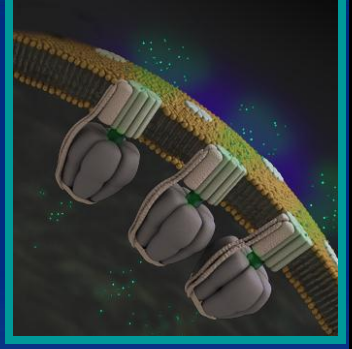
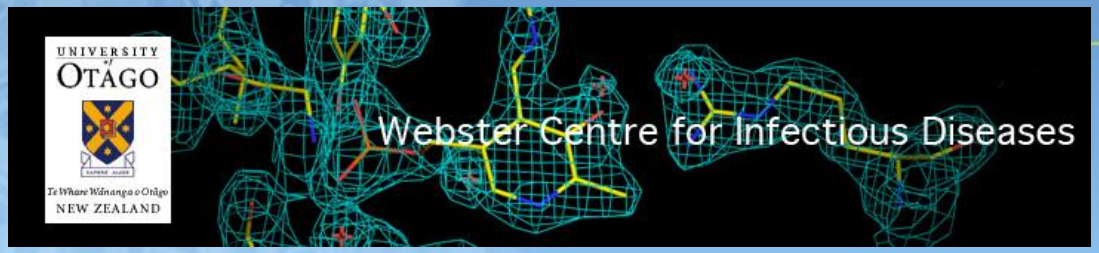
Discovery of inhibitors of energy generation in *Mycobacterium tuberculosis*



A game changer in the fight against MDR and XDR tuberculosis disease

Greg Cook
Department of Microbiology and Immunology

The Maurice Wilkins Centre for Molecular Biodiscovery and the Webster Centre for Infectious Diseases



MAURICE WILKINS CENTRE
FOR MOLECULAR BIODISCOVERY

UNIVERSITY of OTAGO
Te Whare Wānanga o Otāgo
NEW ZEALAND

- Extensive use of antibiotics in hospitals and agriculture to combat bacterial pathogens (36% increase globally, 62% India – 13 Bn units/year)
- Underestimated the ability of bacteria to mutate or transfer resistance genes - MDR, XDR and TDR
- Outbreaks expensive to control ($30 billion/yr – USA, 23,000 deaths, > 2M illnesses)
- Lack of new drugs developed to cope with resistance - “Zyvox” totally synthetic drug released 2000 - April 2001

Tuberculosis disease and drug resistance

- 9.4 million new TB cases per year
- 5.3% TB cases are MDR-TB; XDR-TB and TDR-TB emerging (10 frontline drugs)
- MDR-, XDR-TB difficult to diagnose and expensive to treat (24 months)
- TB is the leading killer of people with HIV: XDR-TB/HIV – 4 week mortality rate
- **No new drugs in 40 years!**

Should NZ be complacent?

NOTABLE CASES

Extensively drug-resistant tuberculosis: New Zealand's first case and the challenges of management in a low-prevalence country

Tze Liang Goh, Cindy R Towns, Katharine L Jones, Joshua T Freeman and Colin S Wong

