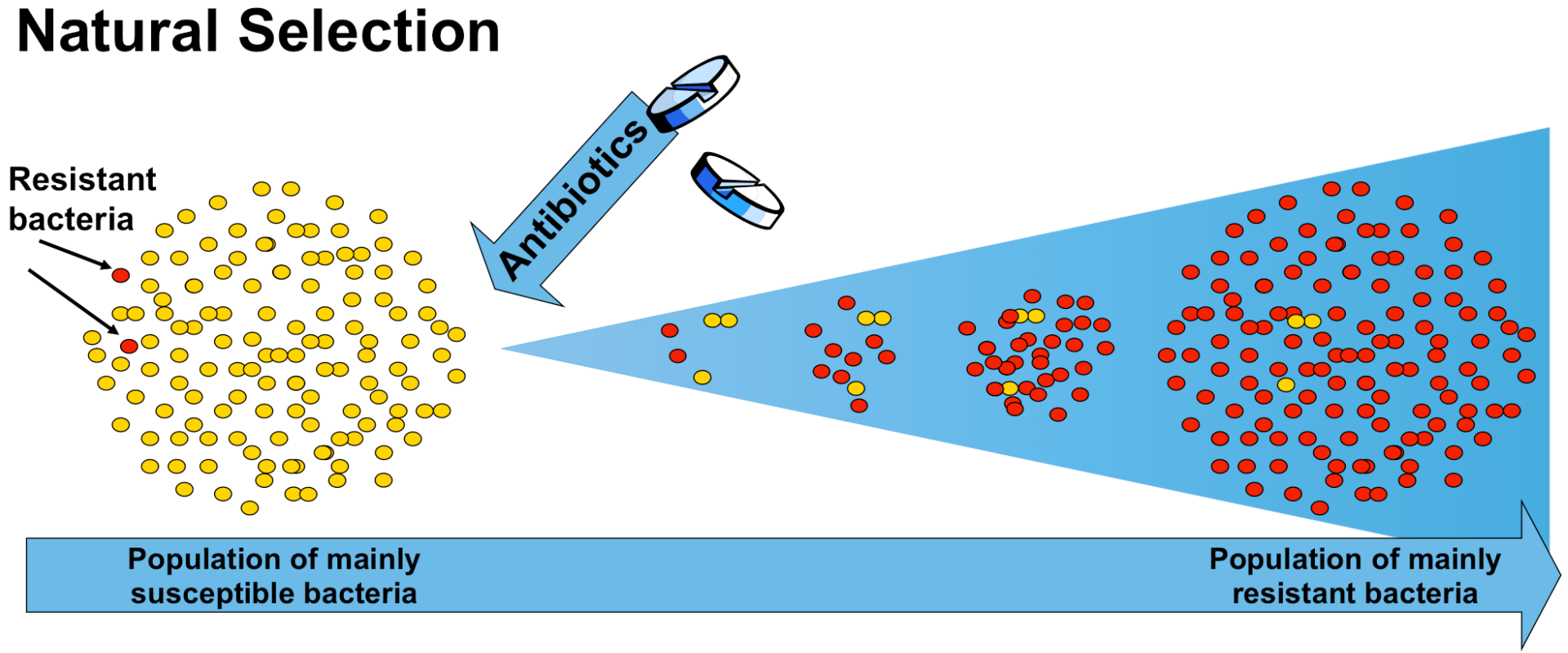
DESFECHO	MDR	XDR
ÊXITO	62%	40%
FALHA/RECIDIVA	7%	22%
MORTE	9%	15%

Contents lists available at ScienceDirect
International Journal of Infectious Diseases
journal homepage: www.elsevier.com/locate/ijid

Therapeutic drug monitoring: how to improve drug dosage and patient safety in tuberculosis treatment

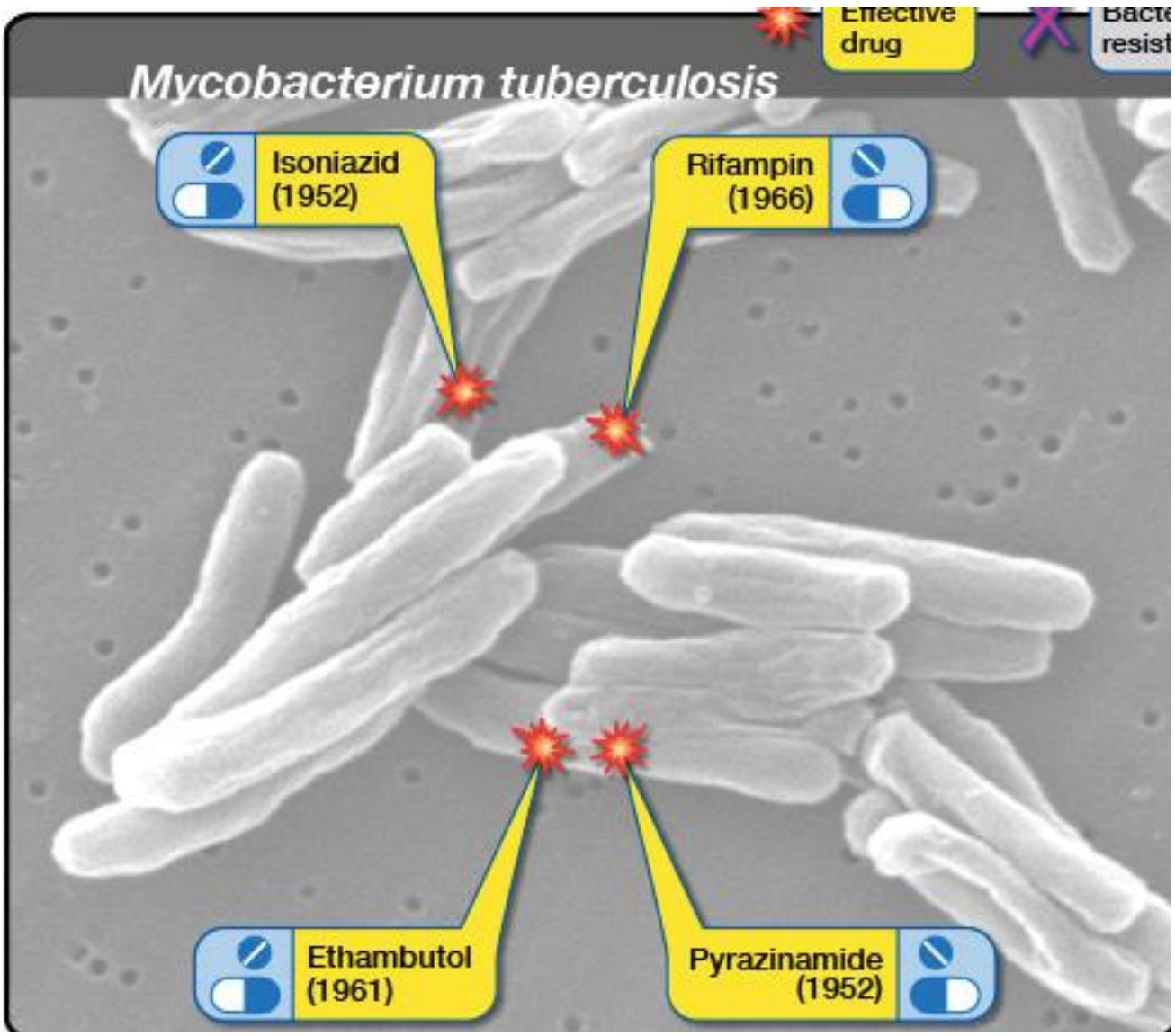
Giovanni Sotgiu [a], Jan-Willem C. Alffenaar [b], Rosella Centis [c], Lia D'Ambrosio [c], Antonio Spanevello [d,e], Andrea Piana [a], Giovanni Battista Migliori [c,*]

Resistência aos atb é um processo evolutivo e antropogênico




IT'S NOT THE STRONGEST OF SPECIES THAT SURVIVES, NOR THE MOST INTELLIGENT.
IT IS THE ONE THAT IS THE MOST ADAPTABLE TO CHANGE.

MECANISMOS DE RESISTÊNCIA AOS CLÁSSICOS ANTIMICROBIANOS


Effective drug
Bacte resist
Mycobacterium tuberculosis
Isoniazid (1952)
Rifampin (1966)
Ethambutol (1961)
Pyrazinamide (1952)

Review

Drug Resistance Mechanisms in *Mycobacterium tuberculosis*

Juan Carlos Palomino * and Anandi Martin

'able 1

irst- and second-line TB drugs, genes involved in their activation and mechanisms involved.

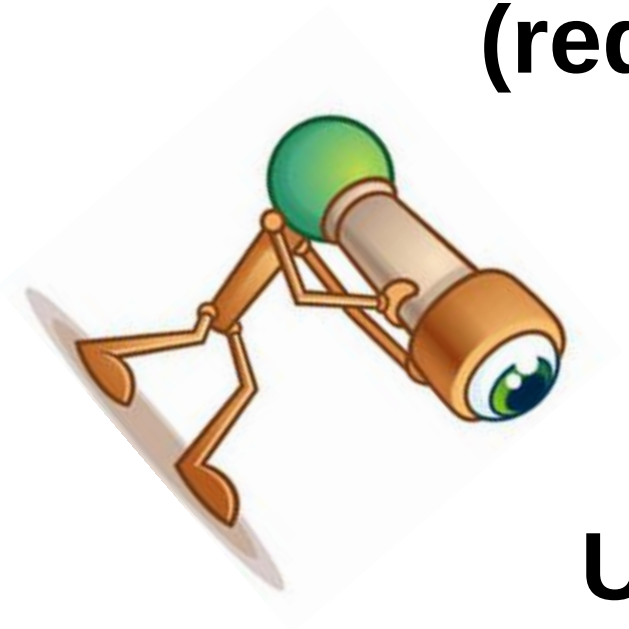
Drug	Gene	Mechanism Involved
Isoniazid	*katG*, *inhA*	Catalase/peroxidase; enoyl reductase
Rifampicin	*rpoB*	RNA polymerase
Pyrazinamide	*pncA*, *rpsA*	Pyrazinamidase; ribosomal protein 1
Ethambutol	*embB*	Arabinosyl transferase
Streptomycin	*rpsL*, *rrs*, *gidB*	S12 ribosomal protein, 16A rRNA, 7-methylguanosine methyltransferase
Quinolones	*gyrA*, *gyrB*	DNA gyrase
Capreomycin	*rrs*, *tlyA*	16S rRNA, rRNA methyltransferase
Kanamycin/Amikacin	*rrs*	16S rRNA
Ethionamide	*ethA*	Enoyl-ACP reductase
ara-aminosalicylic acid	*thyA*, *folC*	Thymidylate synthase A

PARADIGMAS DA RESISTÊNCIA

EM

M. tuberculosis

VISÃO CLÁSSICA (reducionista)

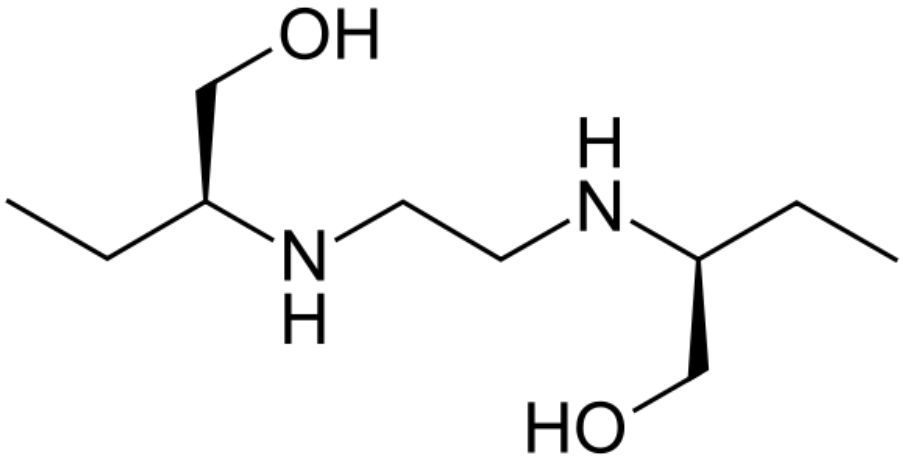


Uma Mutação

Resistência para um ATB

PRIMEIRA QUEBRA DO PARADIGMA

The curious case of embB 306 mutation



Ethambutol

© 1997 Nature Publishing Group http://www.nature.com/naturemedicine

1997

The *emb* operon, a gene cluster of *Mycobacterium tuberculosis* involved in resistance to ethambutol

AMALIO TELENTI[1,2], WOLFGANG J. PHILIPP[1], SRINAND SREEVATSAN[3], CLAUDIA BERNASCONI[1], KATHRYN E. STOCKBAUER[3], BRIGITTE WIELES[1], JAMES M. MUSSER[3] & WILLIAM R. JACOBS, JR.[2]

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1997, p. 1677–1681 Vol. 41, No. 8
0066-4804/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Ethambutol Resistance in *Mycobacterium tuberculosis*: Critical Role of *embB* Mutations

SRINAND SREEVATSAN,[1] KATHRYN E. STOCKBAUER,[1] XI PAN,[1] BARRY N. KREISWIRTH,[2] SORAYA L. MOGHAZEH,[2] WILLIAM R. JACOBS, JR.,[3] AMALIO TELENTI,[4] AND JAMES M. MUSSER[1,5]*

Met306Val	**40 µg/ml**
Met306Ile	**20 µg/ml**

The curious case of embB 306 mutation

2004

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2004, p. 4447–4449 Vol. 48, No. 11
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.11.4447–4449.2004
Copyright © 2004, American Society for Microbiology. All Rights Reserved.

Novel Mutations within the *embB* Gene in Ethambutol-Susceptible Clinical Isolates of *Mycobacterium tuberculosis*

Ann S. G. Lee,[1]* Siti Noor Khadijah Othman,[1] Yu Min Ho,[1] and Sin Yew Wong[2]

Mutações embB306 tb podem estar presentes em cepas sensíveis a EMB

A mutação embB306 pode predispor a resistência a outras drogas

The curious case of embB 306 mutation

2008

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2008, p. 2027–2034 Vol. 52, No. 6
0066-4804/08/$08.00+0 doi:10.1128/AAC.01486-07
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Transfer of *embB* Codon 306 Mutations into Clinical *Mycobacterium tuberculosis* Strains Alters Susceptibility to Ethambutol, Isoniazid, and Rifampin[▽]†

Hassan Safi, Brendan Sayers, Manzour H. Hazbón, and David Alland*

embB 306 mutation is necessary but not sufficient for determining high-level EMBr

embB 306 mutation has higher propensity to develop INH and RIF resistance

TB caused by mutant embB306 could be more prone to evolve into MDR?

Fitness e resistência aos antimicrobianos

Mutações que conferem resistência aos antibióticos estabelecem um benefício (vantagens) quando o antibiótico está presente.

Mas Mutações que conferem resistência aos antibióticos determinam algum custo biológico (menor *fitness*) ?

Cepas resistentes são menos virulentas?

September 11, 1953

SCIENCE, Vol. 118

Some Observations on the Pathogenicity of Isoniazid-Resistant Variants of Tubercle Bacilli[1]

Gardner Middlebrook and Maurice L. Cohn

Department of Research and Laboratories, National Jewish Hospital at Denver and the University of Colorado School of Medicine, Denver

tive H37Rv strain. These experiments indicated that these strains of tubercle bacilli may become partially or completely attenuated for the guinea pig when they become resistant to 10 µg of INH/ml of OA or Tween-albumin liquid medium, under experimental laboratory conditions.

Cepas resistentes a 10 µg / mL INH eram parcialmente ou completamente atenuadas em infecção em cobaio

REVIEW 10.1111/j.1469-0691.2008.02685.x

Fitness cost of drug resistance in *Mycobacterium tuberculosis*

S. Gagneux

Division of Mycobacterial Research, MRC National Institute for Medical Research, Mill Hill, London, UK

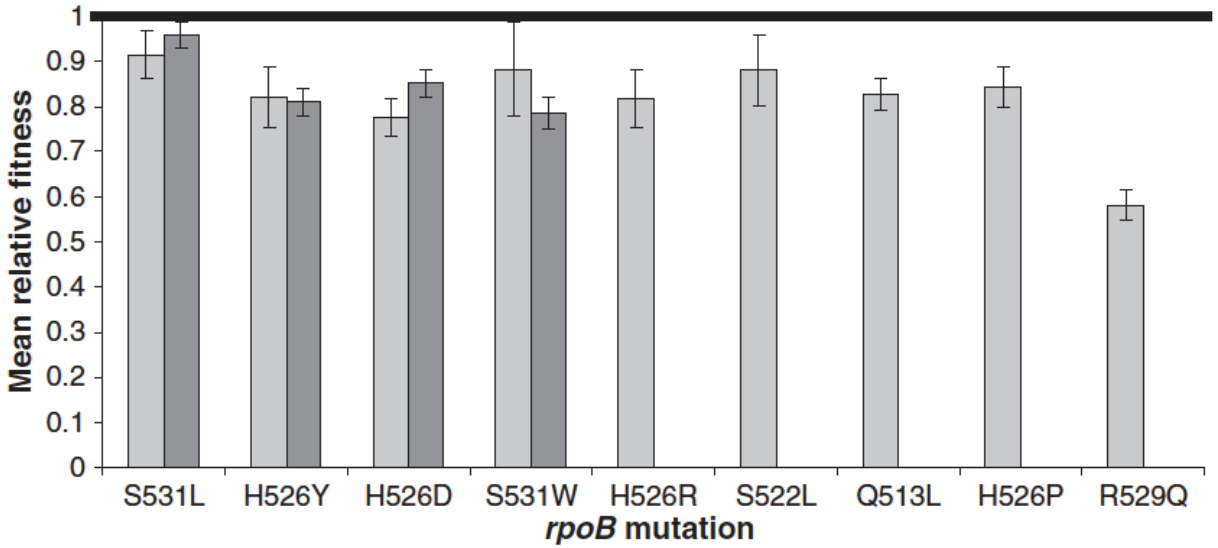
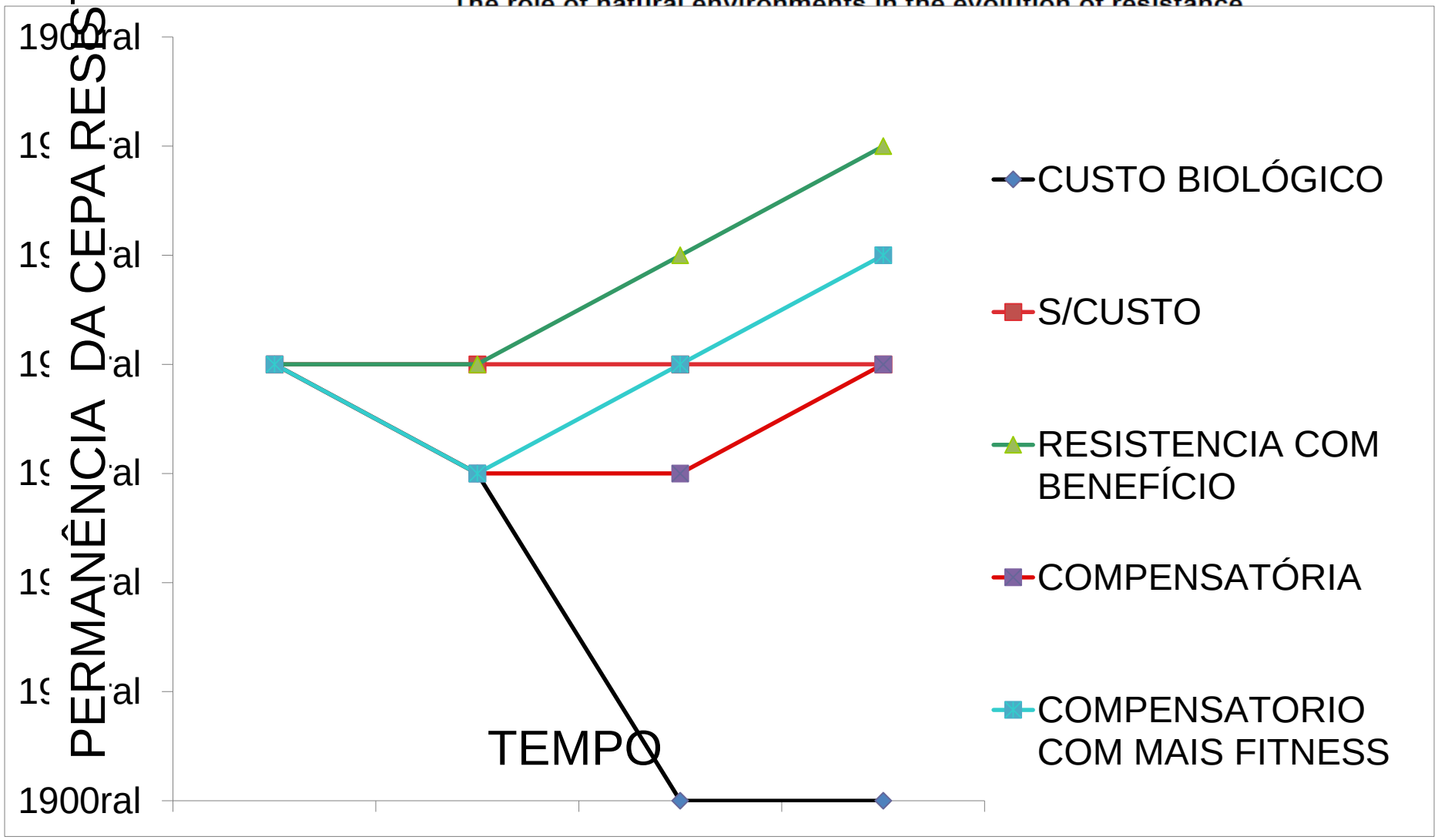


Fig. 1. Relative competitive fitness of laboratory-derived rifampicin-resistant mutants of *Mycobacterium tuberculosis*. The relative fitness of the rifampicin-susceptible ancestor is defined as 1 (black line). All rifampicin-resistant mutants had a statistically significant fitness cost as compared to the rifampicin-susceptible ancestor strain (error bars indicate 95% CIs). This cost was less in *rpoB* S531L mutants than in other *rpoB* mutants, irrespective of the strain background. Light grey bars, CDC1551 mutants; dark grey bars, T85 mutants.

PROCEEDINGS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The role of natural environments in the evolution of resistance



COMO O *M. tuberculosis* RECUPERA O FITNESS?