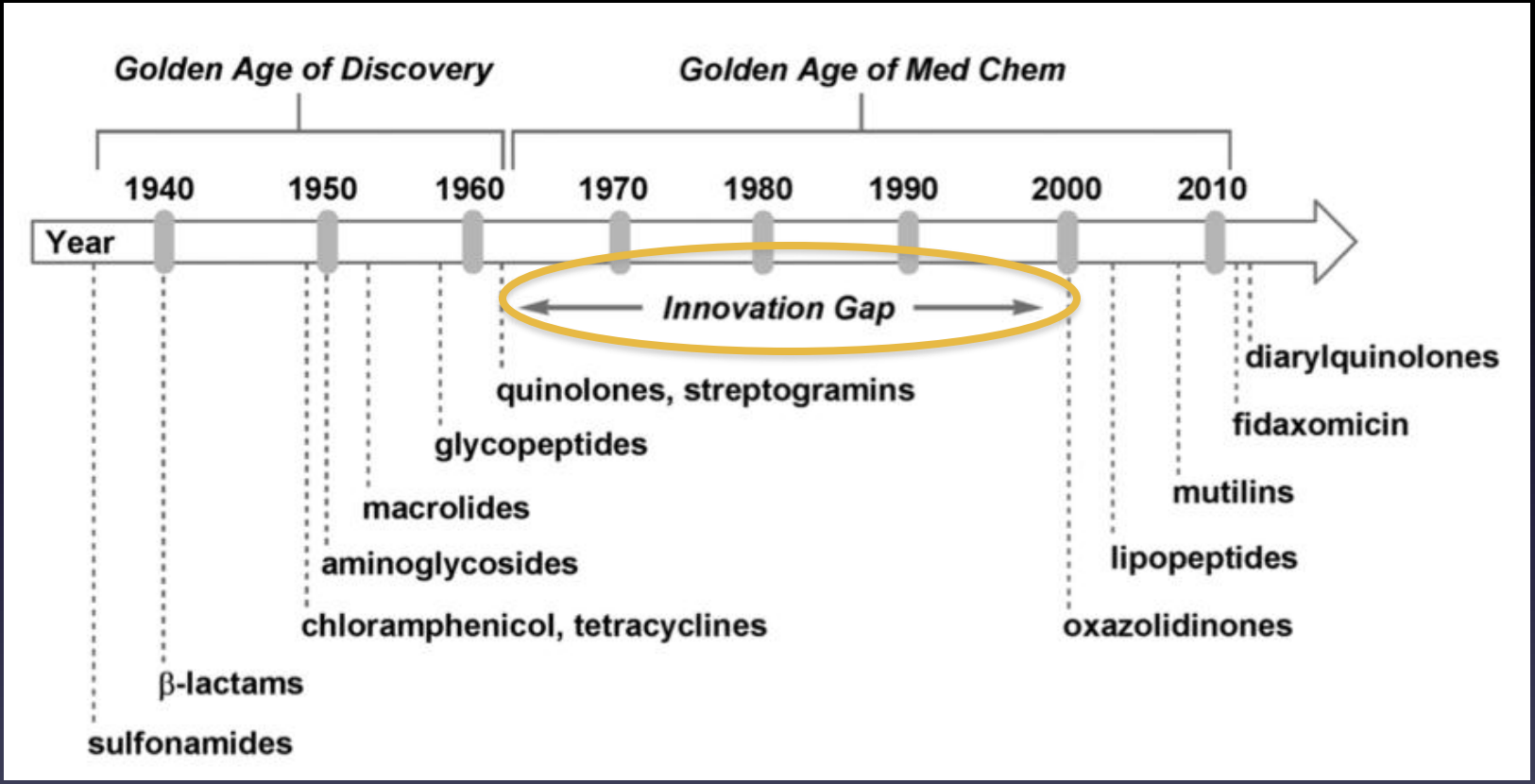
In 2010, an immigrant from Burma was the first person to be diagnosed in New Zealand with extensively drug-resistant tuberculosis (XDR-TB). The strain of Mycobacterium tuberculosis *is the most resistant reported to date in Australasia. Key difficulties of managing this disease in a low-prevalence country were delays from drug-susceptibility testing and in acquiring appropriate medicines, and a lack of evidence-based guidelines. Solutions are needed for New Zealand and the wider region as more cases of XDR-TB are likely to be encountered in the future. (MJA 2011; 194: 602-604)*

M. tuberculosis strain: INH/RIF/PZA/ETH – first-line, Second-line agents: capreomycin, ethionamide, ofloxacin, amikacin 9 different drugs for treatment ($10,000/month, 18 months) – CURED

32 cases of MDR-TB (last 10 years) – an average annual rate of 1.2% among culture-positive TB cases (acquired overseas)

Antimicrobial Drug Discovery and Development

No new structural classes of antibiotics were introduced between 1962 and 2000



Long time to market and expensive $800M – 1.3 Bn
Dalbavacin (1996)……..18 years!

Drug discovery 1917-2005
(too few targets/too much resistance)