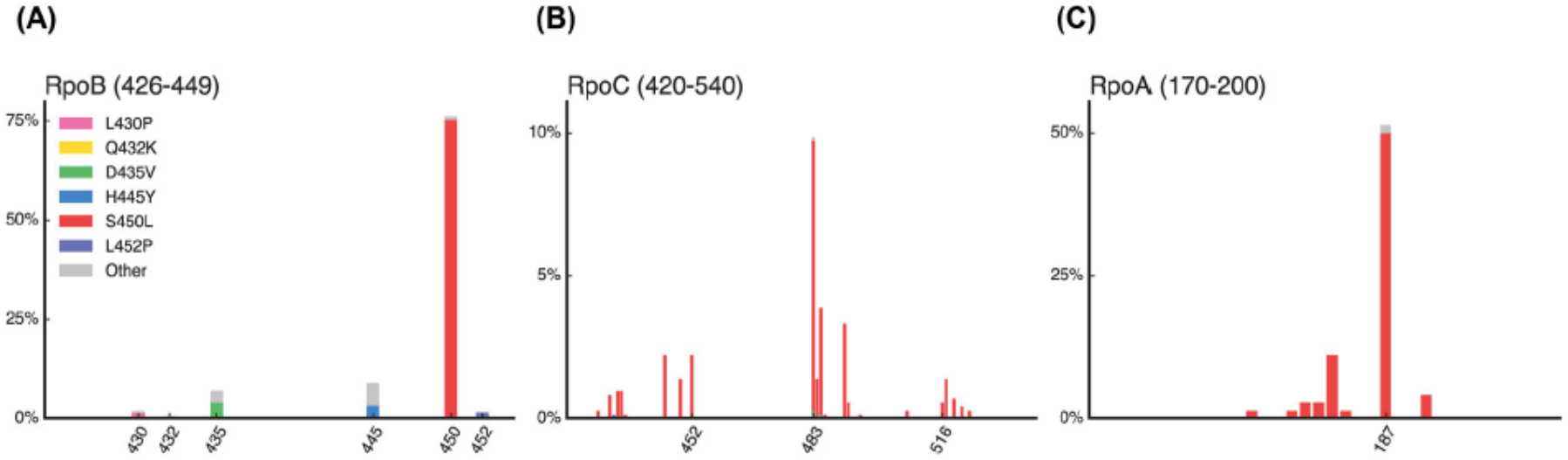
FEMS Microbiology Reviews, fux011, 41, 2017, 354–373

doi: 10.1093/femsre/fux011
Advance Access Publication Date: 25 March 2017
Review article

REVIEW ARTICLE

Antimicrobial resistance in *Mycobacterium tuberculosis*: mechanistic and evolutionary perspectives

Sebastian M. Gygli[1,2], Sonia Borrell[1,2], Andrej Trauner[1,2] and Sebastien Gagneux[1,2,*]



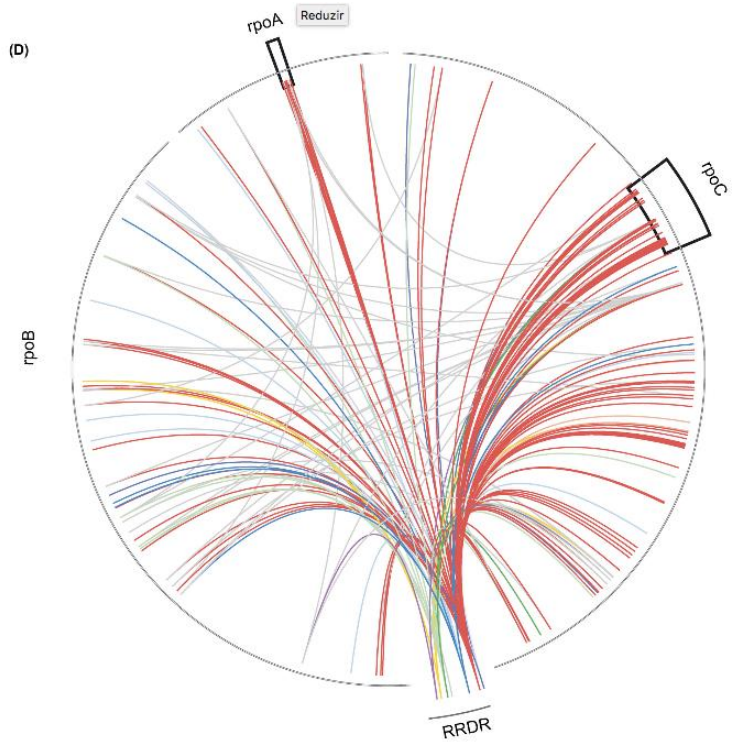


Figure 4. Summary of rifampicin resistance and fitness cost compensatory mutations in *rpoB* and *rpoA/C* respectively. (a) Frequency of rifampicin resistance mutations in *rpoB*. (b) Frequency of putative compensatory mutations in *rpoC* in codons 420–540. (c) Frequency of putative compensatory mutations in *rpoA* in codons 170–200.

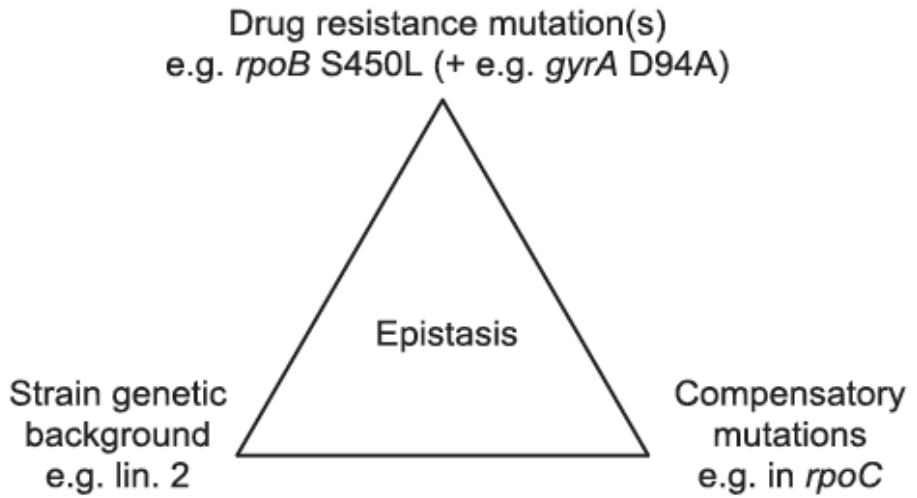


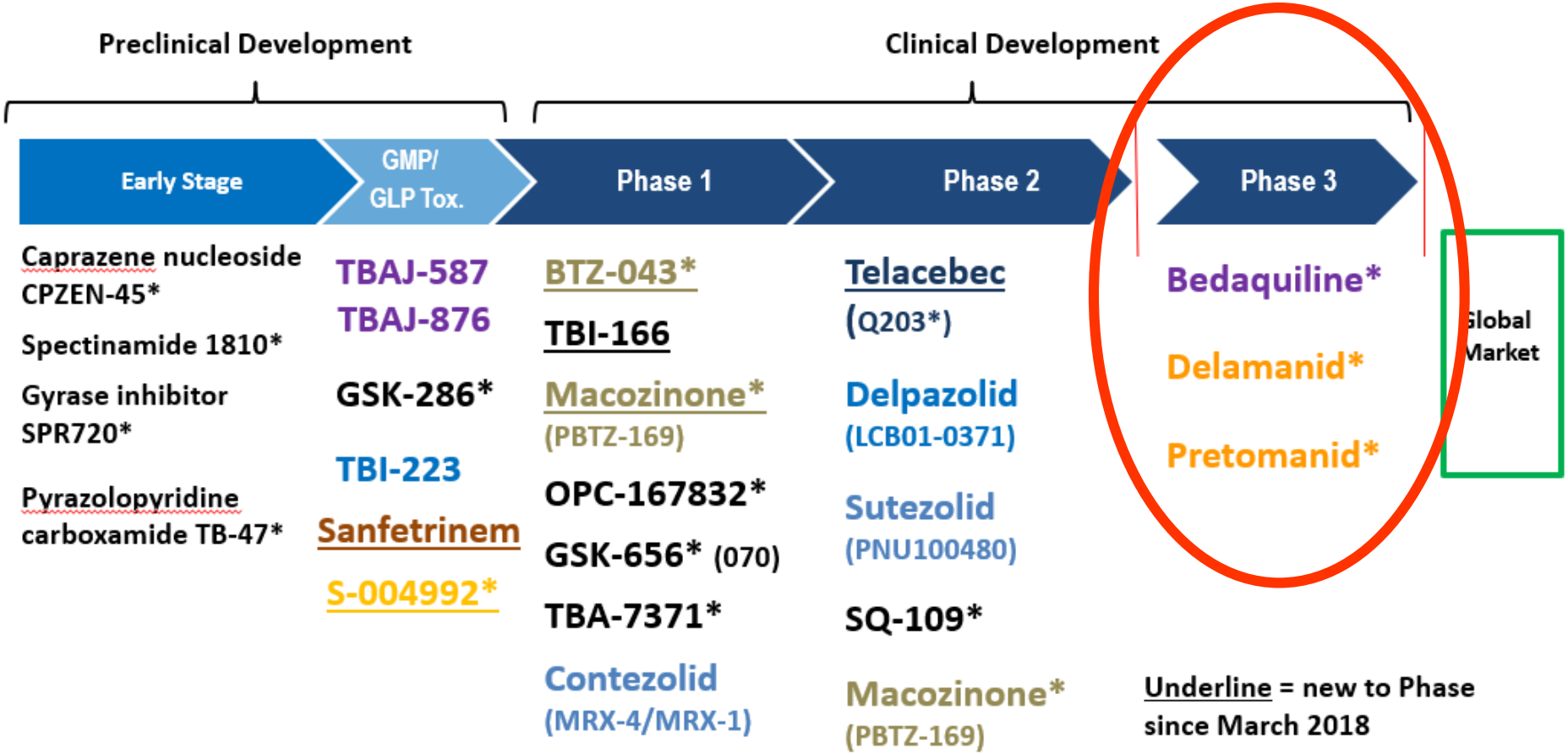
Figure 3. Pervasive epistatic interactions influencing the physiology and population biology of drug-resistant *M. tuberculosis* strains. The effect of drug resistance mutations alone or in combination as well as the presence of compensatory mutations affects the fitness of drug-resistant *M. tuberculosis* strains as a function of the strain genetic background.

NOVOS

ANTIMICROBIANOS E REGIMES

ANTI-TB

2018 Global New TB Drug Pipeline [1]



New chemical class* Known chemical classes for any indication are color coded: fluoroquinolone, rifamycin, oxazolidinone, nitroimidazole, diarylquinoline, benzothiazinone, imidazopyridine amide, beta-lactam.

[1] New Molecular Entities not yet approved, being developed for TB or only conditionally approved for TB. Showing most advanced stage reported for each. Details for projects listed can be found at http://www.newtbdrugs.org/pipeline/clinical

Ongoing projects without a lead compound series identified: http://www.newtbdrugs.org/pipeline/discovery

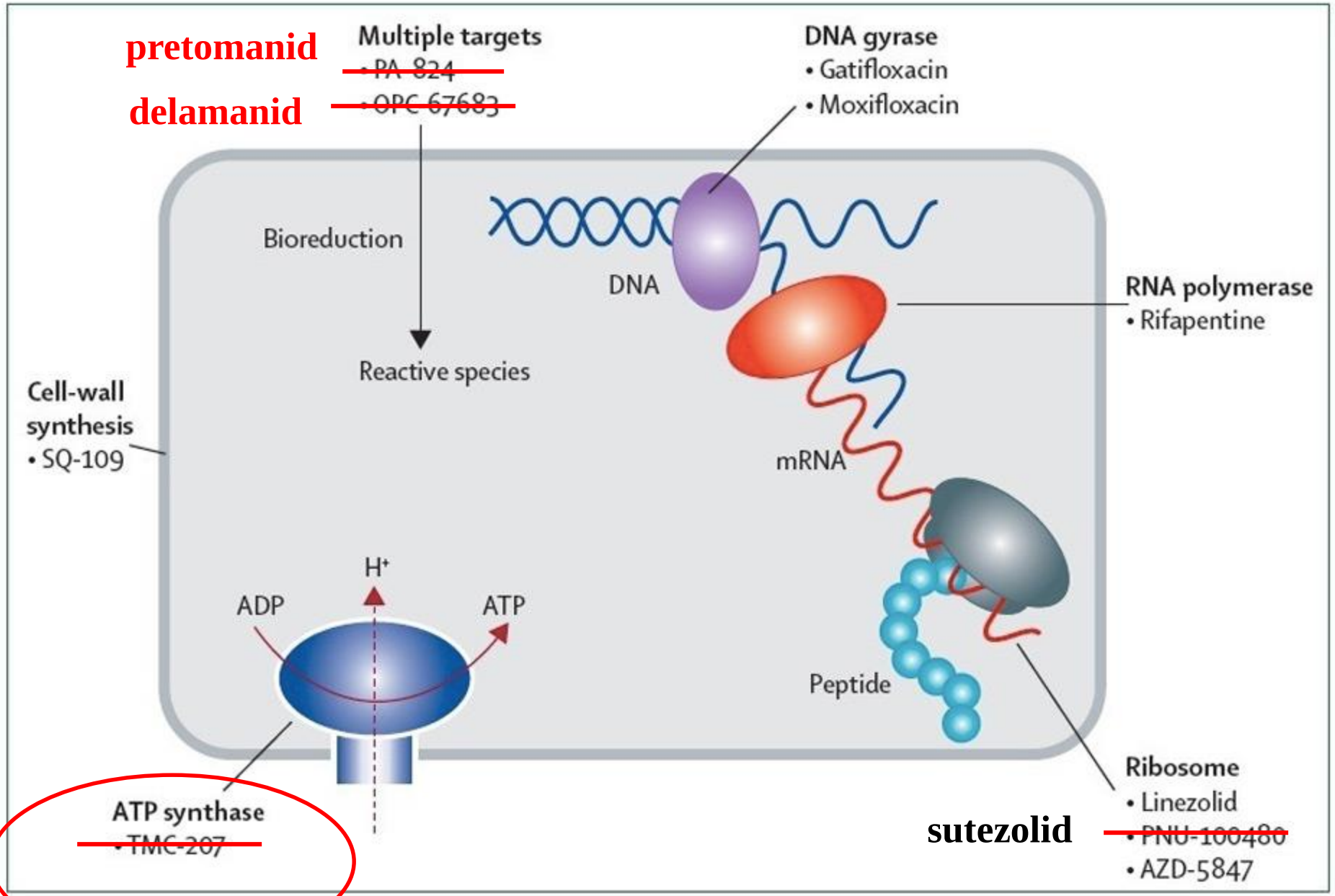
WORKING GROUP ON NEW TB DRUGS

www.newtbdrugs.org

Updated: October 2018

Source: 'The Antibiotic Resistance Crisis', C. Lee Ventola, Data from CDC & FDA Center for Drug Evaluation and Research, Office of Health Economics

ALJAZEERA


pretomanid
delamanid
Multiple targets
• PA-824
• OPC-67683
DNA gyrase
• Gatifloxacin
• Moxifloxacin
Bioreduction
DNA
RNA polymerase
• Rifapentine
Reactive species
Cell-wall synthesis
• SQ-109
mRNA
H+
ADP
ATP
Peptide
ATP synthase
• TMC-207
bedaquiline
Ribosome
• Linezolid
• PNU-100480
• AZD-5847
sutezolid

FOR HEALTHCARE PROFESSIONALS IN THE US AND PUERTO RICO ONLY

Sirturo™ bedaquiline 100mg tablets

Multi-drug Resistant TB
About SIRTURO™
SIRTURO™ Clinical Trials

SIRTURO™ Is the First Medication for Pulmonary MDR-TB With a Novel Mechanism of Action in Over 40 Years

Indications

Phase III

GOOD NEWS!!!!!

BDQ reduced about 30% the median time to sputum culture conversion

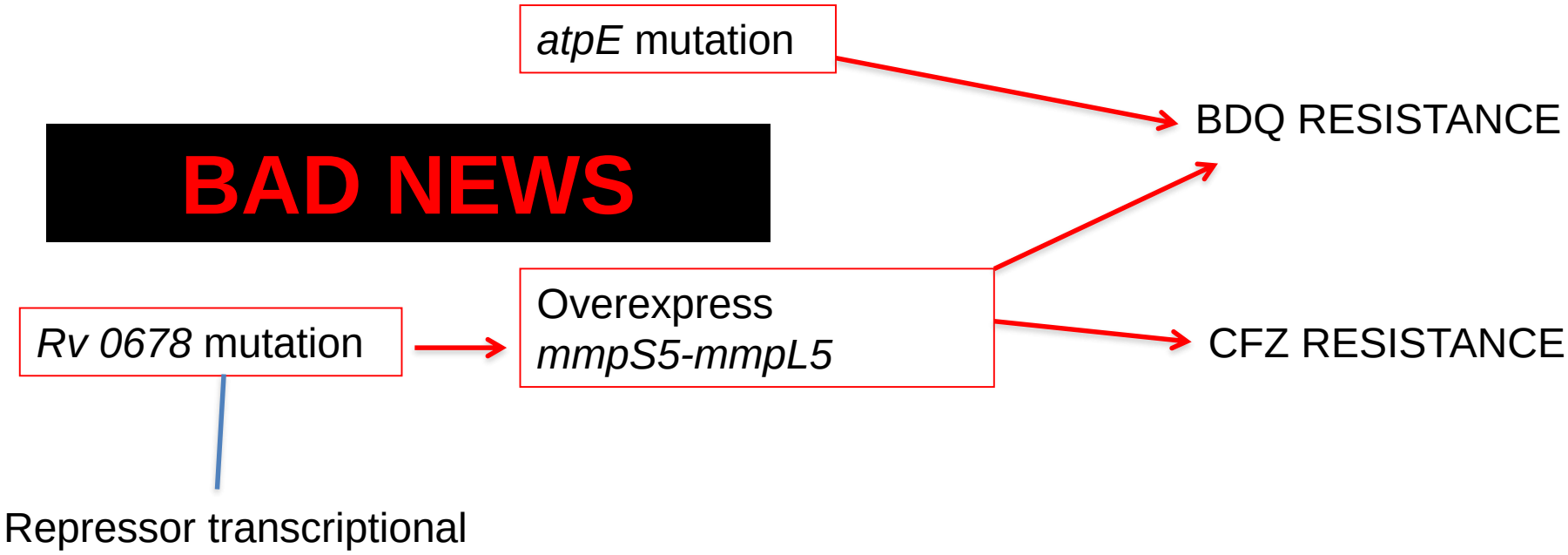
increased the rate of culture conversion

OPEN ACCESS Freely available online

Acquired Resistance of *Mycobacterium tuberculosis* to Bedaquiline

Koen Andries[1]*, Cristina Villellas[1], Nele Coeck[2], Kim Thys[1], Tom Gevers[1], Luc Vranckx[1], Nacer Lounis[1], Bouke C. de Jong[2], Anil Koul[1]

1 Department of Infectious Diseases, Janssen Pharmaceutica, Beerse, Belgium, **2** Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium



OVEREXPRESS EFFLUX SYSTEM = MULTIPLE DRUG RESISTANCE

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JUNE 4, 2009 VOL. 360 NO. 23

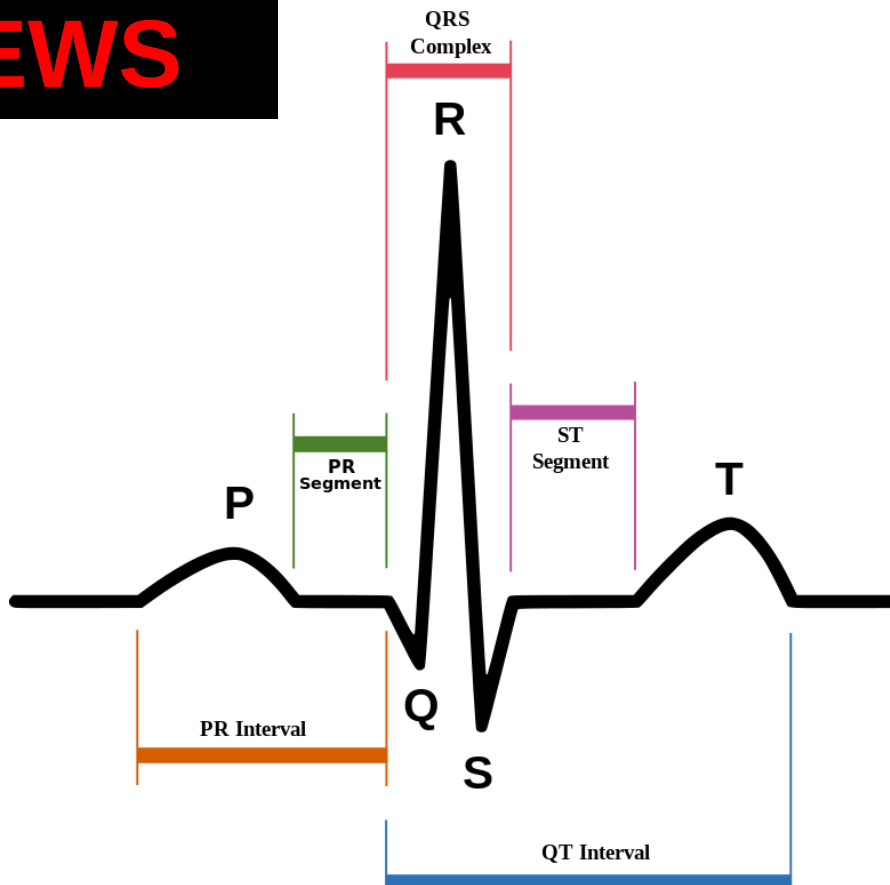
The Diarylquinoline TMC207 for Multidrug-Resistant Tuberculosis

Andreas H. Diacon, M.D., Ph.D., Alexander Pym, M.D., Ph.D., Martin Grobusch, M.D., D.T.M.&H., Ramonde Patientia, M.D., Roxana Rustomjee, M.D., Ph.D., Liesl Page-Shipp, M.D., Christoffel Pistorius, M.D., Rene Krause, M.D., Mampedi Bogoshi, M.D., Gavin Churchyard, M.B., Ch.B., Amour Venter, Nat.Dip.Med.Tech.(Micro), Jenny Allen, B.Sc., Juan Carlos Palomino, Ph.D., Tine De Marez, Ph.D., Rolf P.G. van Heeswijk, Pharm.D., Ph.D., Nacer Lounis, Ph.D., Paul Meyvisch, M.Sc., Johan Verbeeck, D.V.M., Ph.D., Wim Parys, M.D., Karel de Beule, Pharm.D., Koen Andries, D.V.M., Ph.D., and David F. Mc Neeley, M.D., M.P.H.T.M.

MORE BAD NEWS

INCREASES IN THE QT INTERVAL

.



OUTRAS PREOCUPAÇÕES

METABOLIZADO PELA CYP450 3A4 NÃO PODE SER ADMINISTRADO JUNTO COM A RIF

- **DESORDENS HEPÁTICAS (8.8% BDQ X 1.9% PLACEBO)**

- **NÃO DEVE SER USADO JUNTO AO DELAMANIDE (AMBOS CAUSAM AUMENTO DO INTERVALO QT)**

(OPC 67683) DELAMANIDA

in/Register
dd a Project

Last Updated: 24-Jul-2014

PRECLINICAL | PHASE I | PHASE II | PHASE III

PROJECT INFO

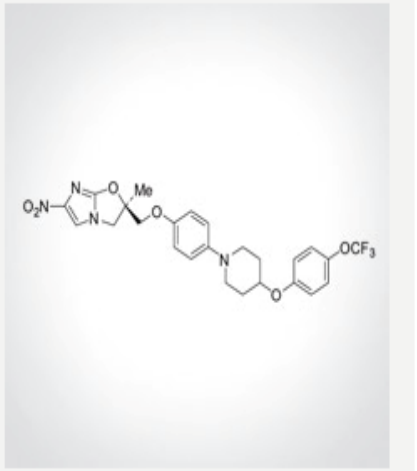
Submitted for registration to a stringent regulatory authority (FDA, EMA, WHO pre-qualification

Yes

Project type

New chemical entity (NCE)

Clinical project description



Name of compound or target

INIBE A SÍNTESE DE ÁCIDOS MICÓLICOS

LIBERA ÓXIDO NÍTRICO

Delamanida

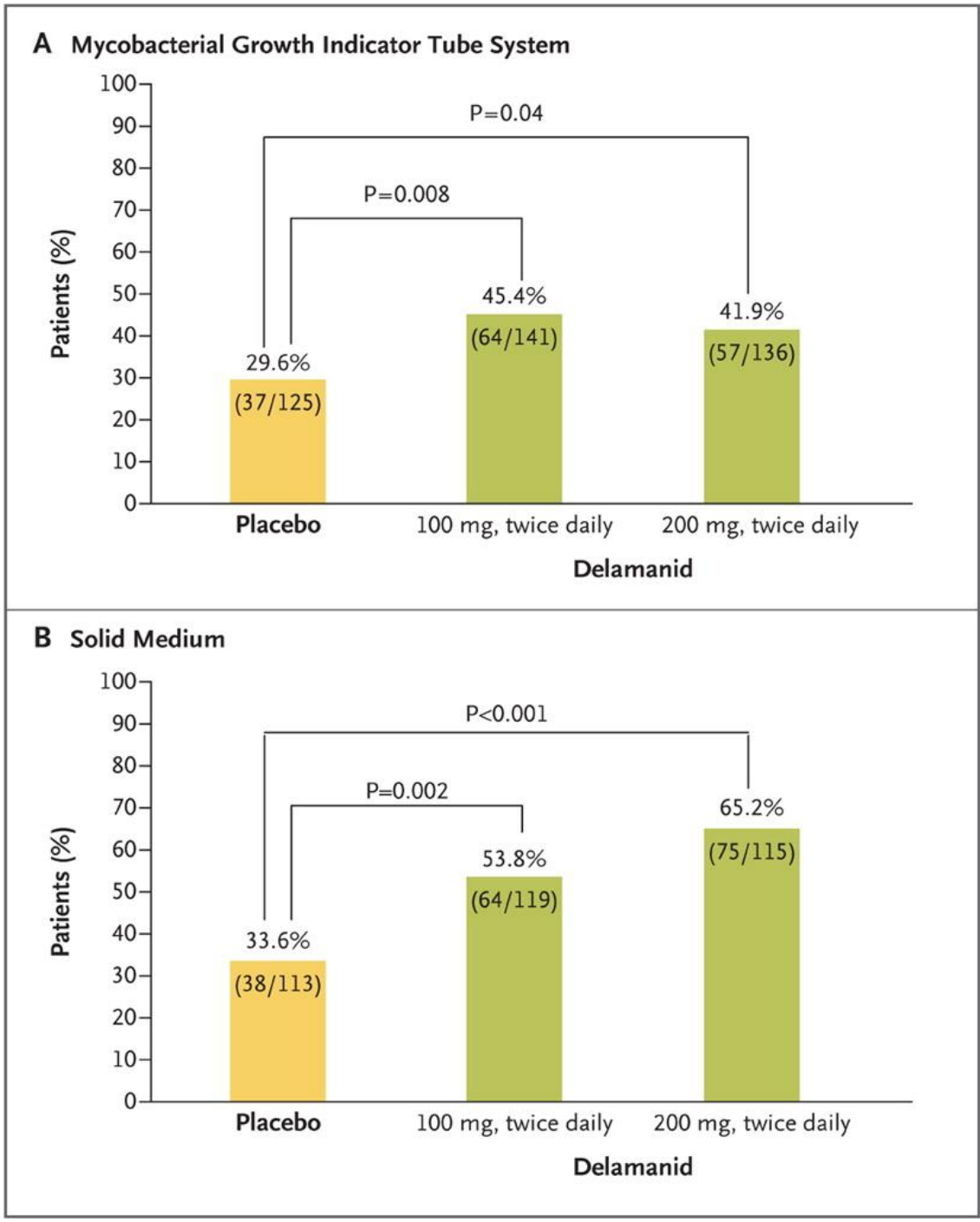
- ***More M. tuberculosis*** **specific→ minimal drug interactions**

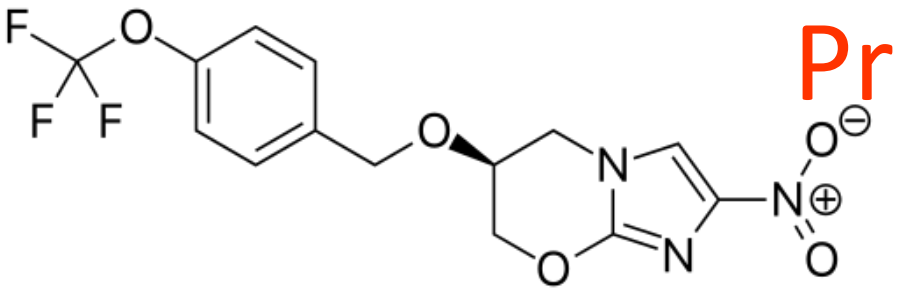
- **Dose dependent activity in vitro similar to rifampin**

Delamanid improved 2 month culture conversion

QT prolongation more common than with placebo

Gler et al. *NEJM* 2012





Pretomanida (PA-824)

- Nitroimidazole (como Delamanid).
- Inibe síntese de parede celular
- Cadeia respiratoria
- Prolongamento do intervalo QT

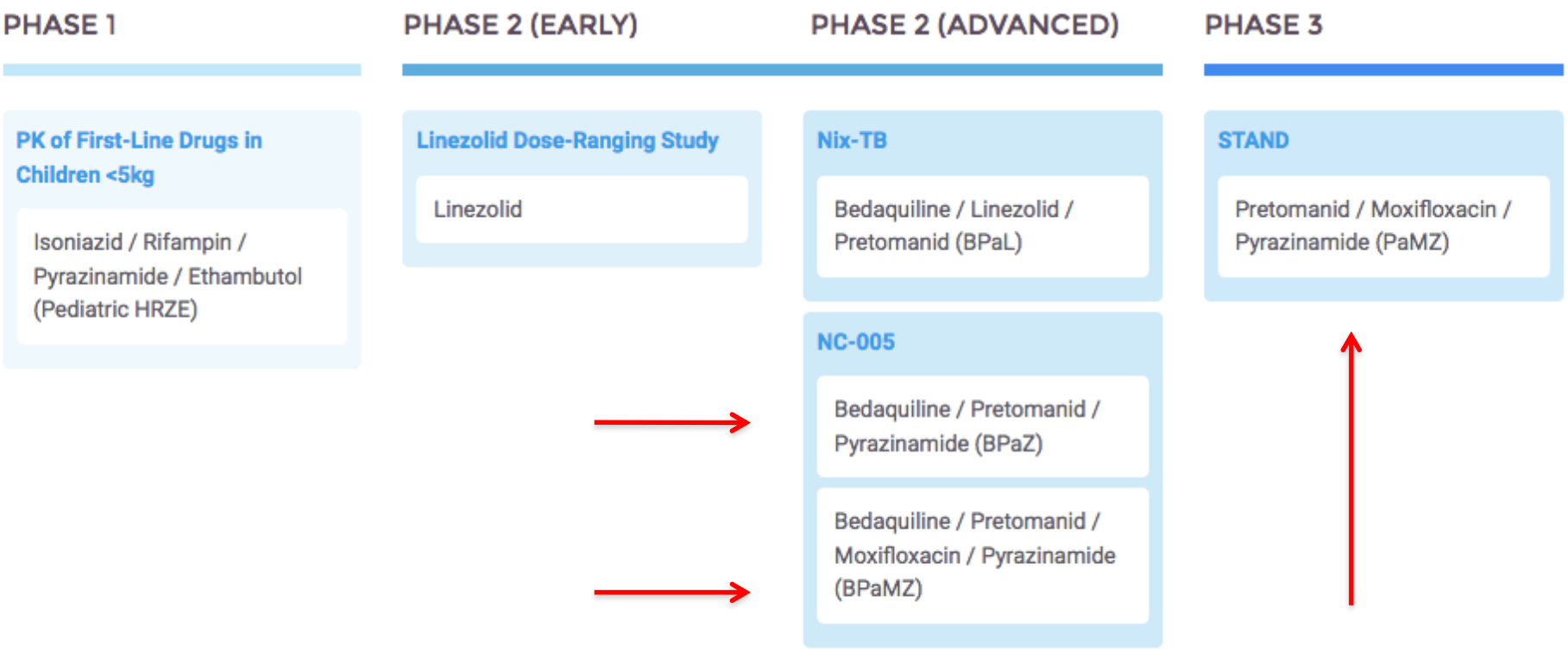
- Atividade contra cepas resistentes a isoniazida com CMI = 0.03 0.2 μg/ml
-
- É um profarmaco que requer ativação

NOVOS REGIMES TERAPÊUTICOS

Discovery

Clinical Development and Marketed Products

TB Alliance manages the largest pipeline of new TB drugs in history and has advanced multiple products to market. Projects with t
greatest impact on the disease, while being cost-effective and simple to administer, are prioritized. For the latest status of the STA
clinicaltrials.gov or contact us to schedule a briefing.



PaMZ

Pretomanid + Moxifloxacin + Pyrazinamide

FASE 2A NC (New Combination 1)-001, eliminou Mtb mais rápido nas duas primeiras semanas de tratamento quando comparado com o regime atua.....REDUÇÃO DO TEMPO???

Tem o potencial para curar a TB DS e algumas formas de MDR-TB

Pode ser administrado com ARV

ADJUVANTES DO TRATAMENTO COM ATB

RESISTÊNCIA A BDQ E O EFLUXO!

HISTORY

1996

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 362–366, January 1996
Microbiology

Efflux pump of the proton antiporter family confers low-level fluoroquinolone resistance in *Mycobacterium smegmatis*

H. E. TAKIFF*†, M. CIMINO*, M. C. MUSSO*, T. WEISBROD‡, R. MARTINEZ*, M. B. DELGADO*, L. SALAZAR*, B. R. BLOOM‡, AND W. R. JACOBS, JR.‡

*Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela; and ‡Howard Hughes Institute, Albert Einstein College of Medicine, Bronx, NY 10461

1998

JOURNAL OF BACTERIOLOGY, Nov. 1998, p. 5836-5843 Vol. 180, No. 22
0021-9193/98/$04.00+0
Copyright © 1998, American Society for Microbiology. All Rights Reserved.

Molecular Cloning and Characterization of Tap, a Putative Multidrug Efflux Pump Present in *Mycobacterium fortuitum* and *Mycobacterium tuberculosis*

JOSÉ A. AÍNSA,[1]† MARIAN C. J. BLOKPOEL,[2] ISABEL OTAL,[1] DOUGLAS B. YOUNG,[2] KOEN A. L. DE SMET,[2] AND CARLOS MARTÍN[1]*

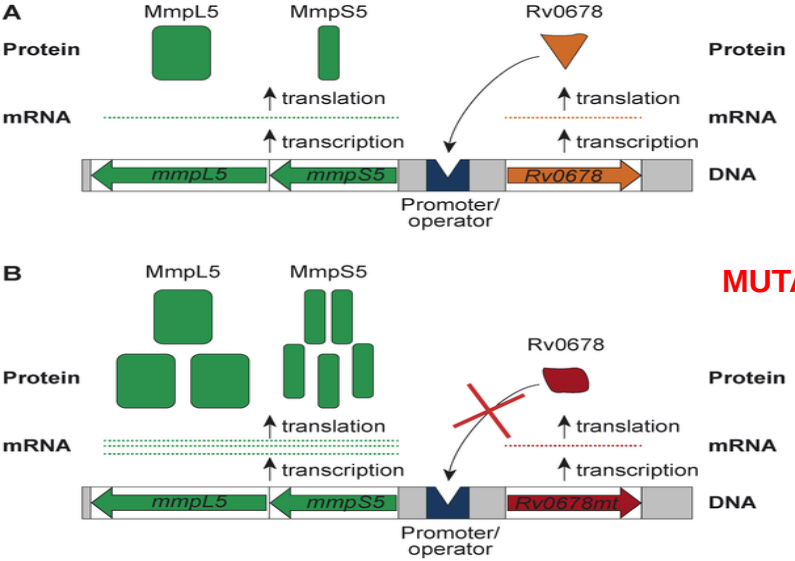
Departamento de Microbiología Medicina Preventiva y Salud Pública, Universidad de Zaragoza, 50009 Zaragoza, Spain,[1] and Department of Infectious Diseases and Microbiology, Imperial College School of Medicine, St. Mary's Campus, London W2 1PG, United Kingdom[2]

2001

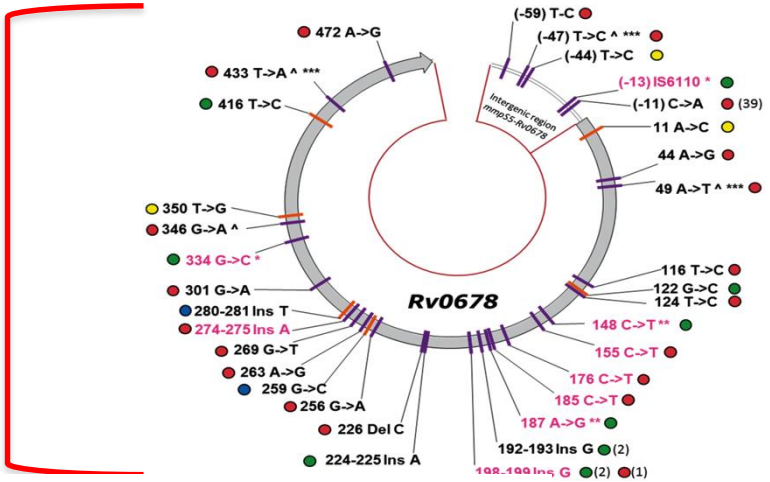
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2001, p. 800–804 Vol. 45, N
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.3.800–804.2001
Copyright © 2001, American Society for Microbiology. All Rights Reserved.

Characterization of P55, a Multidrug Efflux Pump in *Mycobacterium bovis* and *Mycobacterium tuberculosis*

PEDRO E. A. SILVA,[1] FABIANA BIGI,[2] MARÍA DE LA PAZ SANTANGELO,[2] MARIA ISABEL ROMANO,[2] CARLOS MARTÍN,[1] ANGEL CATALDI,[2] AND JOSÉ A. AÍNSA[1]*



MUTATIONS IN RV 0678

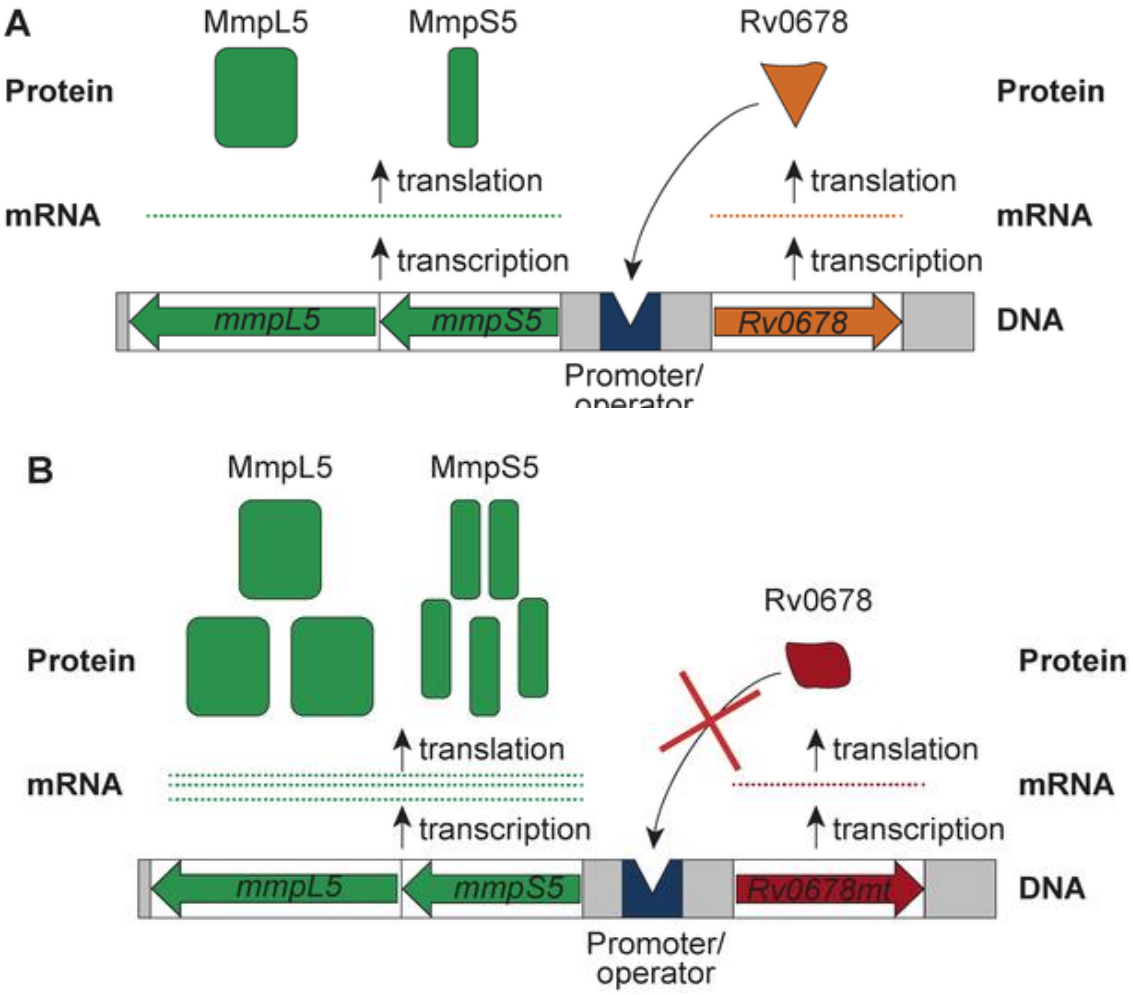


NO MUTATIONS atpE

Strain	Description	BDQ MIC (μg/ml)	
H37Rv	wild type *M. tuberculosis* strain	0.063	
BCLA 2	BDQ-R mutant, H37Rv-derived, no *atpE* mutations	0.250 (×4)	NO MUT
BCA 4	BDQ-R mutant, H37Rv-derived, no *atpE* mutations	0.500 (×8)	
BCA 8	BDQ-R mutant, H37Rv-derived, no *atpE* mutations	0.500 (×8)	NO MUT
BK12	BDQ-R mutant, H37Rv-derived, *atpE* mutation [2]	2.000 (×32)	MUT
LV13	BDQ-R mutant, H37Rv-derived, *atpE* mutation [24]		
EH 3.0	MDR *M. tuberculosis* strain [3]		
EH 3.2	BDQ-R mutant, EH 3.0-derived, no *atpE* mutations [3]		
EH 3.6	BDQ-R mutant, EH 3.0-derived, no *atpE* mutations [3]		
EH 3.3	BDQ-R mutant, EH 3.0-derived, no *atpE* mutations [3]		

The MICs of BDQ-R preclinical strains derived from either the drug susceptible H37Rv or th
represent the difference between resistant and wild-type MICs. **wt**: wild-type; **Ins**: insertion
doi:10.1371/journal.pone.0102135.t001

Figure 3. Mechanism of BDQ and CFZ resistance in Rv0678 mutants.



Andries K, Villellas C, Coeck N, Thys K, Gevers T, et al. (2014) Acquired Resistance of Mycobacterium tuberculosis to Bedaquiline. PLoS ONE 9(7): e102135. doi:10.1371/journal.pone.0102135
http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0102135

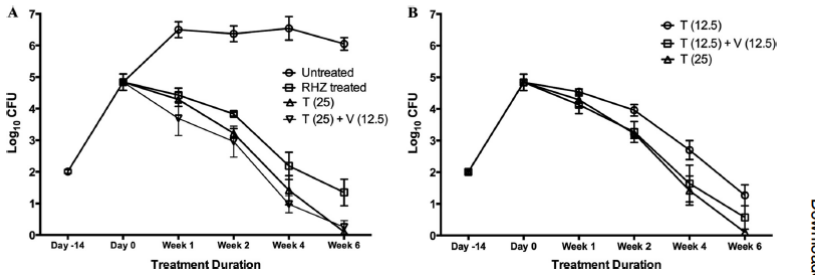
AAC Journals.ASM.org

2015

Verapamil Increases the Bactericidal Activity of Bedaquiline against *Mycobacterium tuberculosis* in a Mouse Model

Shashank Gupta,[a,b] Sandeep Tyagi,[a] William R. Bishai[a,b]

Gupta et al.



GOOD NEWS!!!!!

Verapamil potentiates the activity of bedaquiline,

Verapamil may be cardioprotective, reducing the risk of QT prolongation

EDITORIAL

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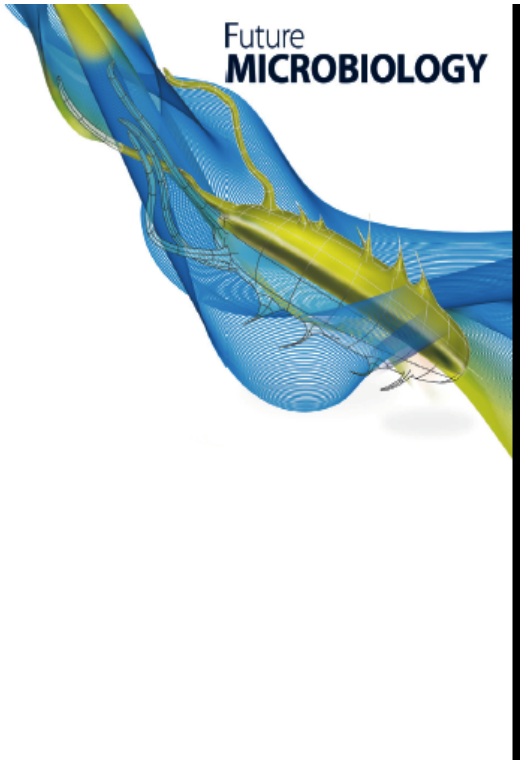


Can the addition of verapamil to bedaquiline-containing regimens improve tuberculosis treatment outcomes? A novel approach to optimizing TB treatment

"…with very few anti-tuberculosis drugs in the pipeline, repurposing existing approved drugs as adjuvants to shorten treatment duration has emerged as a promising alternative strategy to outpace the evolution of drug resistance."