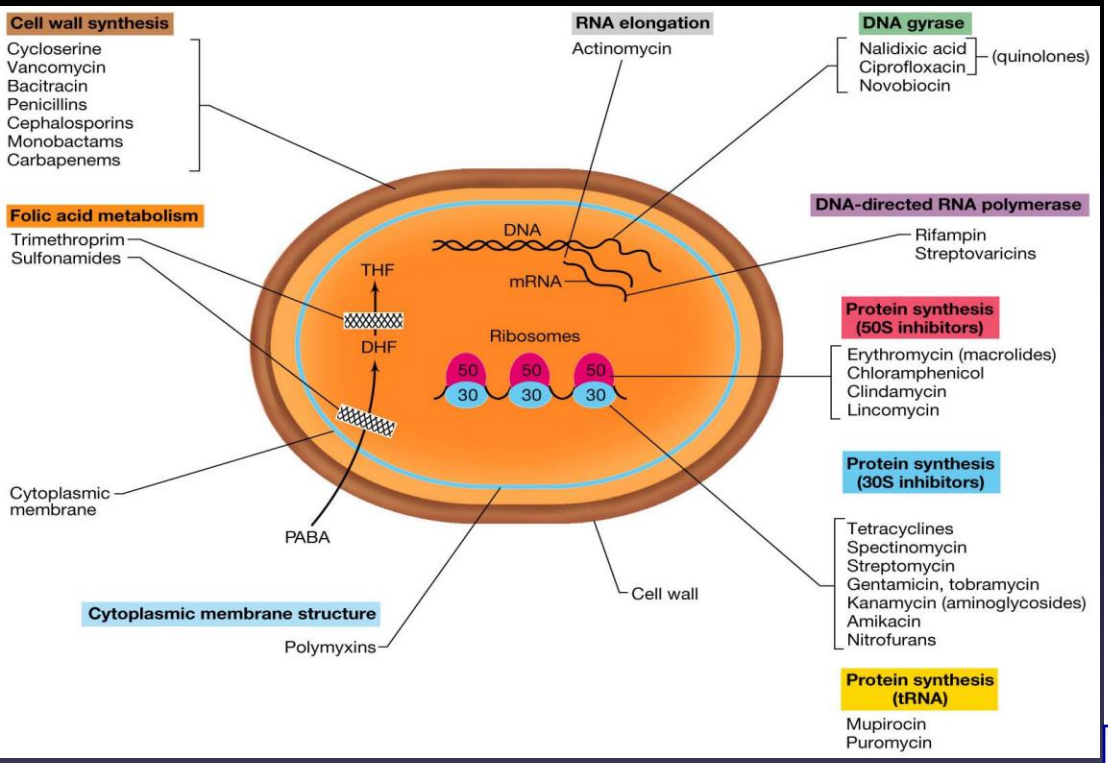
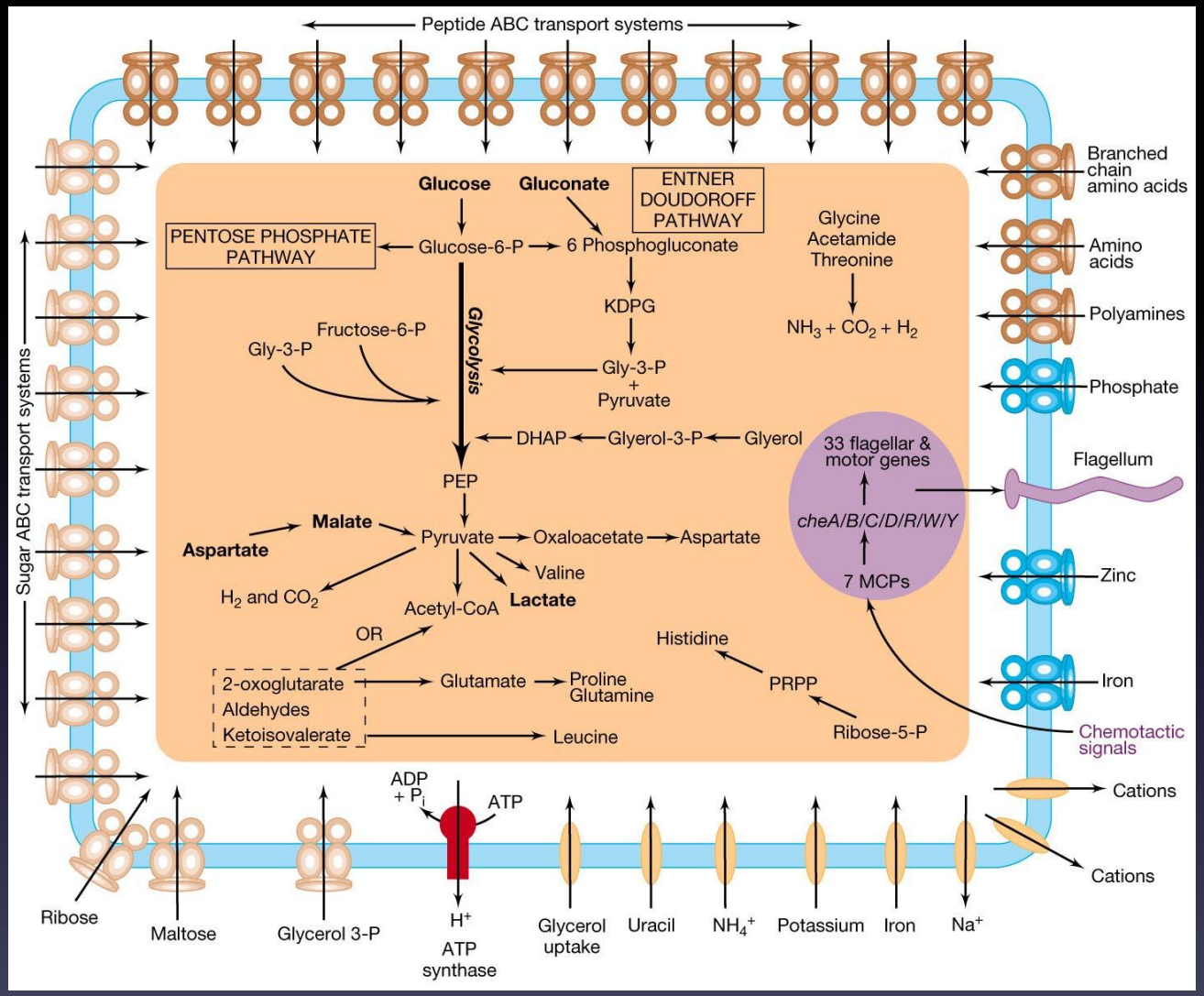