J Antimicrob Chemother 2016; **71**: 17–26
doi:10.1093/jac/dkv316 Advance Access publication 15 October 2015

Journal of Antimicrobial Chemotherapy

Efflux pump inhibitors: targeting mycobacterial efflux systems to enhance TB therapy

Caroline M. Pule, Samantha L. Sampson*, Robin M. Warren, Philippa A. Black, Paul D. van Helden, Tommie C. Victor and Gail E. Louw

MORE EXPERIMENTAL EVIDENCES THAT EIP CAN WORK AS ADJUVANT IN THERAPEUTIC

Acceleration of Tuberculosis Treatment by Adjunctive Therapy with Verapamil as an Efflux Inhibitor

2013

Shashank Gupta[1,2], Sandeep Tyagi[1], Deepak V. Almeida[1,3], Mariama C. Maiga[1,2], Nicole C. Ammerman[1,3], and William R. Bishai[1,2,3]

[1]Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University, Baltimore, Maryland; [2]Howard Hughes Medical Institute Chevy Chase, Maryland; and [3]KwaZulu-Natal Research Institute for Tuberculosis and HIV, Durban, South Africa

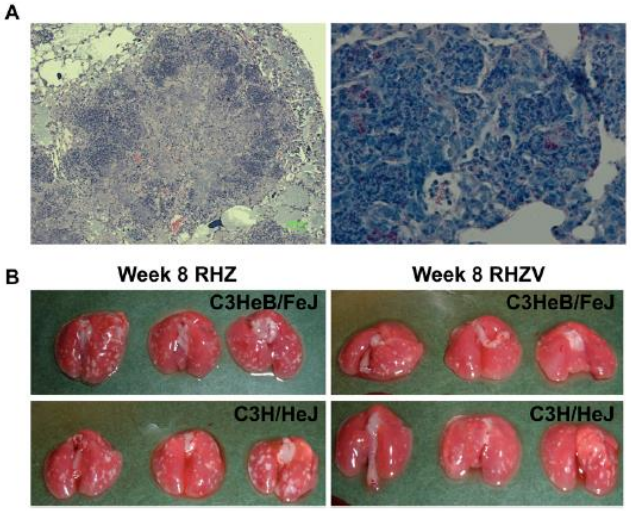


Figure 2. Adjunctive drug treatment with verapamil improves pathology of *Mycobacterium tuberculosis*–infected lungs. (*A*) Microscopic histopathology of lungs at 2 weeks of infection in C3HeB/FeJ mice. The *left panel* is hematoxylin and eosin staining (40×), and the *right panel* is acid fast staining for *M. tuberculosis* H37Rv (500×). (*B*) Gross lung pathology at 8 weeks of treatment in different groups, as indicated.

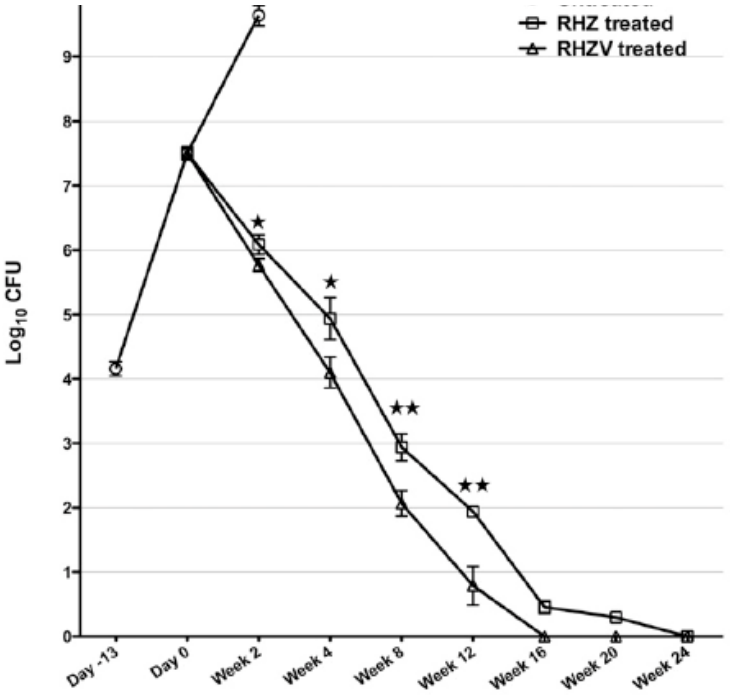


Figure 3. Adjunctive drug treatment with verapamil reduces bacterial counts during active disease. (*A*) Timeline and experimental scheme for the 9 months of the experiment. A total of 10 mice from each group were held at Weeks 16, 20, and 24 of treatment for an additional 3 months without any treatment for relapse study. (*B*) C3HeB/FeJ and C3H/HeJ mice were infected with Log_{10} 4.2 *Mycobacterium tuberculosis* H37Rv, and the treatment started at Day 14 after infection. The mice were treated with rifampin (R; 10 mg/kg), isoniazid (H; 10 mg/kg), pyrazinamide (Z; 150 mg/kg), and verapamil (V; 9.40 mg/kg) daily for 5 d/wk. The lungs were homogenized, diluted, and plated for cfu counts and expressed as Log_{10} cfu (±SD). $^{\star}P < 0.01$ and $^{\star\star}P < 0.001$ for RHZ versus RHZV groups.

NÚCLEO DE PESQUISA EM MICROBIOLOGIA MÉDICA

- CARACTERIZAÇÃO DO EFLUXO EM MICOBACTÉRIAS
- NOVOS ANTIBÓTICOS
- INIBIDORES DO EFLUXO

antibiotics

MDPI

Article

Efflux Activity Differentially Modulates the Levels of Isoniazid and Rifampicin Resistance among Multidrug Resistant and Monoresistant *Mycobacterium tuberculosis* Strains

frontiers in Microbiology

ORIGINAL RESEARCH
published: 27 April 2017
doi: 10.3389/fmicb.2017.00711

Check for updates

Interplay between Mutations and Efflux in Drug Resistant Clinical Isolates of *Mycobacterium tuberculosis*

Diana Machado[1†], Tatiane S. Coelho[2,3†], João Perdigão[4], Catarina Pereira[4], Isabel Couto[1], Isabel Portugal[4], Raquel De Abreu Maschmann[2,5], Daniela F. Ramos[3], Andrea von Groll[3], Maria L. R. Rossetti[5,6], Pedro A. Silva[2,3‡] and Miguel Viveiros[1‡]*

HIPÓTESE DE COMO OCORRE O EFLUXO ?

CAMINHOS PARA INIBIR O EFLUXO

International Journal of Antimicrobial Agents 49 (2017) 308–314

Contents lists available at ScienceDirect

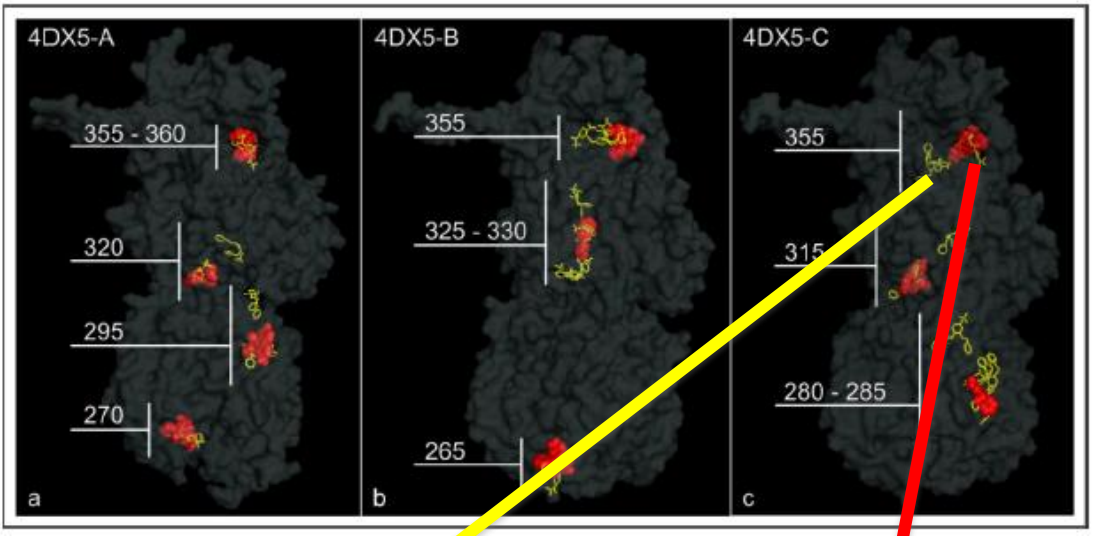
International Journal of Antimicrobial Agents

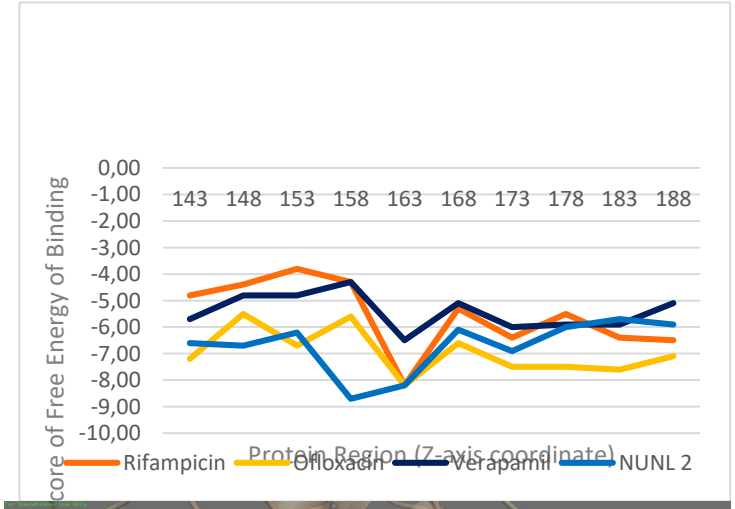
journal homepage: www.elsevier.com/locate/ijantimicag

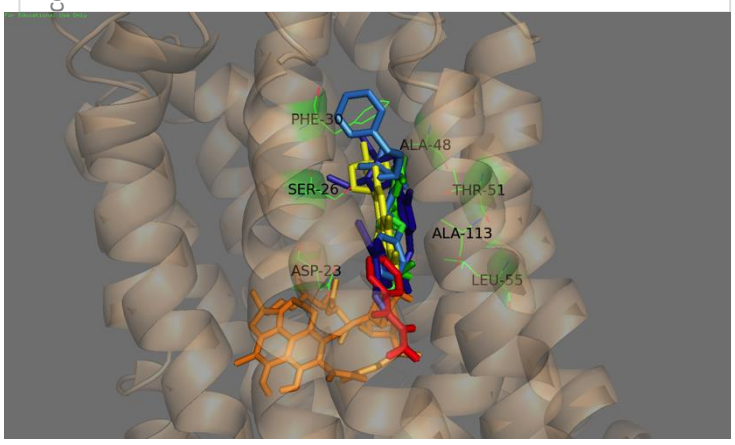
2017

In vitro and in silico analysis of the efficiency of tetrahydropyridines as drug efflux inhibitors in *Escherichia coli*

Lande Silva Jr [a], Lillian Lucas Carrion [a], Andrea von Groll [a], Sofia Santos Costa [b], Elisabete Junqueira [b], Daniela Fernandes Ramos [a], Jéssica Cantos [a], Vinicius Rosa Seus [c], Isabel Couto [b], Liana da Silva Fernandes [d], Hélio Gauze Bonacorso [d], Marcos Antônio Pinto Martins [d], Nilo Zanatta [d], Miguel Viveiros [b], Karina S. Machado [c], Pedro Eduardo Almeida da Silva [a,*]

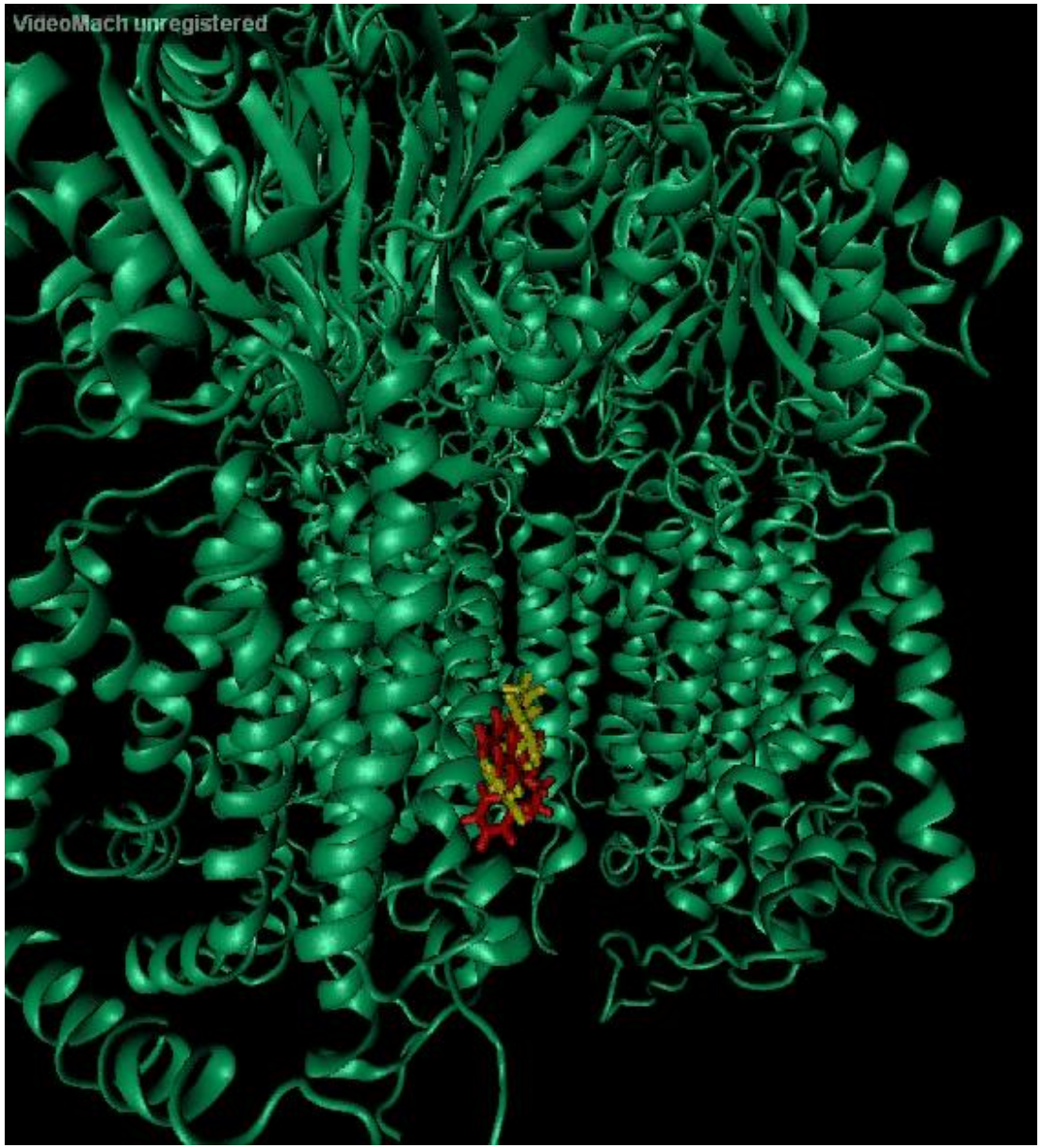




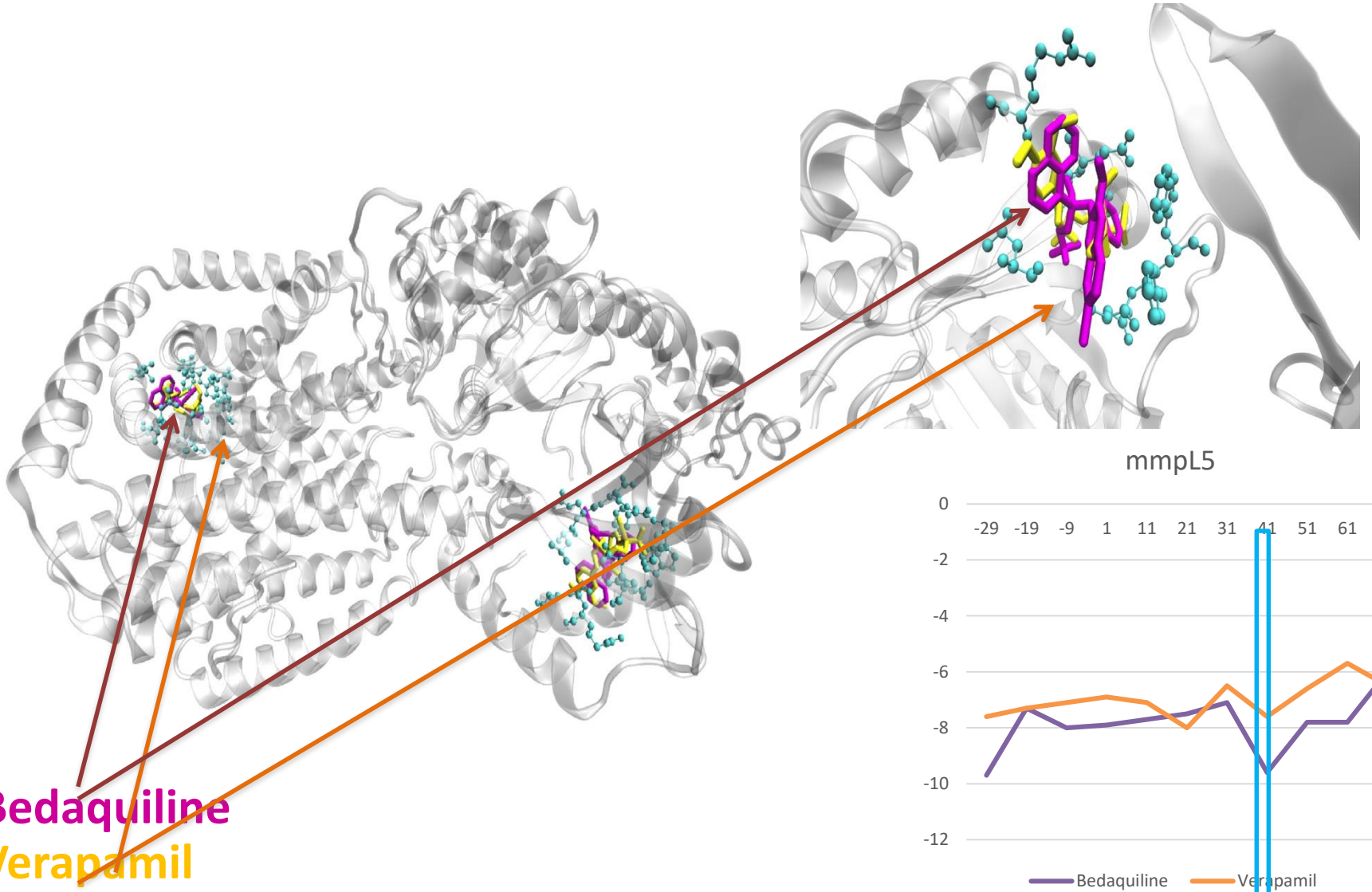


EFFLUX INHIBITOR

ANTIBIOTIC


VideoMach unregistered

Screening Docking mmpL5



Unplubished data

NOVOS ATB E INIBIDORES DE EFLUXO

Tuberculosis 111 (2018) 198–201

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Tuberculosis

journal homepage: www.elsevier.com/locate/tube

Drug Discovery and Resistance

Anti-*Mycobacterium tuberculosis* activity of naphthoimidazoles combined with isoniazid and rifampicin

Lélia Pacheco Corrêa Barros[a], Karina Pena Del Rio[b], Tatiane dos Santos Conceição Carvalho[b], Maria do Carmo Freire Ribeiro Pinto[b], Kelly Cristina Gallan de Moura[b], Priscila Cristina Bartolomeu Halicki[a], Daniela Fernandes Ramos[a], Pedro Eduardo Almeida da Silva[a,*]

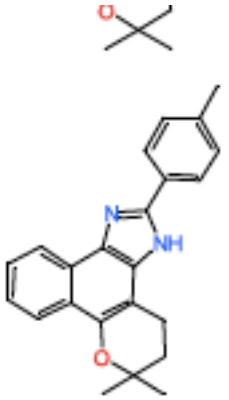
A2		$C_{23}H_{23}N_2O$	6,6-dimethyl-2-(p-tolyl)-3,4,5,6-tetrahydrobenzo[7,8]chromeno[5,6-d]imidazole

Table 2
Interaction of INH/RIF with naphthoimidazoles against *M. tuberculosis* H37Rv. Given that MIC of INH alone 0.03 μg/mL; MIC of RIF alone = 0.5 μg/mL; FICI = Fractional Inhibitory Concentration Index; (A) = Additivity and (I) = Indifference. INH Interaction with RMP: additivity (FICI = 1).

Compound	MIC (μg/mL)	FIC			
		MIC Compound/INH(μg/mL)	FICI/Interaction	MIC Compound/RIF(μg/mL)	FICI/Interaction
A1	3.12	1.56/0.016	**1 (A)**	1.56/0.25	**1 (A)**
A2	1.56	1.56/0.016	**1.5 (I)**	0.78/0.06	**0.62 (A)**
B1	3.12	1.56/0.016	**1 (A)**	1.56/0.125	**0.75 (A)**
B2	25	25/0.016	**1.5 (I)**	12.5/0.125	**0.75 (A)**
B3	1.56	1.56/0.016	**1.5 (I)**	1.56/0.25	**1.5 (I)**
C1	3.12	1.56/0.016	**1 (A)**	1.56/0.5	**1.5 (I)**
C2	3.12	1.56/0.016	**1 (A)**	1.56/0.25	**1 (A)**
C3	> 100	> 100/0.03	**ND**	> 100/0.5	**ND**
D1	6.25	3.12/0.016	**1 (A)**	3.12/0.25	**1 (A)**

*FICI ≤ 0.5: Synergism; 0.5 < FICI ≤ 1; Additivity; 1 < FICI ≤ 2, Indifference; and FICI > 2, Antagonism.

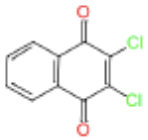
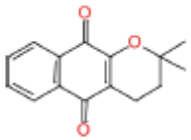
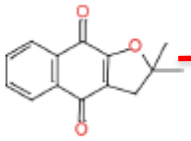
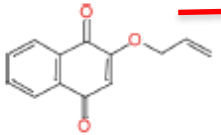
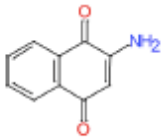
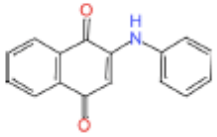
frontiers in Microbiology

ORIGINAL RESEARCH
published: 09 April 2018
doi: 10.3389/fmicb.2018.00673

Naphthoquinone Derivatives as Scaffold to Develop New Drugs for Tuberculosis Treatment

*Priscila C. B. Halicki[1], Laís A. Ferreira[1], Kelly C. G. De Moura[2], Paula F. Carneiro[2], Karina P. Del Rio[2], Tatiane dos S. C. Carvalho[2], Maria do C. F. R. Pinto[2], Pedro E. A. da Silva[1] and Daniela F. Ramos[1]**

TABLE 1 | Characterization of the 1,4-naphthoquinone derivatives.