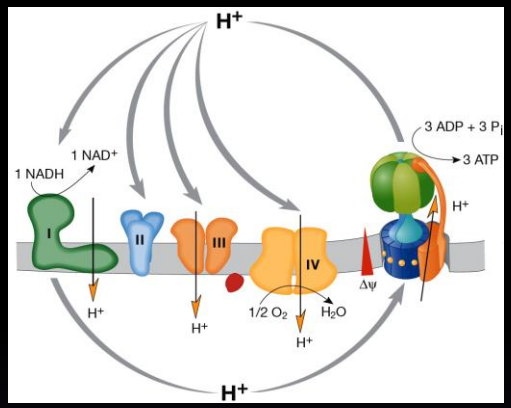
Table 1 Evolution of resistance to clinical antibiotics

Antibiotic	*Year deployed*	*Clinical resistance observed*[a]
Sulfonamides	1930s	1940s
Penicillin	1943	1946
Streptomycin	1943	1959
Chloramphenicol	1947	1959
Tetracycline	1948	1953
Erythromycin	1952	1988
Vancomycin	1956	1988
Methicillin	1960	1961
Ampicillin	1961	1973
Cephalosporins	1960s	Late 1960s
Nalidixic acid	1962	1962
Fluoroquinolones	1980s	1980s
Linezolid[b]	1999	1999
Daptomycin[b]	2003	2003
Retapamulin[b,c,d]	2007	2007
Fidaxomicin	2011	2011
Bedaquiline[b,e]	2013	?

The demand for new drugs has never been greater!

We need a *game changer* for drug discovery





Many metabolic genes (30% of genome) are essential for growth

Cellular energetics as a new target space for drug development

- Development of antibiotics to target metabolism = "*metabiotics*"
- Novel targets = unique mode of action/new molecules. Narrow spectrum agents
- Overcome current resistance determinants. Efflux pumps – powered by energy
- Host electron acceptor availability (tetrathionate) – *Salmonella*, Electron donors (H_2) *Helicobacter*
- Sodium bioenergetics - *Vibrio cholerae*, *Fusobacterium*, *Staphylococcus*

Cook et al. 2014 Advances in Pathogen Biology IN PRESS

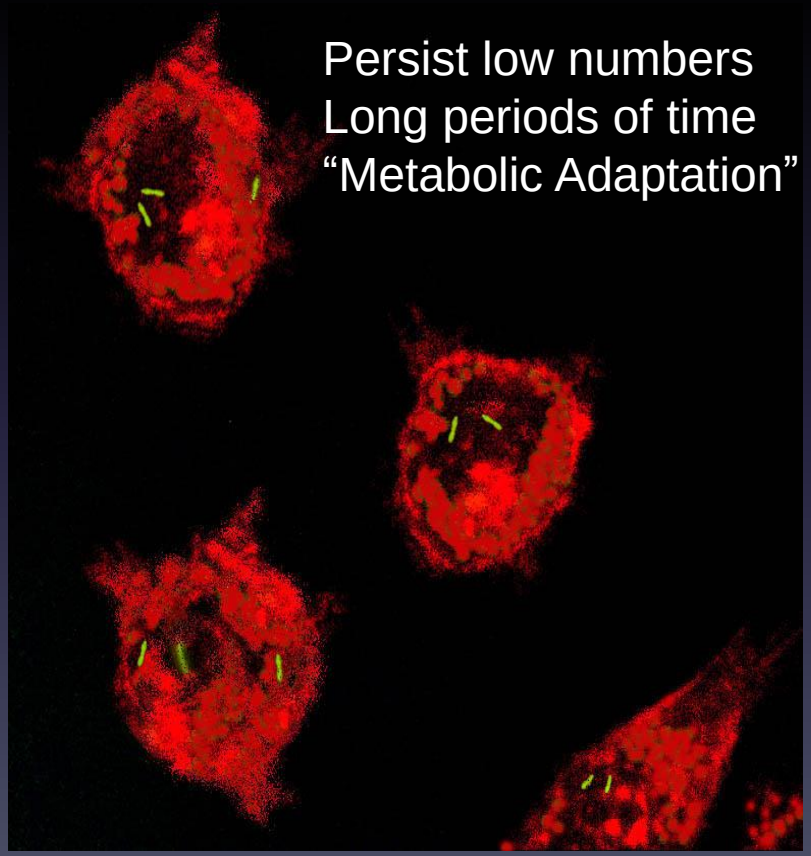
Need to understand how pathogens metabolize and persist in host tissues

What is the physiological state of growing and slow growing-non-replicating/metabolizing cells?

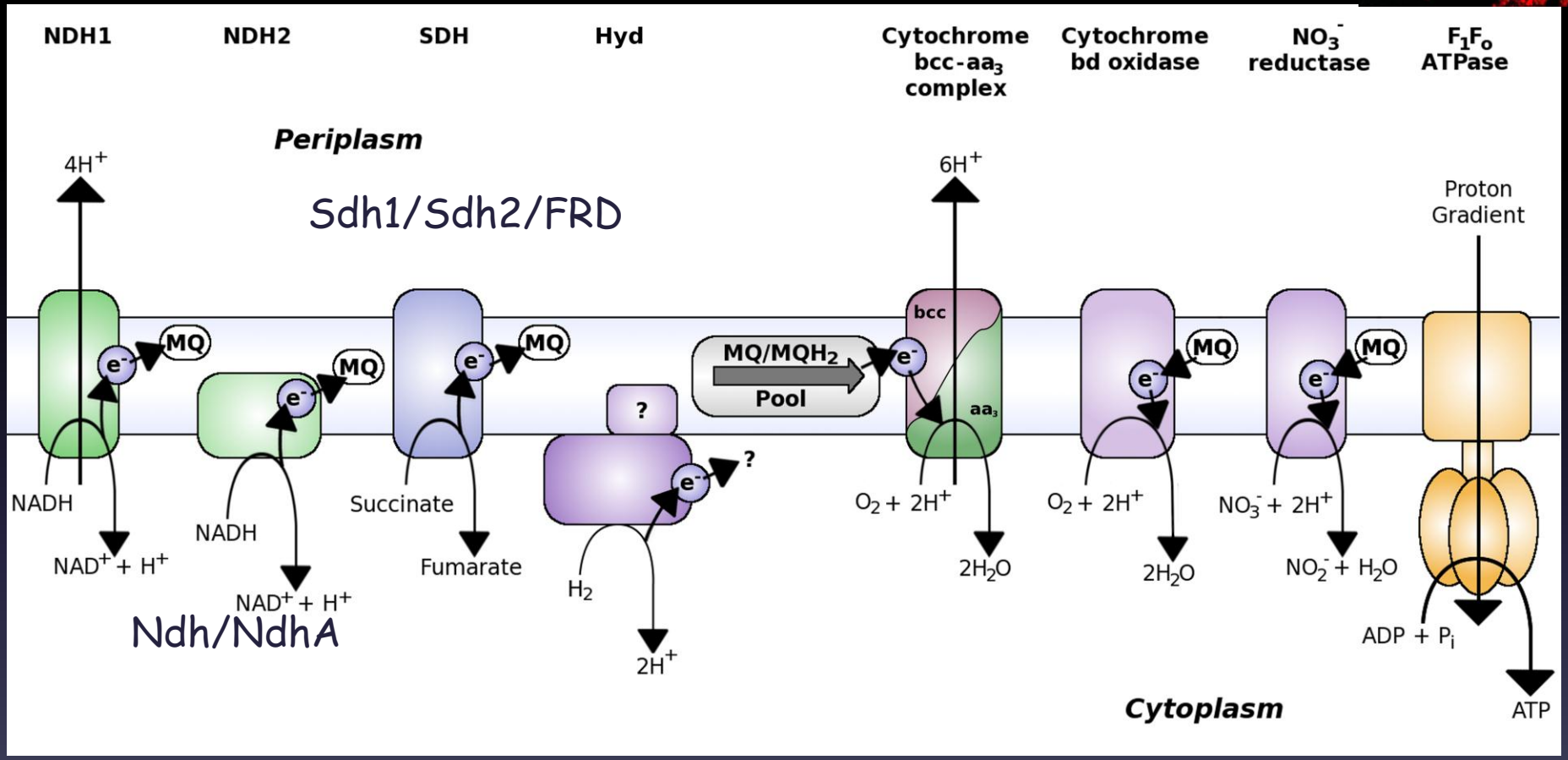
What are the key metabolic, energetic and regulatory processes?