	Chemical structure	Chemical formula	Nomenclature
1		$C_{10}H_4Cl_2O_2$	2,3-Dichloronaphthalene-1,4-dione
2		$C_{15}H_{14}O_3$	2,2-Dimethyl-3,4-dihydro-2H-benzo[g]chromene-5,10-dione
3		$C_{14}H_{12}O_3$	2,2-Dimethyl-2,3-dihydronaphtho[2,3-b]furan-4,9-dione
4		$C_{13}H_{10}O_3$	2-(Allyloxy)naphthalene-1,4-dione
5		$C_{10}H_7NO_2$	2-Aminonaphthalene-1,4-dione
6		$C_{16}H_{11}NO_2$	2-(Phenylamino)naphthalene-1,4-dione

Compound	MIC (μM)		
	H37Rv	INH_R	RMP_R
1	110.6	110.6	110.6
2	103.3	206.6	206.6
3	54.8	54.8	54.8
4	58.4	234	234
5	72.2	36.1	36.1
6	100.4	100.4	12.5
INH	0.438	14.6	≤0.219
RIF	0.608	0.304	622.2

ECOSSITEMA COSTEIRO COMO FONTE DE NOVOS FÁRMACOS

MICROALGAS

- ***Conticribra weissflogii***
- ***Nannochloropsis oceânica***
- ***Chaetoceros mulleri***

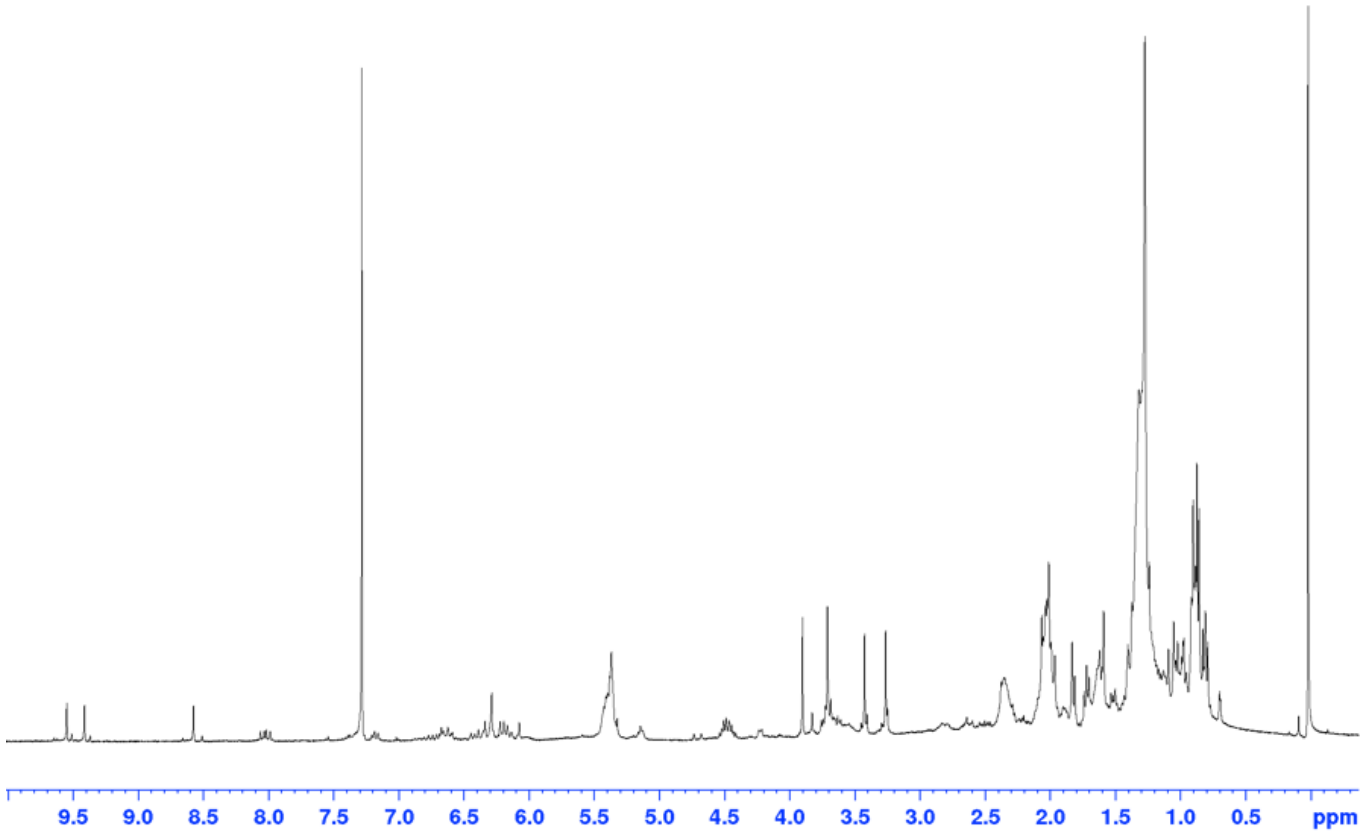
Conticribra weissflogii

Extrato	Rendimento (%)	*S.aureus*	*S. pneumoniae**	*P. aeruginosa*	*A. baumannii*
Água	47,29	Inativo	Inativo	Inativo	Inativo
Metanol	21,3	Inativo	Inativo	Inativo	Inativo
Acetona	2,7	200 µg/mL	Inativo	Inativo	Inativo
Acetato de Etila	3,6	200 µg/mL	400 µg/mL	Inativo	Inativo
Diclorometano	4,1	200 µg/mL (Rx)	Inativo	Inativo	Inativo
Clorofórmio	5,5	200 µg/mL	Inativo	Inativo	Inativo
Hexano	1,7	100 µg/mL	Inativo	Inativo	400 µg/mL
Ciprofloxacino	-	≤0,0625	-	≤0,0625	0,125 (RX)
Amikacina	-	>2	-	1	>2

Extrato	*E. coli**	*Serratia liquefaciens**	*M. marinum**	*M. tuberculosis*
Água	Não ativo	Não ativo	Não ativo	Não ativo
Metanol	Não ativo	Não ativo	800 µg/mL	400 µg/mL
Acetona	Não ativo	Não ativo	Não ativo	100 µg/mL
Acetato de Etila	Não ativo	Não ativo	100 µg/mL	100 µg/mL
Diclorometano	Não ativo	Não ativo	800 µg/mL	200 µg/mL
Clorofórmio	Não ativo	Não ativo	800 µg/mL	100 µg/mL
Hexano	Ativo a 800 µg/mL Rx	Não ativo	Não ativo	≤ 50 µg/mL

Ressonância Magnética Nuclear

Extrato Hexano: picos relativos a ácidos graxos



Pharmaceutical Biology, 2010, 1–4, Early Online

informa healthcare 2010

RESEARCH ARTICLE

Antimycobacterial activity of usnic acid against resistant and susceptible strains of *Mycobacterium tuberculosis* and non-tuberculous mycobacteria

Daniela Fernandes Ramos[1,2], Pedro Eduardo Almeida da Silva[1,2]

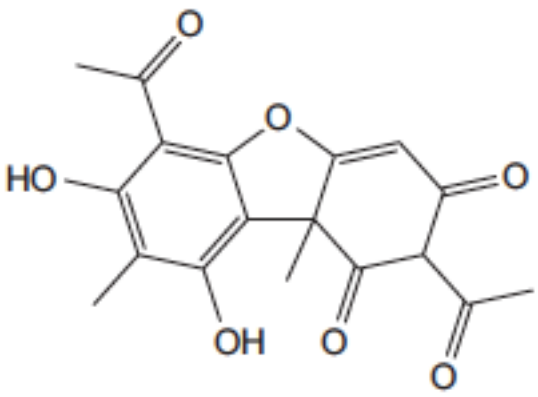


Figure 1. Structure of usnic acid.

Table 1. Usnic acid antimicrobial activity.

Usnic acid	CCCP	Verapamil
12.25 µg/mL	12.5 µg/mL	12.5 µg/mL
1.56 µg/mL	NR	NR
6.25 µg/mL	NR	NR
12.5 µg/mL	NR	NR
12.5 µg/mL	12.5 µg/mL	6.5 µg/mL
100 µg/mL	100 µg/mL	100 µg/mL
50 µg/mL	25 µg/mL	25 µg/mL
25µg/mL	50 µg/mL	25 µg/mL

f usnic acid against isoniazid (INH), streptomycin fampicin (RMP) resistant and susceptible strains ; and NMT with and without inhibitor efflux.

Thieme

Antimicrobial and Efflux Inhibitor Activity of Usnic Acid Against *Mycobacterium abscessus*

2018

Authors
Ivy B. Ramis[1], Júlia S. Vianna[1], Ana Júlia Reis[1], Andrea von Groll[1], Daniela F. Ramos[1], Miguel Viveiros[2],

▶ **Table 1** MICs of the antimicrobials, usnic acid, EIs, and ethidium bromide for the *M. abscessus* strains.

		MIC (μM)							
Strain	AMI	CIP	CLA[a]	CLA[b]	CLA[c]	UA	CCCP	VP	EtBr
ATCC 19977	1.71	3.02	0.67	2.67	5.35	18.15	1.91	687.41	20.29
AT 07	3.41	6.03	0.17	2.67	10.69	9.07	1.91	687.41	81.16
AT 46	1.71	12.07	0.33	42.78	–	9.07	7.64	687.41	20.29
AT 52	6.83	24.14	171.13	–	–	9.07	3.82	687.41	20.29

AMI: amikacin; CIP: ciprofloxacin; CLA: clarithromycin; UA: usnic acid; CCCP: carbonyl cyanide m-chlorophenyl hydrazone; VP: verapamil; EtBr: ethidium bromide; [a] Value by visual reading in day 3; [b] Value by visual reading in day 5; [c] Value by visual reading in day 7. The assays were performed in triplicate.

▶ **Table 2** Interaction between the EIs in combination with usnic acid against *M. abscessus* strains.

	EI	Strains							
		ATCC 19977		AT 07		AT 46		AT 52	
		MIC (μM)	MF	MIC (μM)	MF	MIC (μM)	MF	MIC (μM)	MF
UA	No EI	18.15	–	9.07	–	9.07	–	9.07	–
	+CCCP	9.07	2	4.54	2	9.07	1	4.54	2
	+VP	4.54	4	4.54	2	4.54	2	2.27	4

UA: usnic acid; CCCP: carbonyl cyanide m-chlorophenyl hydrazone; VP: verapamil. The assays were performed in triplicate

Outros Estudos em fase pré-clínica

- Tetrahidropiridinas
- Metalofármacos
- fenazinas
- Extratos de halófitas
- Compostos bioativos marinhos

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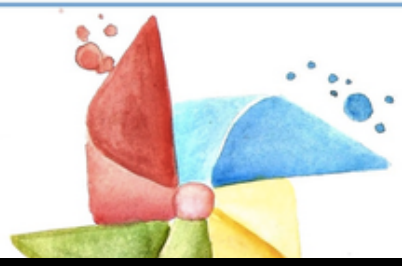

II WORKSHOP DA REDE SUL DE MICOBACTÉRIAS
II MOSTRA ESTADUAL DA ATENÇÃO À SAÚDE PRISIONAL
IV ENCONTRO REGIONAL DE TUBERCULOSE

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II Workshop da Rede Sul de Micobactérias, II Mostra de Experiências em [illegible] de

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- Dr. Luliano Lacava (Technician)
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- Priscila Halick (PhD student)
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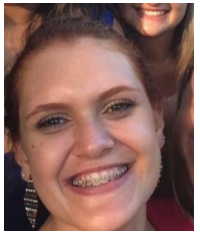
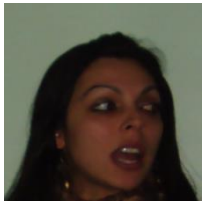
- Vanessa Radim (Master Student)









Sebastião Salgado

Partners

- Prof. Paulo Abreu - FURG
- Profa. Karina Machado – FURG
- Profa. Carla Vitola Gonçalves - FURG
- Prof. Maria Lucia Rossetti – FEPPS e ULBRA – RS
- Dra. Elis Regina Dalla-Rosa - FEPPS – RS
- Prof. Lia Possuelo – UNISC
- Profa. Andreia Valin –UNISC
- Prof. Nilo Zanatta – UFSM
- Prof. Mariana Valença – UCPEL
- Prof. Lande Silva - UCPEL
- Prof. Miguel Viveiros - Universidade Nova de Lisboa
- Prof. Kyle Rhode - University Central of Florida
- Prof. Juan Palomino – University of Ghent
- Profa. Anandi Martin - University of Ghent
- Prof. Terry P. Lybrand – University of Vanderbilt

CAN BE WORSE

EFFLUX AND VIRULENCE!!!!!!

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

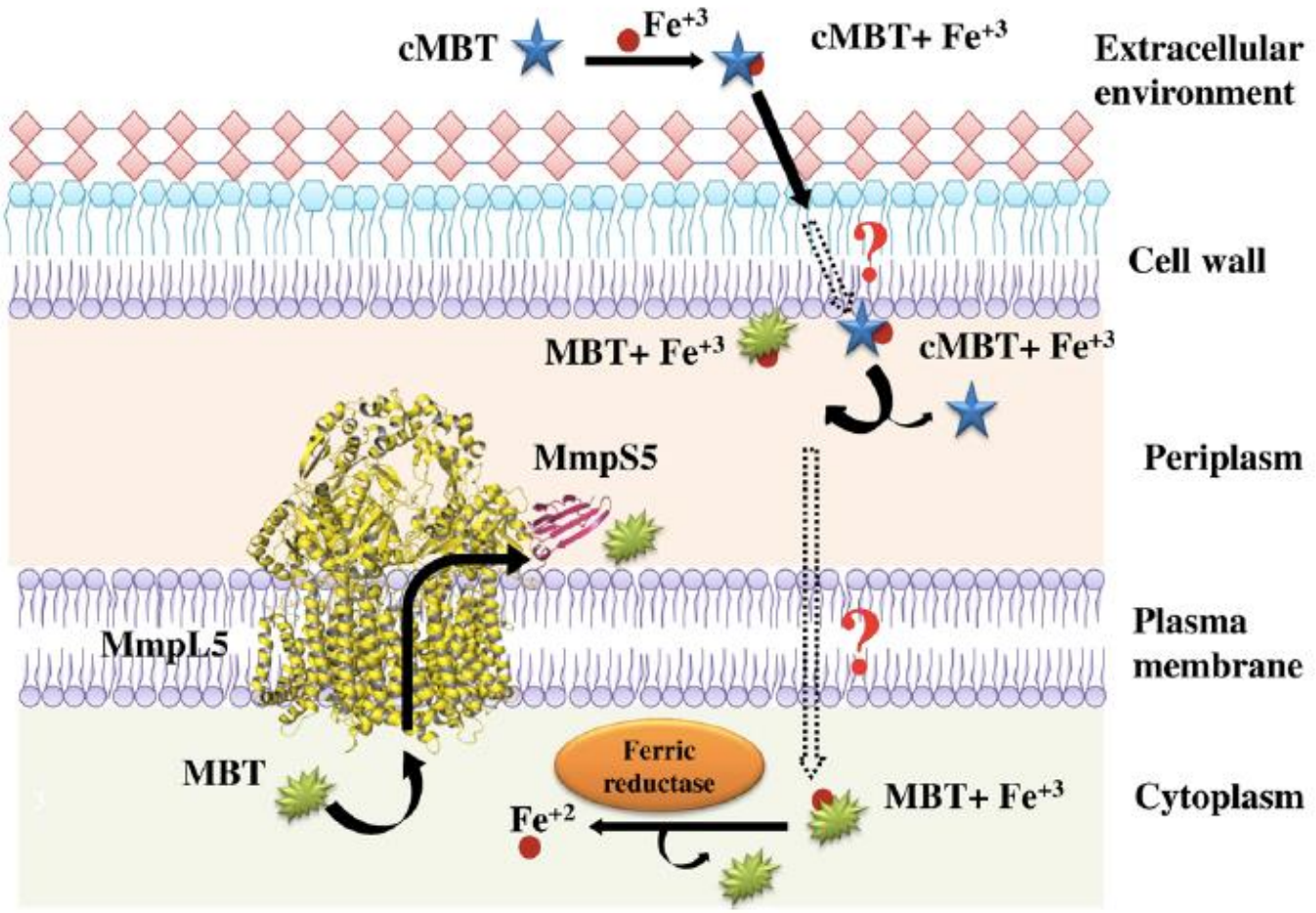
journal homepage: www.elsevier.com/locate/jinorgbio

Siderophore transport by MmpL5–MmpS5 protein complex in *Mycobacterium tuberculosis*

Padmani Sandhu, Yusuf Akhter *

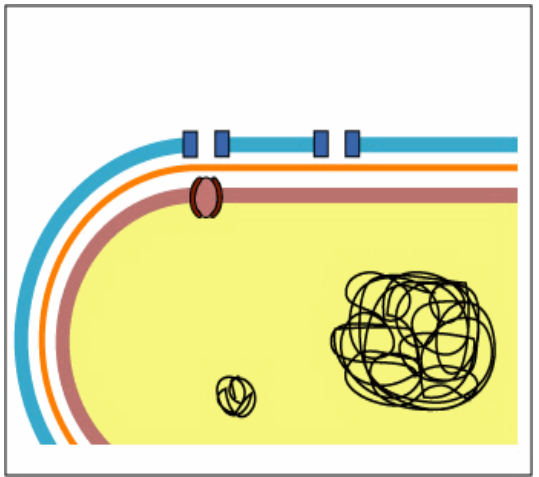
School of Life Sciences, Central University of Himachal Pradesh, District – Kangra, Himachal Pradesh 176206, India

P. Sandhu, Y. Akhter / Journal of Inorganic Biochemistry 170 (2017) 75–84



Roadmap

- Evidences of drug efflux in *Mtb*
- MmL5/MmlS5, the new pump star
- Inibition of the efflux mechanism
- Efflux in *M. abscessus*



Main mechanisms of action of EIP

- Competition Inhibitor x antibiotic
- Supress expression efflux pump
- Disrupt pmf
- Alter ATB structure (no regonised by pump)
- Disrupted pump assembly (RND -Mycobacteria and Gram neg)
- Block Porine (RND - Mycobacteria and Gram neg)