Identify the appropriate pathway/target to kill these cells

Diverse pathology



M. tuberculosis OXPHOS machinery



Targets implicated in TB drug resistance/persistence – NDH-2 (INH), SDH-1 (RIF/INH) and cytochrome bd (BDQ)

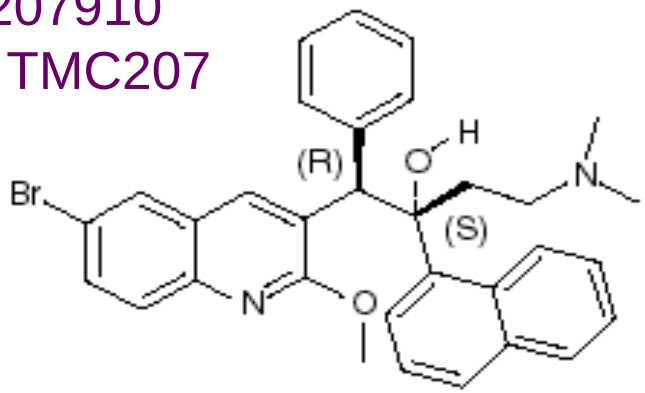
Cook et al. 2014 Energetics of Respiration and Oxidative Phosphorylation in Mycobacteria.
In *Molecular Genetics of Mycobacteria*, Hatfull, G. and Jacobs, Jr., W.R. (Eds) ASM Press, Washington DC.

Most significant discovery in TB research – last 50 years

A Diarylquinoline Drug Active on the ATP Synthase of *Mycobacterium tuberculosis*

Koen Andries,[1*] Peter Verhasselt,[1] Jerome Guillemont,[2] Hinrich W. H. Göhlmann,[1] Jean-Marc Neefs,[1] Hans Winkler,[1] Jef Van Gestel,[1] Philip Timmerman,[1] Min Zhu,[3] Ennis Lee,[4] Peter Williams,[4] Didier de Chaffoy,[1] Emma Huitric,[5] Sven Hoffner,[5] Emmanuelle Cambau,[6] Chantal Truffot-Pernot,[6] Nacer Lounis,[6]† Vincent Jarlier[6]

R207910
or TMC207



MIC = 0.06 µg/ml (nM)
No effect on mitochondrial ATP synthase (20,000-fold)

Shortens anti-tuberculosis treatment (8 weeks) and effective (bactericidal-sterilizing) in patients with drug-susceptible or drug-resistant TB (2009)

Depletes cell of ATP: Kills growing and non-growing cells (hypoxia)

Binds oligomeric c ring of F_1F_o ATP synthase (K_d 500 nM)

F.D.A. Approves Drug for Resistant Tuberculosis

By KATIE THOMAS
Published: December 31, 2012

The Food and Drug Administration announced on Monday that it had approved a new treatment for multidrug-resistant tuberculosis that can be used as an alternative when other drugs fail.

Enlarge This Image

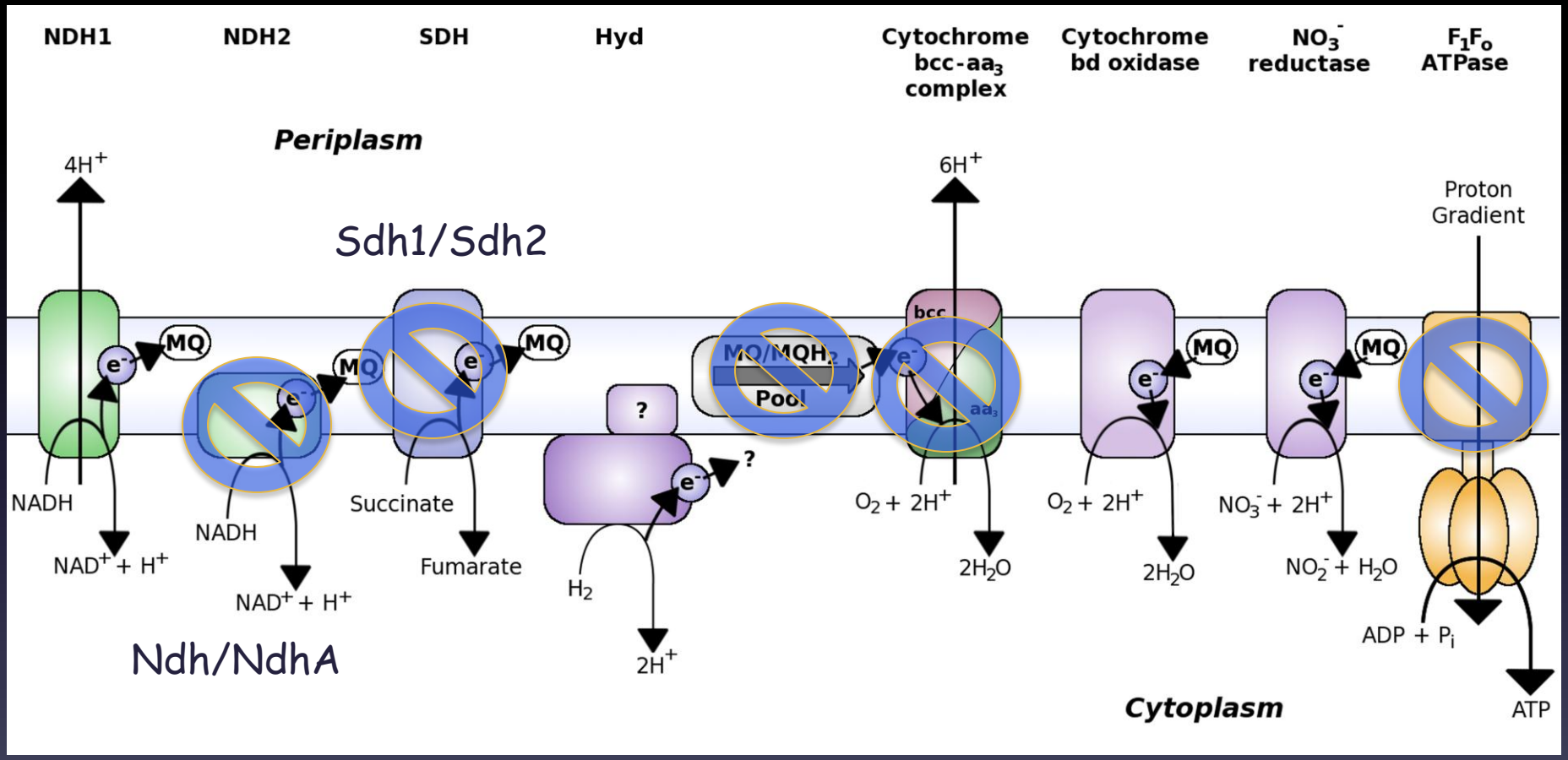
Janssen Research & Development

Koen Andries, a Janssen scientist involved in the discovery and

The drug, to be called Sirturo, was discovered by scientists at Janssen, the pharmaceuticals unit of Johnson & Johnson, and is the first in a new class of drugs that aims to treat the drug-resistant strain of the disease.

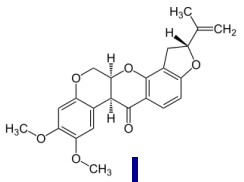
What other OXPHOS components are essential?