SOME STUDIES AND EXAMPLES

Rational design

Case of specific pumps: Tetracycline pumps

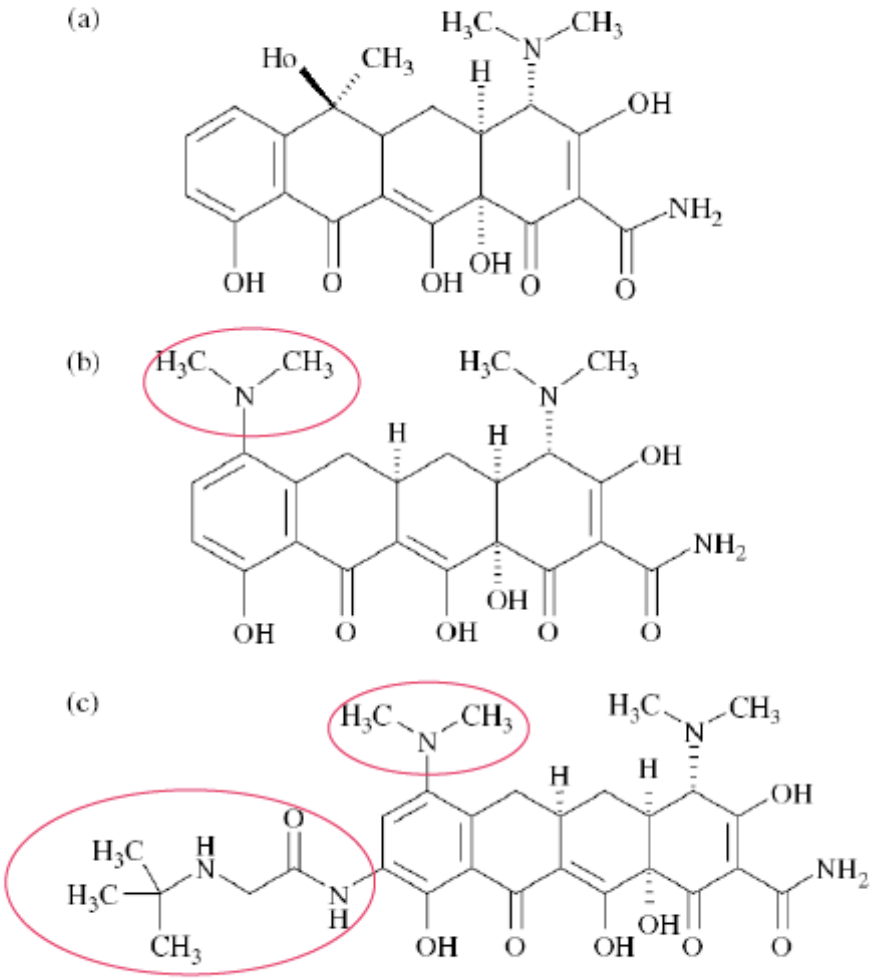
↳By-passing efflux pumps

Tetracycline

Minocycline

Substitution that impairs efflux

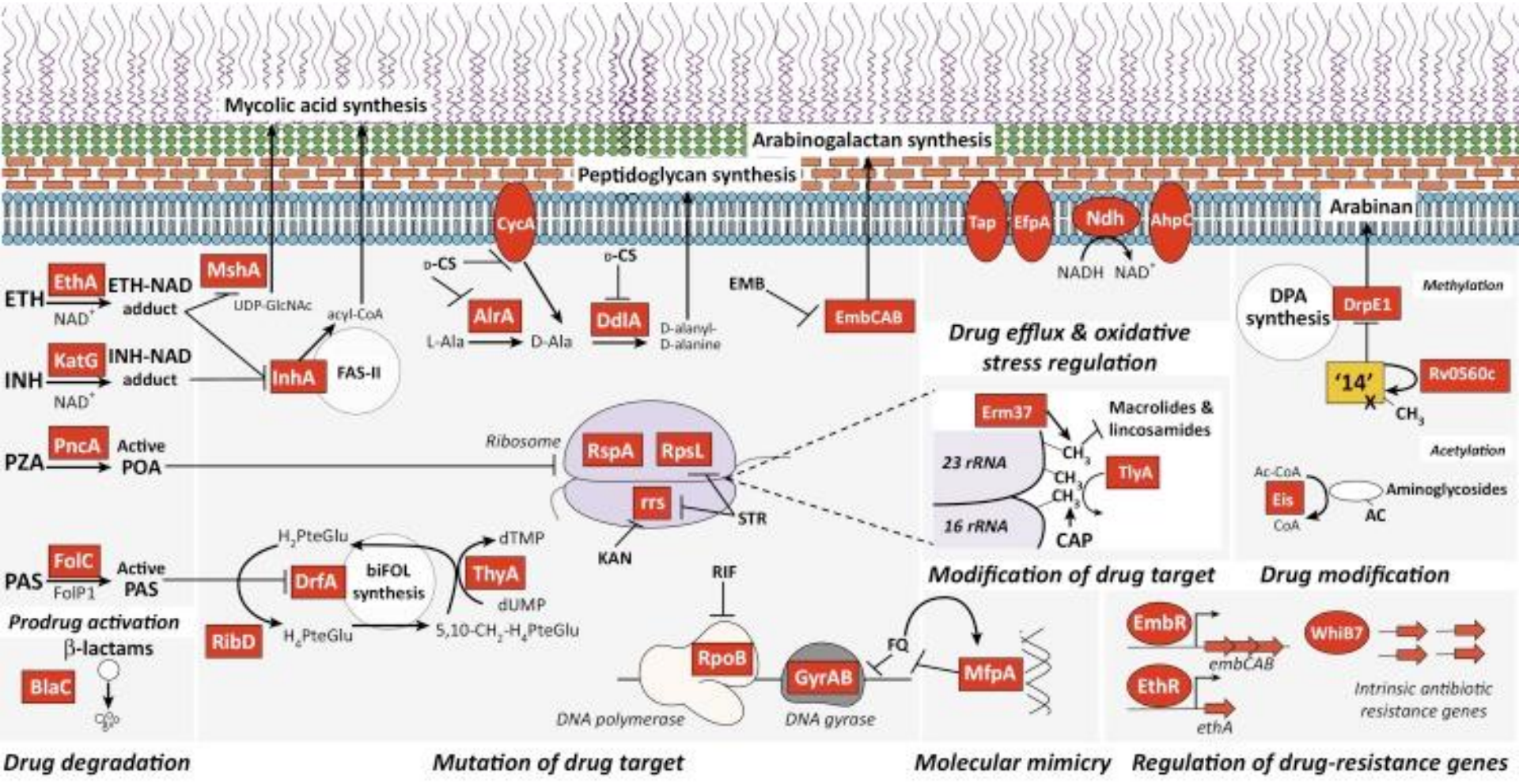
Tigecycline



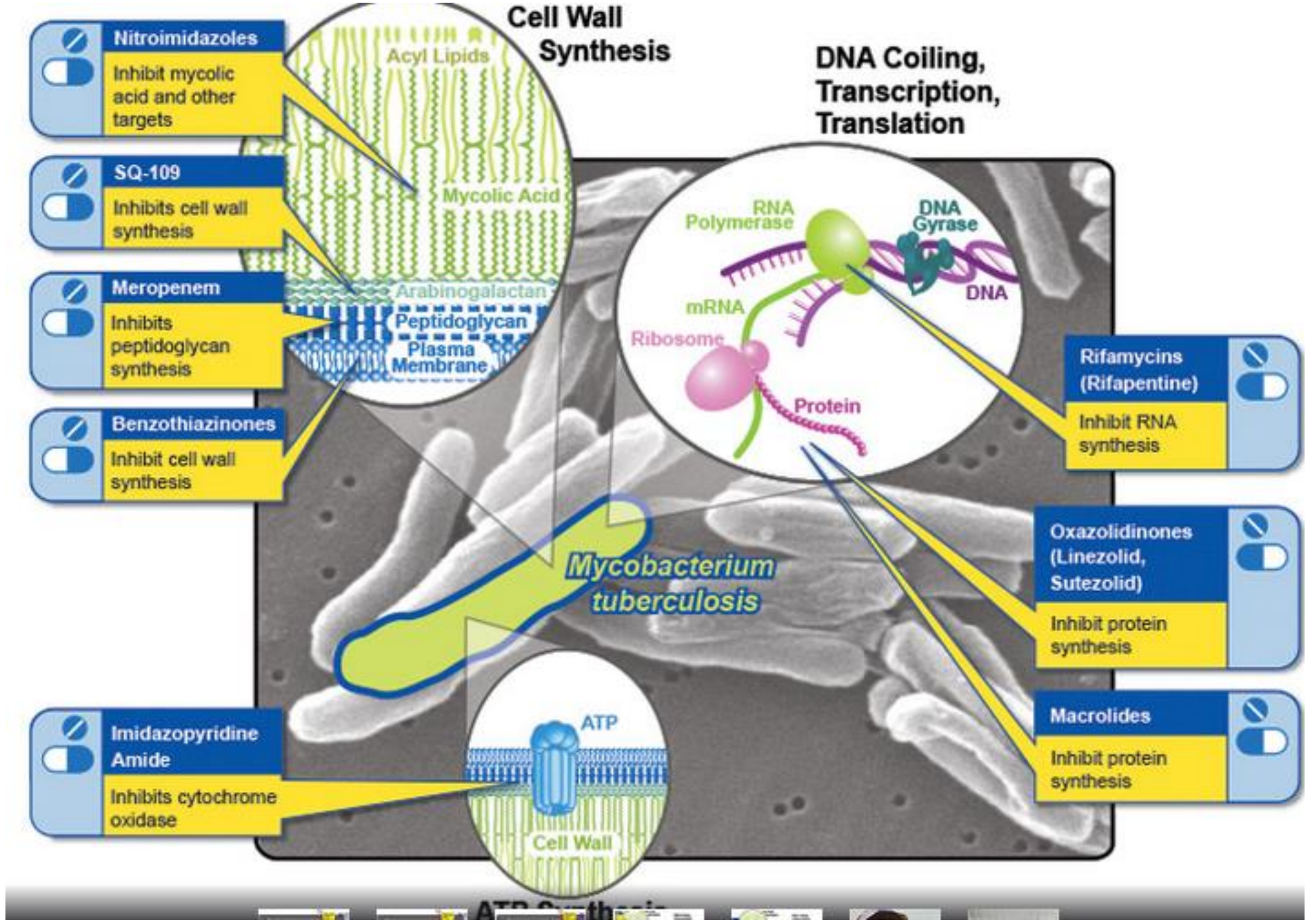
Nelson and Levy, *Antimicrob. Agents Chemother.* 1999, **43**: 1719

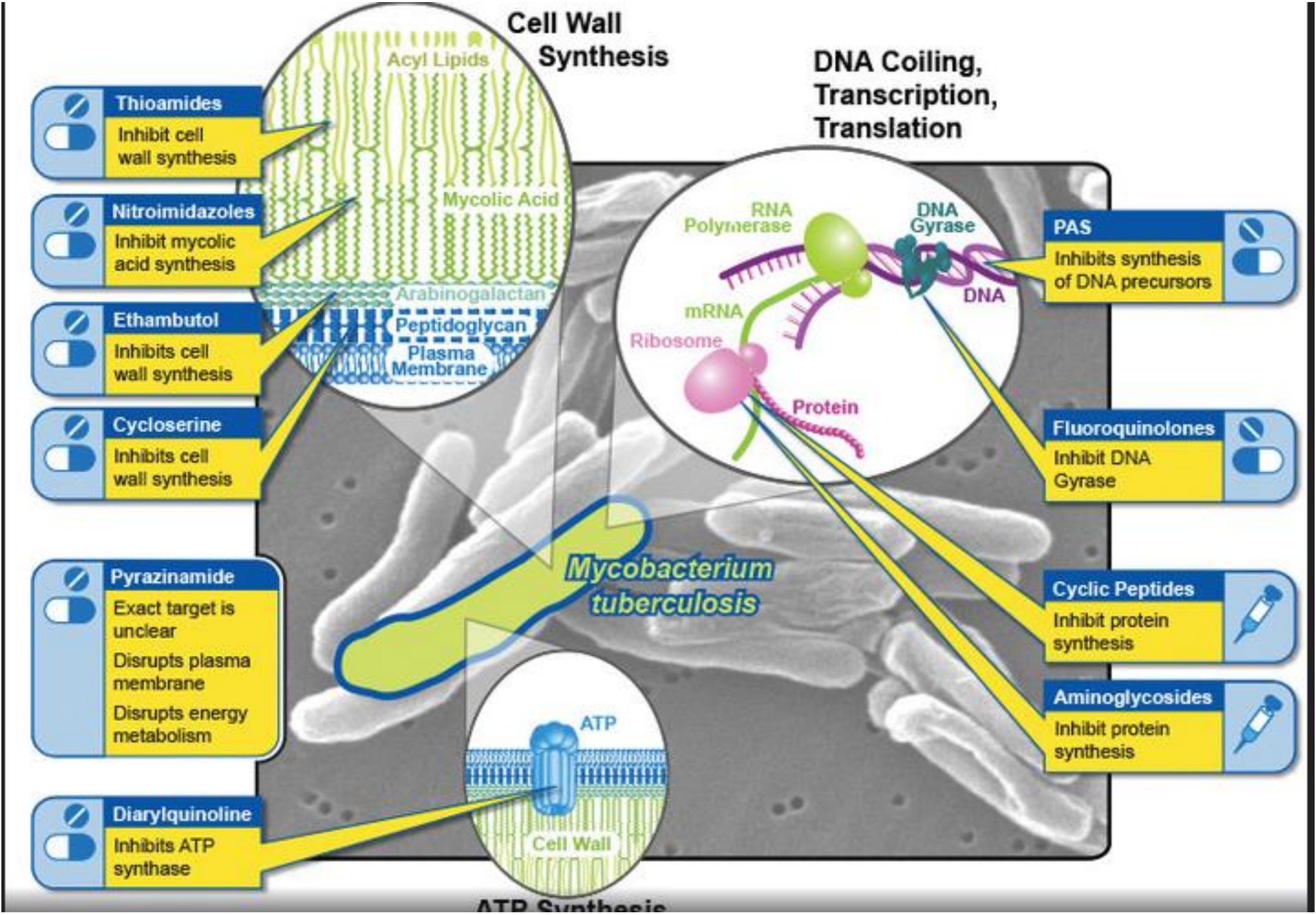
DOCKING (EIP AND ATB) IN TWO EFFLUX PUMP OF Mtb

mmpL5



Trends in Microbiology


Cell Wall Synthesis
DNA Coiling, Transcription, Translation
Nitroimidazoles
Inhibit mycolic acid and other targets
SQ-109
Inhibits cell wall synthesis
Meropenem
Inhibits peptidoglycan synthesis
Benzothiazinones
Inhibit cell wall synthesis
Acyl Lipids
Mycolic Acid
Arabinogalactan
Peptidoglycan
Plasma Membrane
RNA Polymerase
DNA Gyrase
DNA
mRNA
Ribosome
Protein
Rifamycins (Rifapentine)
Inhibit RNA synthesis
Oxazolidinones (Linezolid, Sutezolid)
Inhibit protein synthesis
Macrolides
Inhibit protein synthesis
Mycobacterium tuberculosis
ATP
Cell Wall
Imidazopyridine Amide
Inhibits cytochrome oxidase


Cell Wall Synthesis
Acyl Lipids
Mycolic Acid
Arabinogalactan
Peptidoglycan
Plasma Membrane
DNA Coiling, Transcription, Translation
RNA Polymerase
DNA Gyrase
DNA
mRNA
Ribosome
Protein
Mycobacterium tuberculosis
ATP
Cell Wall
ATP Synthesis
Thioamides
Inhibit cell wall synthesis
Nitroimidazoles
Inhibit mycolic acid synthesis
Ethambutol
Inhibits cell wall synthesis
Cycloserine
Inhibits cell wall synthesis
Pyrazinamide
Exact target is unclear
Disrupts plasma membrane
Disrupts energy metabolism
Diarylquinoline
Inhibits ATP synthase
PAS
Inhibits synthesis of DNA precursors
Fluoroquinolones
Inhibit DNA Gyrase
Cyclic Peptides
Inhibit protein synthesis
Aminoglycosides
Inhibit protein synthesis

Drug	MIC (mg/L)	Gene	Role of gene product
Isoniazid	0.02–0.2 (7H9/7H10)	*katG*	catalase/peroxidase
		inhA	enoyl reductase
		ahpC	alkyl hydroperoxide reductase
Rifampicin	0.05–0.1 (7H9/7H10)	*rpoB*	β-subunit of RNA polymerase
Pyrazinimide	16–50 (LJ)	*pncA*	PZase
Streptomycin	2–8 (7H9/7H10)	*rpsL*	S12 ribosomal protein
		rrs	16S rRNA
		gidB	7-methylguanosine methyltransferase
Ethambutol	1–5 (7H9/7H10)	*embB*	arabinosyl transferase
Fluoroquinolones	0.5–2.0 (7H9/7H10)	*gyrA/gyrB*	DNA gyrase
Kanamycin/amikacin	2–4 (7H9/7H10)	*rrs*	16S rRNA
Capreomycin/viomycin	2–4	*tlyA*	rRNA methyltransferase
Ethionamide	10 (7H11)	*inhA*	enoyl reductase
p-amino salicylic acid	0.5 (LJ)	*thyA*	thymidylate synthase A
PA-824 and OPC-67683	0.03 (7H9/7H10)	Rv3547	hypothetical 16.4 kDa
TMC207	0.03 (7H9/7H10)	*atpE*	ATP synthase

Drug	Class	Sponsor(s)	Phase
bedaquiline	diarylquinoline	Janssen, TB Alliance, NIAID, SAMRC, the Union, Unitaid, USAID	III
delamanid	nitroimidazole	Otsuka, NIAID, Unitaid	III
pretomanid	nitroimidazole	TB Alliance	III
sutezolid	oxazolidinone	Sequella, NIAID, TB Alliance	IIa (developers will have to repeat early stage studies, see text)
Q203	imidazopyridine	Qurient, Infectex, PanACEA	II
SQ109	1,2-ethylene diamine	Infectex, Sequella, PanACEA	II (phase III controversially claimed in Russia, see text)
PBTZ169	DprE1 inhibitor	Nearmedic, iM4TB, BMGF	II
OPC-167832	carbostyril	Otsuka, BMGF	I
LCB01-0371	oxazolidinone	LegoChem Biosciences	II

Respirology

INVITED REVIEW SERIES:
TUBERCULOSIS UPDATES 2018
SERIES EDITORS: CHI CHIU LEUNG, CYNTHIA CHEE AND YING ZHANG

Drug resistance mechanisms and drug susceptibility testing for tuberculosis

PAOLO MIOTTO,[1] YING ZHANG,[2] DANIELA MARIA CIRILLO[1] AND WING CHEONG YAM[3]

Table 2 Overview of fitness cost conferred by drug resistance-related mutations in RIF, INH and EMB

Drug	Mutation[†]	Experiment conditions[‡]	Relative fitness[§]	Reference
RIF	*rpoA* T187A	N/A	~1.00	36
	rpoA T187P	N/A	~1.20	36
	rpoB S531L (S450L)	Competition	>1.00	37
	rpoB S531W (S450W)	Competition	0.67–0.88	37,38
		Independent	0.71	38
		In macrophage	0.28	38
	rpoB H526Y (H445Y)	Competition	0.81–0.89	37,38
		Independent	0.86	38
		In macrophage	0.63	38
	rpoB S522L (S441L)	Competition	0.54–0.88	38
		Independent	0.95	38
		In macrophage	0.50	38
	rpoB S531L (S450L)	Competition	0.91, 0.96,	37,38
	rpoB H526D (H445D)	Competition	0.78–0.81	37,38
	rpoB H526R (H445R)	Competition	0.82	37,38
	rpoB Q513L (Q432L)	Competition	0.83	37,38
	rpoB H526P (H445P)	Competition	0.84	37,38
	rpoB R529Q (R448Q)	Competition	0.58	38
	rpoC D485N	N/A	~1.00	36
INH	*ahpC* downregulation	Animal model[¶]	Reduced[¶]	39,40
	inhA C-15T	Independent	0.82–1.01	41
	katG S315T	Independent	0.82–0.96	41
EMB	*embB* M306V	Competition	0.80–0.90	13
	ubiA A237V	Competition	1.00	13
	Rv3792 L198L	Competition	0.95–1.00	13
	embB M306V + *ubiA* A237V	Competition	0.80–0.90	13
	embB M306 V + *Rv3792* L198L	Competition	0.95–1.00	13

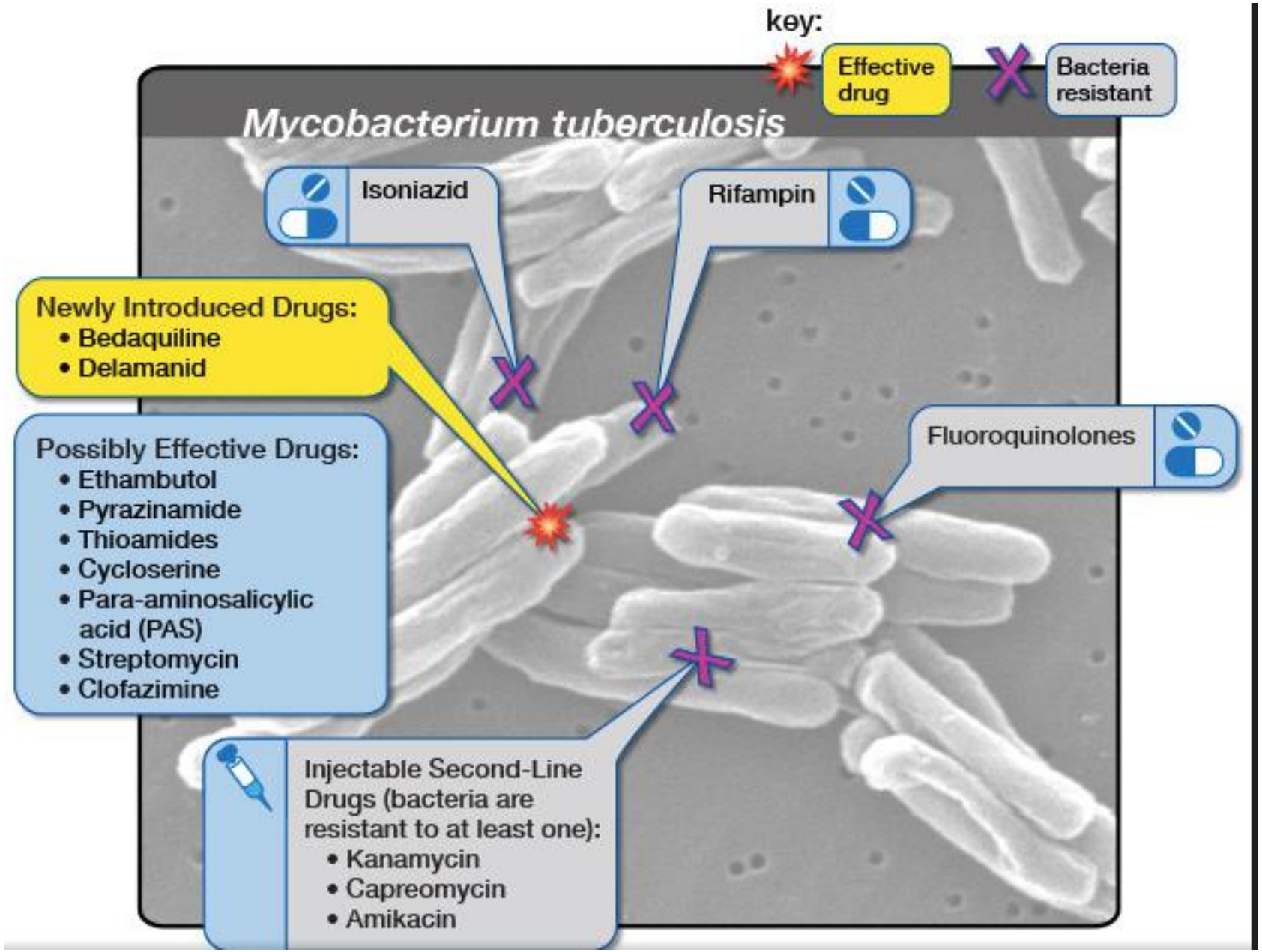
[†]Refer Andre *et al.*[42] for *rpoB* MTB numbering system reported in parenthesis.

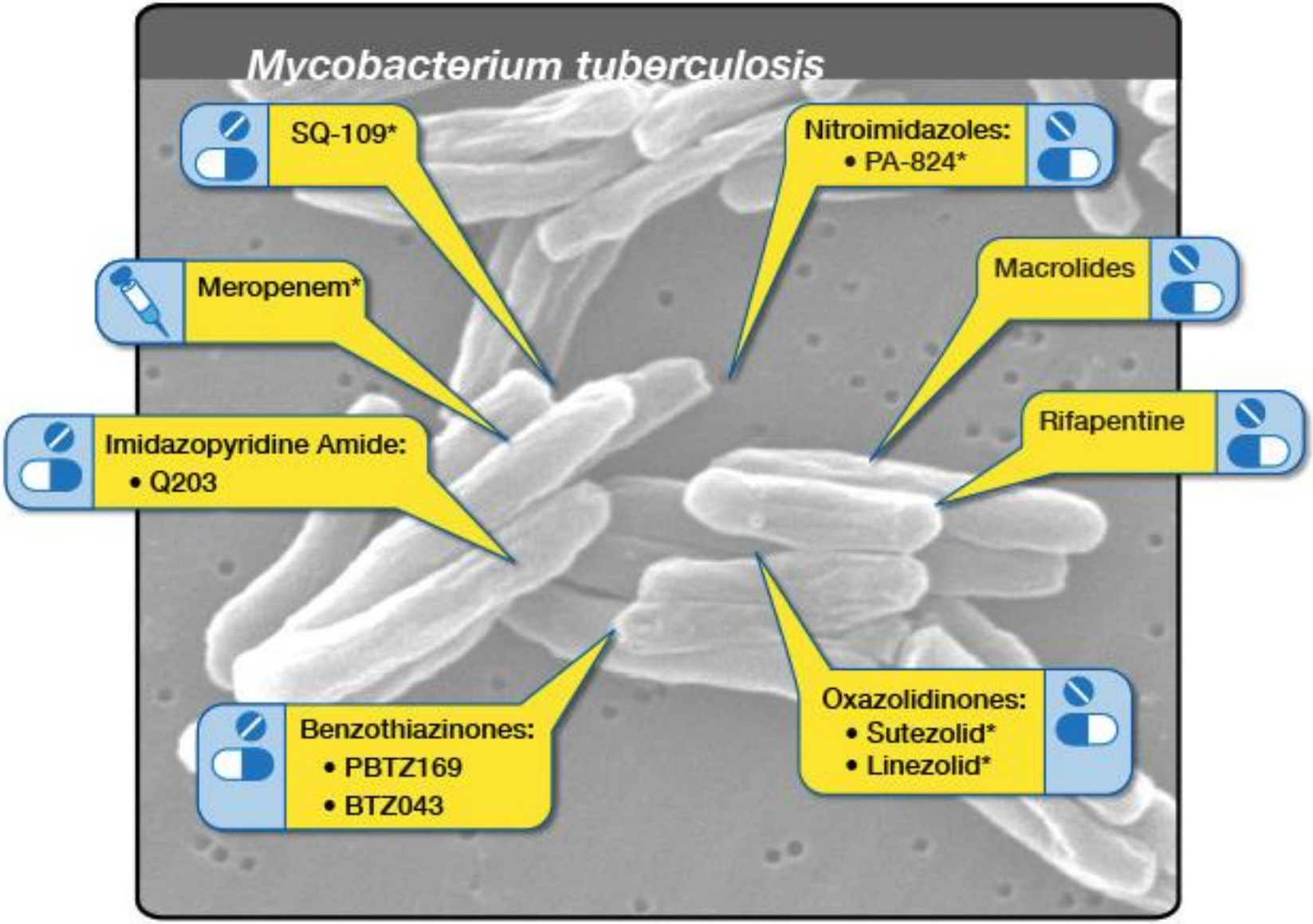
[‡]Experimental conditions were referred as competition, pairwise competition assay; independent, independent mtb growth assay; in macrophage, macrophage challenge experiment and N/A, not available.

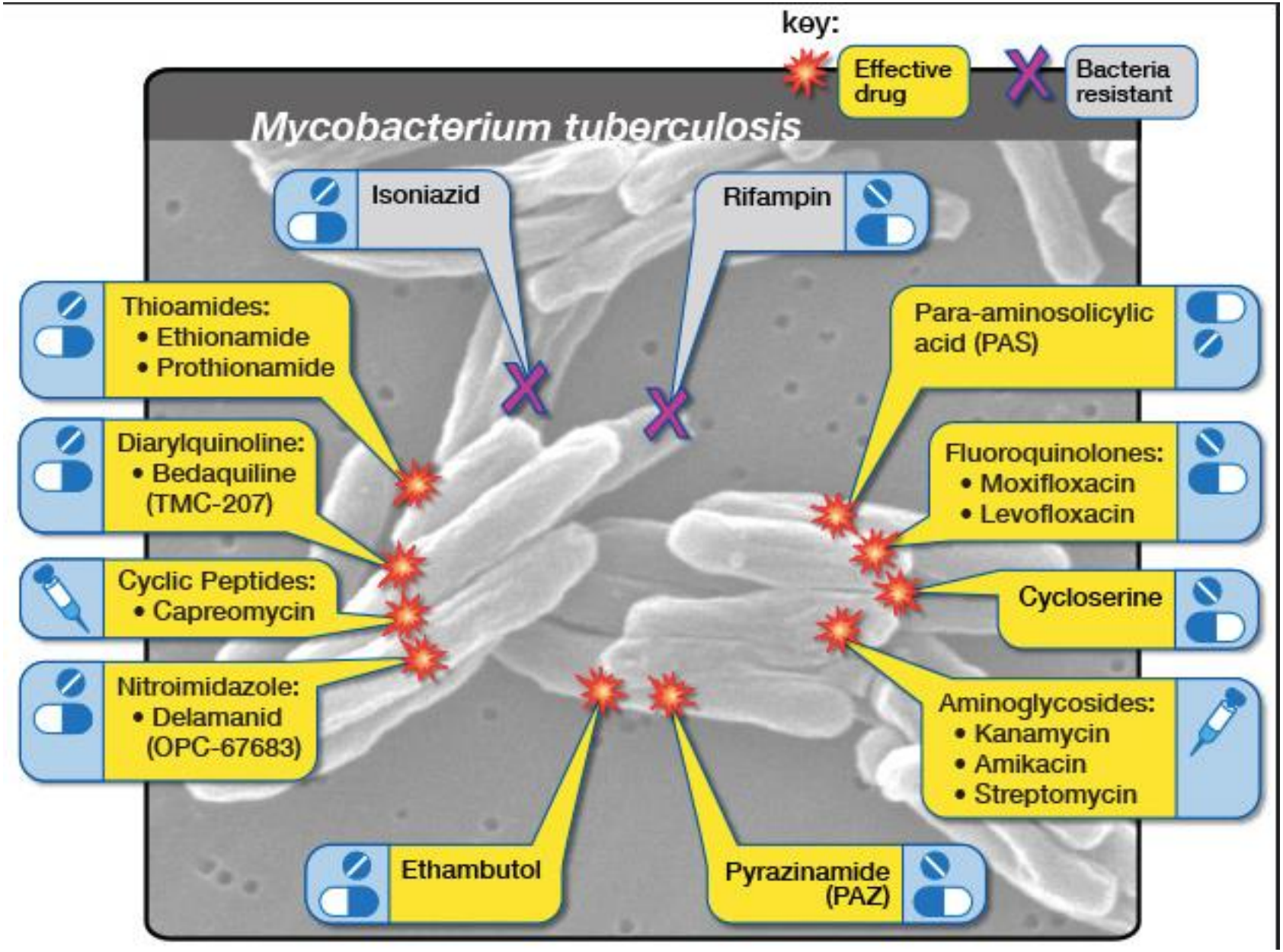
[§]Relative fitness was calculated by (growth rate of mutated strain)/(growth rate of reference strains).

[¶]For study conducted to understand the effect of *ahpC* downregulations, the study was conducted to evaluate the virulence of *ahpC* knockdown MTB in immunocompromised mice.

EMB, ethambutol; INH, isoniazid; MTB, *Mycobacterium tuberculosis*; RIF, rifampicin.


key:
Effective drug
Bacteria resistant
Mycobacterium tuberculosis
Isoniazid
Rifampin
Newly Introduced Drugs:
• Bedaquiline
• Delamanid
Fluoroquinolones
Possibly Effective Drugs:
• Ethambutol
• Pyrazinamide
• Thioamides
• Cycloserine
• Para-aminosalicylic acid (PAS)
• Streptomycin
• Clofazimine
Injectable Second-Line Drugs (bacteria are resistant to at least one):
• Kanamycin
• Capreomycin
• Amikacin


Mycobacterium tuberculosis
SQ-109*
Nitroimidazoles:
• PA-824*
Meropenem*
Macrolides
Imidazopyridine Amide:
• Q203
Rifapentine
Benzothiazinones:
• PBTZ169
• BTZ043
Oxazolidinones:
• Sutezolid*
• Linezolid*


key:
Effective drug
Bacteria resistant
Mycobacterium tuberculosis
Isoniazid
Rifampin
Thioamides:
• Ethionamide
• Prothionamide
Para-aminosolicylic acid (PAS)
Diarylquinoline:
• Bedaquiline (TMC-207)
Fluoroquinolones:
• Moxifloxacin
• Levofloxacin
Cyclic Peptides:
• Capreomycin
Cycloserine
Nitroimidazole:
• Delamanid (OPC-67683)
Aminoglycosides:
• Kanamycin
• Amikacin
• Streptomycin
Ethambutol
Pyrazinamide (PAZ)

2018 Global New TB Drug Pipeline [1]

Discovery | Preclinical Development | Clinical Development

Lead Optimization → Early Stage Development → GMP/ GLP Tox. → Phase 1 → Phase 2 → Phase 3

Diarylthiazoles

DprE1 Inhibitors

InhA Inhibitor

Mtb energy metabolism

Macrolides

Mycobacterial Gyrase Inhibitors

Arylsulfonamides

Inhibitors of MmpL3, Translocase-1, Clp, PKS13

Oxazolidinones

Squaramides

CPZEN-45*

Spectinamide - 1810*

SPR720*

TB-47*

TBAJ-587

TBAJ-876

GSK-286*

TBI-223

Sanfetrinem

S-004992*

BTZ-043*

TBI-166

Macozinone* (PBTZ-169)

OPC-167832*

GSK-656* (070)

TBA-7371*

Contezolid (MRX-4/MRX-1)

Telacebec (Q203*)

Delpazolid (LCB01-0371)

Sutezolid (PNU100480)

SQ-109*

Macozinone* (PBTZ-169)

Bedaquiline* (TMC-207)

Delamanid* (OPC-67683)

Pretomanid* (PA-824)

*New chemical class. Known chemical classes for any indication are color coded: fluoroquinolone, rifamycin, oxazolidinone, nitroimidazole, diarylquinoline, benzothiazinone, imidazopyridine amide, beta-lactam.

[1] New Molecular Entities not yet approved, being developed for TB or only conditionally approved for TB. Showing most advanced stage reported for each. Details for projects listed can be found at http://www.newtbdrugs.org/pipeline/clinical

Ongoing projects without a lead compound series identified: http://www.newtbdrugs.org/pipeline/discovery

Underline = new to Phase since March 2018

WORKING GROUP ON NEW TB DRUGS

www.newtbdrugs.org

Updated: October 2018

2018 Global New TB Drug Pipeline [1]

Small Pharma | Non Profits

Preclinical Development | Clinical Development

Early Stage

- Caprazene nucleoside CPZEN-45*
- Spectinamide 1810*
- Gyrase inhibitor SPR720*
- Pyrazolopyridine carboxamide TB-47*

GMP/ GLP Tox.

- TBAJ-587
- TBAJ-876
- GSK-286*
- TBI-223
- Sanfetrinem
- S-004992*

Phase 1

- BTZ-043*
- TBI-166
- Macozinone* (PBTZ-169)
- OPC-167832*
- GSK-656* (070)
- TBA-7371*
- Contezolid (MRX-4/MRX-1)

Phase 2

- Telacebec (Q203*)
- Delpazolid (LCB01-0371)
- Sutezolid (PNU100480)
- SQ-109*
- Macozinone* (PBTZ-169)

Phase 3

- Bedaquiline*
- Delamanid*
- Pretomanid*

Global Market

Underline = new to Phase since March 2018

New chemical class* Known chemical classes for any indication are color coded: fluoroquinolone, rifamycin, oxazolidinone, nitroimidazole, diarylquinoline, benzothiazinone, imidazopyridine amide, beta-lactam.

[1] New Molecular Entities not yet approved, being developed for TB or only conditionally approved for TB. Showing most advanced stage reported for each. Details for projects listed can be found at http://www.newtbdrugs.org/pipeline/clinical

Ongoing projects without a lead compound series identified: http://www.newtbdrugs.org/pipeline/discovery

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2018 Global TB Drug and Regimen Clinical Research [1]

Ongoing Clinical Development Research: Strategy / Optimization / Regimen Development

Phase 2	Phase 3 Regimens	Optimization/Post Market
Bedaquiline-Delamanid (ACTG 5343)	Bedaquiline-STREAM MDR-TB Trial Stage 2 with oral OBR (9 mo) or OBR with injectables (6 mo)	Bedaquiline-Linezolid with OBR for MDR-TB (NExT Trial)
Bedaquiline-Pretomanid-Moxifloxacin-PZA (SimpliciTB Trial, NC-008)	Bedaquiline-Pretomanid-Linezolid (Nix-TB)	endTB 5-Regimen Trial for MDR TB
Bedaquiline - Pretomanid – Moxifloxacin - Pyrazinamide (BPaMZ) (NC-005)	Delamanid with OBR for MDR-TB	PredictTB – PET/CT, biomarkers DS-TB, 4 mo
Levofloxacin with OBR for MDR-TB (OPTI-Q)	High Dose Rifampicin for DS-TB (RIFASHORT)	TRUNCATE-TB Trial, 2 mo
Linezolid Dose-Ranging	Rifapentine - Moxifloxacin for DS-TB (CDC TBTC 31)	
Nitazoxanide	Pretomanid-Moxifloxacin-Pyrazinamide (STAND)	
Beta-Lactams		
High Dose Rifampicin (PANACEA)		
TB PRACTECAL - regimens with Bedaquiline-Pretomanid-Linezolid		

Known chemical classes are color coded: fluoroquinolone, rifamycin, oxazolidinone, nitroimidazole, diarylquinoline, benzothiazinone , imidazopyridine amide, beta-lactam.

[1] Strategy trials, regimen development, open label, repurposed drug studies. Details for projects listed can be found at http://www.newtbdrugs.org/pipeline/clinical

[2] OBR = Optimized Background Regimen

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2018 Global TB Drug Discovery Pipeline [1]

Hit-to-Lead	Lead Optimization
Actinomycete Metabolites (U ILL Chicago, Myongii U)	**Diarylthiazoles** (TBDA)
Novel Hit-to-Lead Programs (Lilly DDI) GATB	**InhA Inhibitors (**GATB/GHDDI)
Adamantanids (U ILL Chicago)	**Spectinamides** (St. Jude, U Tenn, CSU, UZ, Microbiotix)
Whole-Cell Hit-to-Lead (GSK, GATB)	**Macrolides** (GATB, Evotec)
Menaquinone Synthase Inhibitors (CSU)	**Clp** (SPRINT TB / A* Star)
M. tb Energy Metabolism Inhibitors (GATB, TBDA, J&J/CSIR-Imtech, Univ. of Notre Dame	**Indolcarboxamides / MmpL3 inhibitors** (GATB, TBDA)
Isoprenoid Biosynthesis Inhibitors (Lilly DDI)	**Oxazolidinones** (IMM)
Whole-Cell Hit-to-Lead (GATB, Evotec)	**Aryl Sulfonamides** (GATB, GSK, TBDA)
RNA Polymerase Inhibitors (GATB)	**PKS13 inhibitors** (GATB, DDU, TAMU, GSK, TBDA)
ClpC/P1P2 (GATB)	**Squaramides** (GATB, TBDA, Evotec)

[1] Details for projects listed can be found at http://www.newtbdrugs.org/pipeline-discovery.php and clinical development projects can be viewed at http://www.newtbdrugs.org/pipeline.php.

Abbreviations of Developers: **A*Star**- Agency for Science Technology and Research **CSU**-Colorado State University; **FAPESP**-São Paulo Research Foundation; **GATB**-Global Alliance for TB Drug Development (TB Alliance); **GSK**-GlaxoSmithKline; **Lilly DDI**-Lilly TB Drug Discovery Initiative; **RI**-Research Institute; **SPRINT TB**-Singapore Programme of Research Investigating New Approaches to Treatment of TB; **St. Jude**-St. Jude Children's Research Hospital; **TAMU**-Texas A&M University; **TBDA**-TB Drug Accelerator; **U**-University; **U ILL**-University of Illinois; **UPenn**-University of Pennsylvania; **U Tenn**-University of Tennessee; **UZ**-University of Zurich

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Updated: October 2018

Clinical Pipeline

Trials for Market Approval

Download PPT

Advancing Yes | Marketed | NCE | Repurposed | Trials for Market Approval | Show Biologics

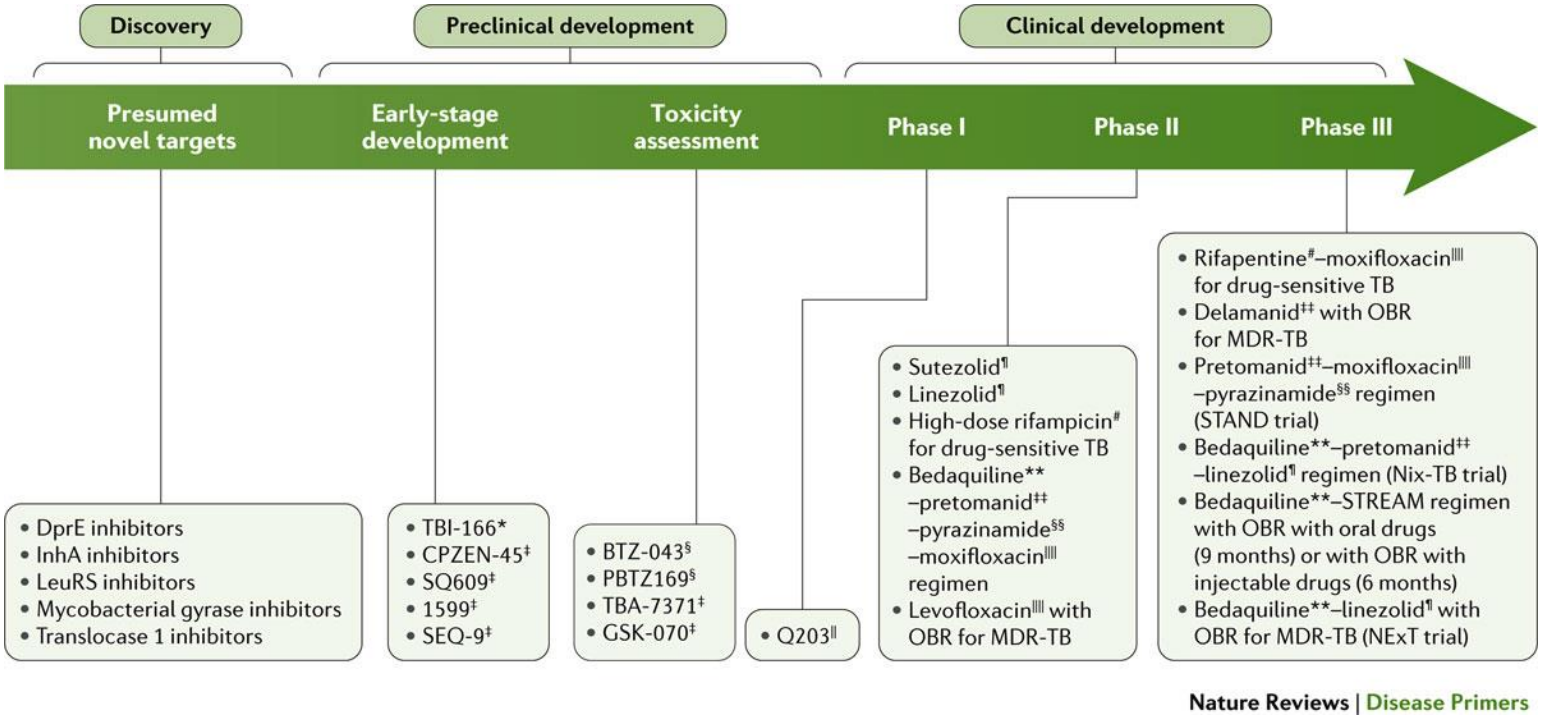
Pre-Clinical (GLP)	Phase 1	Phase 2	Phase 3
		Auranofin The Aurum Institute NPC, Calibr, The Scripps Research Institute	**Bedaquiline - Pretomanid - Linezolid** TB Alliance
		TB Host Directed Therapy (TBHDT)	Nix-TB (B-Pa-L)
			ZeNix (B-Pa-L) NC-007
		Nitazoxanide Weill Medical College of Cornell University	**Clofazimine** Novartis
		Nitzoxanide NZT001 14-day EBA	**Pretomanid, Moxifloxacin, Pyrazidamide (PaMZ)** TB Alliance
			STAND

UPDATES ON NEW COMPOUNDS IN DEVELOPMENT

Table 1. Drugs in development for tuberculosis

Drug	Class	Sponsor(s)	Phase
bedaquiline	diarylquinoline	Janssen, TB Alliance, NIAID, SAMRC, the Union, Unitaid, USAID	III
delamanid	nitroimidazole	Otsuka, NIAID, Unitaid	III
pretomanid	nitroimidazole	TB Alliance	III
sutezolid	oxazolidinone	Sequella, NIAID, TB Alliance	IIa (developers will have to repeat early stage studies, see text)
Q203	imidazopyridine	Qurient, Infectex, PanACEA	II
SQ109	1,2-ethylene diamine	Infectex, Sequella, PanACEA	II (phase III controversially claimed in Russia, see text)
PBTZ169	DprE1 inhibitor	Nearmedic, iM4TB, BMGF	II
OPC-167832	carbostyril	Otsuka, BMGF	I
LCB01-0371	oxazolidinone	LegoChem Biosciences	II

Figure 5 The global TB drug pipeline



Pai, M. *et al.* (2016) Tuberculosis
Nat. Rev. Dis. Primers doi:10.1038/nrdp.2016.76

Review

Drug Resistance Mechanisms in *Mycobacterium tuberculosis*

Juan Carlos Palomino * and Anandi Martin

'able 1

irst- and second-line TB drugs, genes involved in their activation and mechanisms involved.

Drug	Gene	Mechanism Involved
Isoniazid	*katG*, *inhA*	Catalase/peroxidase; enoyl reductase
Rifampicin	*rpoB*	RNA polymerase
Pyrazinamide	*pncA*, *rpsA*	Pyrazinamidase; ribosomal protein 1
Ethambutol	*embB*	Arabinosyl transferase
Streptomycin	*rpsL*, *rrs*, *gidB*	S12 ribosomal protein, 16A rRNA, 7-methylguanosine methyltransferase
Quinolones	*gyrA*, *gyrB*	DNA gyrase
Capreomycin	*rrs*, *tlyA*	16S rRNA, rRNA methyltransferase
Kanamycin/Amikacin	*rrs*	16S rRNA
Ethionamide	*ethA*	Enoyl-ACP reductase
'ara-aminosalicylic acid	*thyA*, *folC*	Thymidylate synthase A

The recognized mechanism of resistance to ethambutol has been linked to mutations in the gene *embB* with mutations at position *embB*306 as the most prevalent in most of the studies performed [47,48]. Some studies, however, have also found mutations in *embB*306 in ethambutol susceptible isolates [49]. Moreover, a study with a large number of *M. tuberculosis* isolates found that mutations in *embB*306 were not necessarily associated with resistance to ethambutol but with a predisposition to develop resistance to increasing number of drugs and to be transmitted [50]. In fact, allelic exchange studies have shown that individual mutations causing certain amino acid substitutions produced ethambutol resistance, while other amino acid substitutions had little or no effect on ethambutol resistance [51]. The same authors have more recently reported that mutations in the decaprenylphosphoryl-B-D-arabinose (DPA) biosynthetic and utilization pathway genes, Rv3806c and Rv3792, together with mutations in *embB* and *embC* accumulate, giving rise to a range of MICs of ethambutol depending on mutation type and number [52]. These findings could have influence on the correct detection of ethambutol resistance by current molecular methods. Mutations in *embB*306 then, cause variable degrees of ethambutol resistance and are required but are not enough to cause high-level resistance to ethambutol. There remain about 30% ethambutol resistant strains that do not present any mutation in embB stressing the need to identify other possible mechanisms of drug resistance to this drug.

Consequently, mutations in *rpsL* and rrs are the major mechanisms of resistance to streptomycin but account for 60%–70% of the resistance found [69]. Among the mutations reported in *rpsL*, a substitution in codon 43 from lysine to arginine has been the most commonly reported. This mutation produces high-level resistance to streptomycin. In *rrs* the most common mutations occur around nucleotides 530 and 915. There remain an important percentage of strains resistant to streptomycin that lack mutations in either of these two genes, suggesting additional mechanisms of resistance.

In the last years, it has also been reported that mutations in *gidB*, a gene encoding a conserved 7-methylguanosine methyltransferase specific for the 16S rRNA, confers low-level resistance to streptomycin [70,71].

is a tetramer formed by two α and β subunits, coded by *gyrA* and *gyrB*, respectively, which catalyzes the supercoiling of DNA [77]. The main mechanism of development of fluoroquinolone resistance in *M. tuberculosis* is by chromosomal mutations in the quinolone resistance-determining region of *gyrA* or *gyrB*. The most frequent mutations found are at position 90 and 94 of *gyrA* but mutations at position 74, 88 and 91 have also been reported [78,79]. A recent systematic review of fluoroquinolone-resistance-associated gyrase mutations in *M. tuberculosis* has been published [80].

One interesting finding in *M. tuberculosis* is the presence of a natural polymorphism at position 95 in *gyrA* that is not related to fluoroquinolone resistance since it is also found in fluoroquinolone-susceptible strains [81]. Another interesting finding has been the report that the simultaneous occurrence of mutations T80A and A90G in *gyrA* led to hypersusceptibility to several quinolones [82]. This finding could point out that the problem of fluoroquinolone resistance in *M. tuberculosis* might be more complex than was thought initially.

Cross-resistance is assumed to occur between fluoroquinolones although isolated reports have acknowledged the presence of strains resistant to gatifloxacin and moxifloxacin that were still susceptible to ofloxacin [83]. Also, the involvement of efflux mechanisms has been suggested as a possible cause for fluoroquinolone resistance in *M. tuberculosis* [84].

These four antibiotics have the same mechanism of action by inhibiting the protein synthesis but, while kanamycin and amikacin are aminoglycosides, capreomycin and viomycin are cyclic peptide antibiotics. All four are second-line drugs used in the management of MDR-TB.

Kanamycin and amikacin inhibit protein synthesis by alteration at the level of 16S rRNA. The most common mutations found in kanamycin-resistant strains are at position 1400 and 1401 of the *rrs* gene, conferring high-level resistance to kanamycin and amikacin. However, mutations at position 1483 have also been reported [85,86]. Full cross-resistance between kanamycin and amikacin is not complete, as previously thought. Some studies have shown variable levels and patterns of resistance suggesting that other mechanisms of resistance might be possible [87]. In concordance with this, a low-level resistance to kanamycin has been associated with mutations in the promoter region of the *eis* gene, encoding an aminoglycoside acetyltransferase [88]. Mutations at position −10 and −35 of the *eis* promoter led to an overexpression of the protein and low-level resistance to kanamycin but not to amikacin. These mutations were found in up to 80% of clinical isolates showing low-level resistance to kanamycin [88,89].

Capreomycin and viomycin, on the other hand, have a similar structure and bind at the same site in the ribosome, at the interface of the small and large subunits [90]. They show full cross-resistance as reported in previous studies [91]. Mutations in the *tlyA* gene have also been associated with resistance to capreomycin and viomycin. TlyA is an rRNA methyltransferase specific for 2'-O-methylation of ribose in rRNA. Mutations in t*lyA* determine the absence of methylation activity [92]. Although some studies did not find this association, a recent meta-analysis, evaluating the association of genetic mutations and resistance to second-line drugs, has confirmed the presence of *tlyA* mutations in addition to mutations in *rrs* and *eis* [93].

3.8. Clofazimine

Clofazimine is a riminophenazine compound reported long ago as having anti-TB activity [107]. Due to the availability of other effective anti-TB drugs at the time and some undesirable side-effects, such as pigmentation of the skin, its use was more limited to the treatment of leprosy [108]. It is now considered in the group 5 drugs of the WHO for the management of MDR-TB. Until recently, the exact mode of action of this antibiotic was not completely understood. Recent studies, however, have signalled the outer membrane as the possible target of clofazimine [109]. Another study found that in *M. tuberculosis* clofazimine is reduced by NADH dehydrogenase and subsequently after spontaneous reoxidation liberates bactericidal levels of reactive oxygen species (ROS) [110].

Resistance to clofazimine has not yet been fully characterized; however, a recent study has found that in spontaneous mutants of the reference strain H37Rv, mutations in the transcriptional regulator Rv0678 caused an upregulation of MmpL5, a multisubstrate efflux pump, which not only caused resistance to clofazimine but also to bedaquiline [111].

3.9. Linezolid

Also part of the category 5 drugs of second-line anti-TB drugs, linezolid is an oxazolidinone originally approved for clinical use in the treatment of skin infections and nosocomial pneumonia caused by Gram-positive bacteria [112]. The mode of action of linezolid is by inhibition of an early step in the synthesis of proteins, binding to the 50S ribosomal subunit [101]. Resistance to linezolid in *M. tuberculosis* is still unusual, but a study analyzing 210 MDR strains found 1.9% of strains being resistant to linezolid [113]. Further analysis of *in vitro* selected linezolid-resistant mutants found that strains with mutations in the 23S rRNA had MICs of 16–32 µg/mL, while strains with MICs of 4–8 µg/mL or susceptible strains showed no mutations [114]. A more recent study using next-generation sequencing has also found the mutation T460C in *rplC*, encoding the 50S ribosomal L3 protein, in *in vitro*-selected mutants and clinical isolates of *M. tuberculosis* resistant to linezolid [115]. Previous studies have also found evidence of the possible involvement of efflux pumps in the resistance of *M. tuberculosis* to linezolid [84].

Table 1 gives an overview of the first- and second-line anti-tuberculosis drugs curre
target of action.

Table 1. First- and second-line TB drugs, genes involved in their activation and mechan

Drug	Gene	Mechanism Involved	F
Isoniazid	*katG*, *inhA*	Catalase/peroxidase; enoyl reductase	
Rifampicin	*rpoB*	RNA polymerase	
Pyrazinamide	*pncA*, *rpsA*	Pyrazinamidase; ribosomal protein 1	
Ethambutol	*embB*	Arabinosyl transferase	
Streptomycin	*rpsL*, *rrs*, *gidB*	S12 ribosomal protein, 16A rRNA, 7-methylguanosine methyltransferase	
Quinolones	*gyrA*, *gyrB*	DNA gyrase	
Capreomycin	*rrs*, *tlyA*	16S rRNA, rRNA methyltransferase	
Kanamycin/Amikacin	*rrs*	16S rRNA	
Ethionamide	*ethA*	Enoyl-ACP reductase	
Para-aminosalicylic acid	*thyA*, *folC*	Thymidylate synthase A	

4.2. Delamanid

Delamanid, previously known as OPC-67683, is a derivative of nitro-dihydro-imidazooxazole with activity against *M. tuberculosis* that acts by inhibiting the synthesis of mycolic acid and is undergoing clinical evaluation in a phase III trial [74]. The structure of delamanid is shown in Figure 2. Delamanid was previously shown to have a very good *in vitro* and *in vivo* activity against drug-susceptible and drug-resistant *M. tuberculosis* [128], as well as good early bactericidal activity comparable to that of rifampicin [129]. Delamanid has more recently shown its safety and efficacy in a clinical evaluation for MDR-TB [130].

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Evolution of drug resistance in *Mycobacterium tuberculosis:* a review on the molecular determinants of resistance and implications for personalized care

Navisha Dookie[1]*, Santhuri Rambaran[1], Nesri Padayatchi[1,2], Sharana Mahomed[1] and Kogieleum Naidoo[1,2]

Approximately 30% of ethambutol-resistant isolates lack alteration in *embB*, suggesting a different mechanism of resistance.[23,24] Additive mutations occurring in *ubiA* have been reported to cause high-level ethambutol resistance when they occur with *embB* mutations. The *ubiA* gene encodes decaprenyl-phosphate 5-phosphoribosyltransferase synthase, which is involved in cell wall synthesis. Alteration in *ubiA* is reported to be lineage specific, and is predominant in the African isolates.[87,88]

Fluoroquinolones

Fluoroquinolones are potent bactericidal antibiotics currently used as second-line treatment for DR-TB. Ciprofloxacin and ofloxacin represent an older generation of antibiotics that are derivatives of nalidixic acid.[77] New generation fluoroquinolones, such as moxifloxacin and gatifloxacin, are currently being considered for use in regimens for DR-TB.[3] This class of antibiotics targets the DNA gyrase enzyme, thereby preventing transcription during cell replication. DNA gyrases are encoded by the *gyrA* and *gyrB* genes. Resistance to the fluoroquinolones has been linked to mutations occurring in a conserved region known as the quinolone resistance-determining region in the *gyrA* and *gyrB* genes.[24,104–106] Fluoroquinolone-resistant strains of MTB most frequently display mutations in codons 90, 91 and 94 of the *gyrA* gene. Mutations in codons 74, 88 and 91 have also been associated with fluoroquinolone resistance.[107–109] It has been reported that clinically significant resistance to ciprofloxacin and ofloxacin (MIC of 2 mg/L) is conferred by a single gyrase mutation, whereas double mutations in the *gyrA* or concomitant *gyrA* and *gyrB* mutations result in high MICs.[109] A mutation detected in codon 95 of *gyrA* is a natural polymorphism that has no role in mediating fluoroquinolone resistance.[110] The complexity of fluoroquinolone

Bedaquiline

Bedaquiline is the first drug in a new class of agents, the diarylquinolines, to be used for TB treatment. Bedaquiline acts by targeting mycobacterial ATP synthase, inhibiting bacterial respiration. The drug is therefore active against dormant bacilli, an invaluable characteristic for MTB infection. *In vitro* studies show MIC values of 0.03 mg/L, approximately equivalent to those of rifampicin and isoniazid in DS-MTB.[123,124] Bedaquiline in combination with pyrazinamide has demonstrated remarkable sterilizing activity in a mouse model.[123] Target-based mutations in the *atpE* gene described in strains selected *in vitro* have been associated with high-level resistance to bedaquiline, with up to 4-fold increase in MIC.[125,126] The gene encodes the mycobacterial F_1F_0 proton ATP synthase, a key enzyme in ATP synthesis and membrane potential generation.[123,124] Zimenkov *et al.*[127] recently described the first

occurrence of *atpE* D28N and A63V mutations in two clinical isolates of MTB associated with an MIC of 0.12 and 1.00 mg/L, respectively. Prior to this report, the mutations observed in the *atpE* gene were described for lab-generated strains. Non-target-based mutations, such as the presence of mutations in *rv0678*, result in the upregulation of the MmpL5 efflux pump, resulting in low-level bedaquiline resistance and cross-resistance to clofazimine.[126,128] These mutations have been associated with at least a 4-fold increase in MIC.[126] Veziris *et al.*[129] reported the M139T *rv0678* mutation that resulted in a 16-fold increase in MIC after treatment including bedaquiline. The same study reported a double nucleotide deletion at positions 18–19 and an insertion at position 140 of *rv0678*, corresponding to MICs of 0.5 and 0.25 mg/L, respectively. Zimenkov *et al.* reported the most common mechanism associated with increased bedaquiline MICs was the presence of mutations in *rv0678*. Paired isolates representing bedaquiline pre- and post-treatment for 17 patients, revealed elevated bedaquiline MICs prior to treatment. Four of these patients had mutations in the *rv0678* gene associated with an MIC range of 0.06–0.25 mg/L.[127] This is in keeping with a report of the high frequency of *rv0678* mutations detected in MDR-TB and DS patients with no prior exposure to bedaquiline or clofazimine.[130] Mutations in the second non-target mechanism, *pepQ*, were reported with the association of low-level bedaquiline resistance and cross-resistance to clofazimine. Similar to *rv0678*, mutations in *pepQ* result in modest increases of bedaquiline and clofazimine MICs.[131] However, none of the studies reported above documented *pepQ* mutations in clinical isolates with confirmed resistance to bedaquiline or clofazamine.[127,129,132]

Delamanid and pretomanid

Delamanid and pretomanid belong to the nitroimidazole class of antibiotics. Pretomanid, formerly PA-824 is a prodrug that requires activation by deazaflavin-dependent nitro-reductase, which is encoded by *ddn*. *ddn* converts the prodrug into three metabolites, which include des-nitro-imidazole and two unstable by-products. Des-nitro-imidazole compounds promote the anaerobic activity of these compounds by generating reactive nitrogen species, including nitric oxide, which may then boost the host-macrophage killing of MTB.[133,134] Pretomanid has been reported to be highly active against MTB with an MIC range of 0.015–0.25 mg/L.[135] Resistance to pretomanid has been linked to mutations occurring in the genes associated with prodrug activation (*ddn* and *fgd1*), or in genes associated with the F420 biosynthetic pathway (*fbiA*, *fbiB* and *fbiC*).[133] However, analysis of 65 strains of the MTB complex, representing the various lineages, revealed no significant impact on pretonamid MICs.[136] Delamanid, formerly OPC-67683, inhibits the synthesis of methoxy-mycolic and keto-mycolic acid, components of the mycobacterial cell wall.[137] Delamanid displayed potent *in vitro* activity against lab strains and clinical isolates of MTB, with a reported MIC range of 0.006–0.24 mg/L.[138] Bloemberg *et al.*[132] recently reported D49Y in the *fbiA* gene and a frameshift mutation in codon 49 of the *fdg1* gene that corresponded with increasing phenotypic delamanid resistance. Similar to pretomanid, it is a prodrug that requires activation via the same pathway, and thus, resistance to delamanid is associated with mutations in one of the five genes described above.[133] The exclusive role of the drug in TB treatment regimens makes it a desirable agent for treatment

Compensatory evolution

It has been postulated that resistance mutations bear a fitness cost to the bacterium. This concept emanates from the observation that isoniazid-resistant isolates displayed decreased virulence in the guinea pig model.[144] However, studies have since demonstrated the presence of co-occurrence of secondary mutations that act as compensatory mechanisms for the impaired fitness of the pathogen. These compensatory mutations are believed to occur in genes encoding the same protein or genes involved in similar metabolic pathways.[64] Sherman and Mdluli demonstrated this phenomenon in isoniazid-resistant isolates of MTB with an inactivated *katG* gene.[145] The absence of *katG* catalase–peroxidase activity resulted in mutations in the regulatory region of the *ahpC* (alkyl hydroperoxidase reductase) gene, leading to overexpression of this gene. Mutations of the *ahpC* gene are believed to be compensatory for the loss of *katG* activity.[145] More recently, whole-genome analysis demonstrated that mutations occurring in RNA polymerases *rpoA* and *rpoC* were compensatory for the loss of fitness mediated by mutations in the *rpoB* gene in rifampicin-resistant isolates.[146–148] Reports on the varying levels of capreomycin resistance amongst A1401G laboratory mutants and clinical isolates bearing the same mutation, imply a possible interplay of a compensatory mechanism.[99,149] Similarly, mutations in *gyrB* may account for resistance-conferring mutations found in the *gyrA* gene.[132,150]

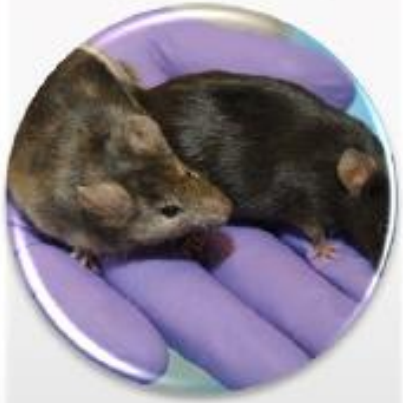
Efflux-mediated resistance

Efflux pump systems are involved in expelling drugs from the bacterial cell, enabling acquisition of resistance mutations in the bacterial genome. MTB presents with one of the largest number of putative efflux pumps with 148 genes coding for membrane transport proteins within its 4.4 Mb genome. The contribution of these efflux systems in acquiring multidrug resistance in MTB has been demonstrated by a number of studies.[151,152] The overexpression of efflux pumps is believed to mediate the build-up of resistance mutations, which confers high-level drug resistance allowing MTB to survive and persist at clinically relevant drug concentrations. The ability of the efflux pumps to extrude a diversity of compounds allows them to expel multiple drugs leading to the MDR phenotype.[151,152] Efflux pump inhibitors are compounds capable of restoring the activity of antibiotics independent of the level of resistance. The inhibitor–antibiotic combination decreases the concentration of antibiotics expelled by efflux pumps, thus decreasing the MIC of the antibiotic. The use of efflux pump inhibitors has been considered as an adjuvant in TB treatment and has the potential to reduce the duration of TB treatment.[64,126,152–154]

FASES DE DESENVOLVIMENTO DE UM NOVO FÁRMACO

Types of tuberculosis clinical trials

Type	Endpoint	Size	Duration of study	What is being studied?
Phase I	Safety/tolerability	small	days-weeks	drug
PK/PD	PK/PD data; drug interactions	small	days-weeks	drug(s)
Phase IIa	EBA	small	days-weeks	drug
Phase IIb	2-month culture conversion; time to conversion	Medium (100-150 patients/arm)	months	regimen
Phase III	Failure/relapse	large	years	regimen
Phase IV	Detection of uncommon side effects	large	years	regimen



Pre-Clinical

- CPZEN-45
  - Caprazene nucleoside
- SQ641
  - Capuramycin
- Lee1599
  - Spectinamide
- SEQ-9
  - Macrolide
- PBTZ 169
  - Benzothiazinone
- BTZ 043
  - Benzothiazinone

Phase I

- TBA-354
  - Nitroimidazole
- Q203
  - Imidazopyridine

Phase II

- Sutezolid
- AZD5847
- SQ109
- Rifapentine
- Bedaquiline*-Pretomanid-Pyrazinamide
- Levofloxacin with OBR(MDR-TB)

Phase III

- Bedaquiline*withOBR (MDR-TB)
- Delamanid*withOBR (MDR-TB)
- Pretomanid-Moxifloxacin-Pyrazinamide

Fig. 4. Drugs that hold clinical promise that are in development and testing. Pipeline info from www.newtbdrugs.org *conditionally approved agents.

The effect of drug resistance on the fitness of *Mycobacterium tuberculosis*

THE LANCET Infectious Diseases Vol 3 January 2003

Resistência a SMR

Mutação *rps*L *e* *16S* RNA → Altera eficiência de tradução

↓

Mutação *rps*D e *rps*E

↓

Compensação da eficiência??

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The potential use of rifabutin for treatment of patients diagnosed with rifampicin-resistant tuberculosis

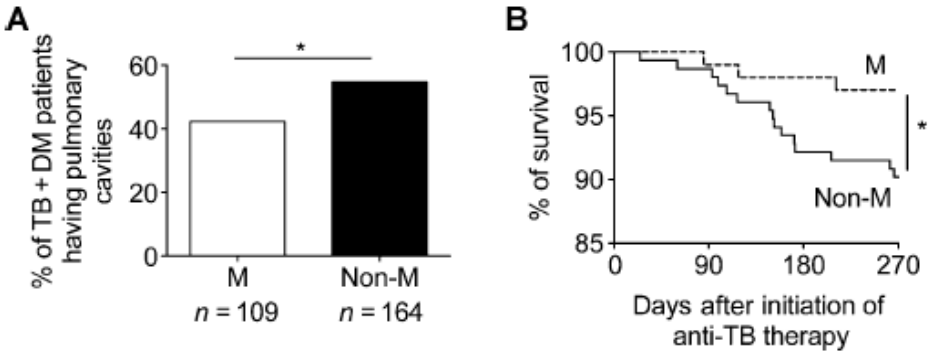
Michael G. Whitfield[1], Robin M. Warren[1]*, Vanessa Mathys[2], Lesley Scott[3,4], Elise De Vos[5], Wendy Stevens[3,4], Elizabeth M. Streicher[1], Guido Groenen[6], Frederick A. Sirgel[1] and Annelies Van Rie[5]

Table 1. *rpoB* polymorphisms and range of rifampicin and rifabutin MICs in 72 isolates selected from clinical isolates from the EXIT-RIF cohort of 349 patients to represent the unique combinations of *rpoB* polymorphisms and rifampicin MICs observed in this cohort

Codon	Nucleotide change	Amino acid change	MIC range (mg/L)		No. of isolates
			rifampicin	rifabutin	
509	deletion 6 bp	frameshift	>1 to ≤5	<0.125	1
511	CTG→CCG	Leu→Pro	<1	<0.125	2
513	CAA→AAA	Gln→Lys	>100	>2	2
513	CAA→CCA	Gln→Pro	>100	0.5–1.0	2
513	CAA→CTA	Gln→Leu	>100	>2.0	2
515	deletion 6 bp	frameshift	>100	>2.0	2
516	GAC→GCC	Asp→Ala	<1.0	<0.125	2
516	GAC→GTC	Asp→Val	>1 to <50	<0.125–0.5	12
516	GAC→TAC	Asp→Tyr	<1 to >10	<0.125–0.25	3
516	GAC→TTC	Asp→Phe	>1 to <5	<0.125	1
516	GAC→TGC	Asp→Cys	<1.0	<0.125	1
516	deletion 3 bp	frameshift	>1 to <20	0.25–0.5	3
517	deletion 3 bp	frameshift	>1 to <10	<0.125	2
518	deletion 3 bp	frameshift	<1.0	<0.125	1
522	TCG→TTG	Ser→Leu	>1 to <5	<0.125	1
526	CAC→AAC	His→Asn	<1.0	<0.125	2
526	CAC→CGC	His→Arg	>100	>2.0	2
526	CAC→CTC	His→Leu	>1 to >100	<0.125 to >2.0	2
526	CAC→GAC	His→Asp	>100	>2.0	4
526	CAC→TAC	His→Tyr	>100	>2.0	2
526	CAC→TGC	His→Cys	>1 to <5	<0.125	1
531	TCG→TTG	Ser→Leu	>5 to >100	>0.025 to >2.0	10
531	TCG→TTT	Ser→Phe	>100	>2.0	2
533	CTG→CCG	Leu→Pro	<1 to <10	<0.125–1.0	4
511 + 516	CTG→CCG + GAC→TAC	Leu→Pro + Asp→Tyr	>5 to 100	0.5 to >2.0	3
511 + 526	CTG→CCG + CAC→TAC	Leu→Pro + His→Tyr	>1 to <5	<0.125	1
512 + 516	AGC→AGG + GAC→GGC	Ser→Arg + Asp→Gly	>1 to <5	<0.125	1
513 + 516	CAA→GAA + GAC→GTC	Gln→Glu + Asp→Val	>100	>2.0	1

Singhal et al, *Sci Trans Med* 2014

Metformin:
Enhances killing of *M. tuberculosis* in the laboratory



HgbA1c to rule-in or rule-out diabetes and refer to care: *don't rely on self-report

***Early therapeutic drug monitoring for diabetics**

***Educational flip-chart**

EVOLUTION, MEDICINE, & PUBLIC HEALTH

Epigenetics, epistasis and epidemics

Fernando Baquero*

Department of Microbiology, Ramón y Cajal University Hospital, Ramón y Cajal Institute for Health Research (IRYCIS), Madrid, Spain

*Correspondence address. Department of Microbiology, Ramón y Cajal University Hospital, Carretera de Colmenar km 9,100. 28034 Madrid, Spain. Tel: +34-913368832; Fax: +34-913368809; E-mail: baquero@bitmailer.net

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KEYWORDS: epigenetics; epistasis; epidemiology; antibiotic resistance

We are already installed in the post-genetic or meta-genetic search for biological causalities. Until recently causation in biology was almost universally attributed to the main genetic factor, the gene, with a close correspondence with the encoded character, the phenotype. The term 'epigenetics' refers to studies 'above the gene' and refers to heritable (reproducible) changes in gene function that cannot be explained by mutations in DNA sequence. The term 'epistasis' etymologically means the 'act of stopping' (any 'on-off' action) and refers to the phenomenon in which one or more genes influences the function of others. The term 'epidemics' (in our case, bacterial epidemics) means 'what is upon the single cell, not a single gene, not a single individual creates the public health problem. As these multiple interactions are to a certain extent of stochastic nature, the complexity of the causal analysis increases significantly, leading to what might be qualified as 'causal relativity' or, in general 'biological relativity' [2].

Epigenetics might influence the evolution of antibiotic resistance. Stochastic variation in the expression of sets of genes is expected to occur even in isogenic populations, due to factors that include DNA methylation, covalent modification of DNA-binding proteins, non-coding DNA or RNA splicing factors. The hypothesis is that these factors, by