Mycobacterial OXPHOS machinery



Membrane potential = 78 ± 12 mV
Transmembrane pH gradient = 43 ± 8 mV
Total PMF = 121 mV (inhibitors of PMF kill cells); aerobic = 180 mV

Druggable targets: Inhibitors of the electron transport chain and ATP synthase in *M. tuberculosis*



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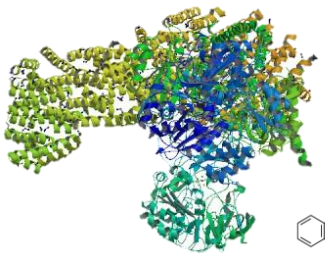

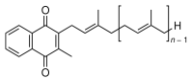

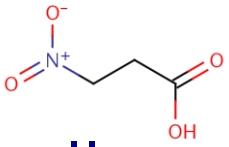

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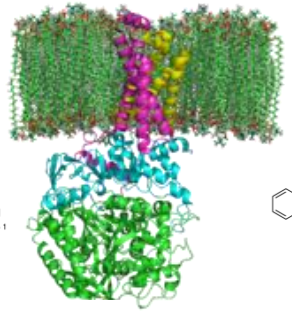

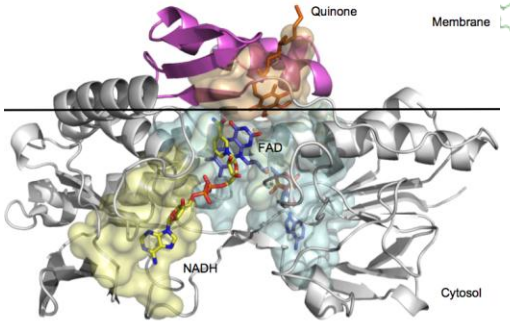

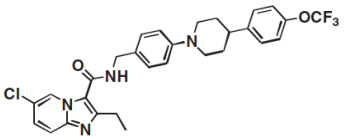

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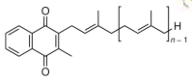

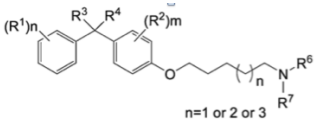

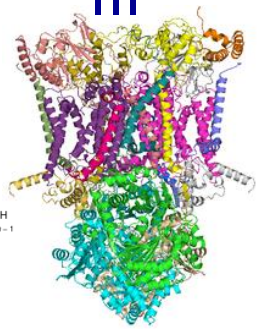

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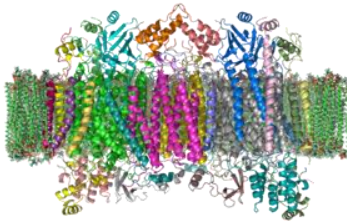
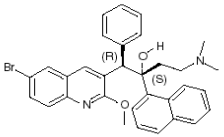
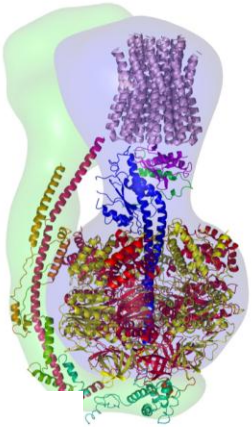
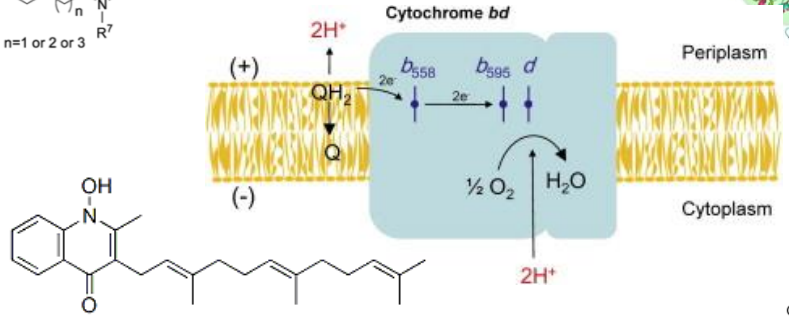
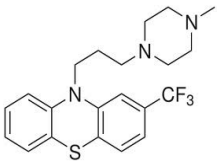


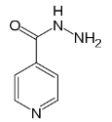


V









NADH dehydrogenase type II - INH^R
Cytochrome bd (persistence)

Need bacterial-specific compounds/drugs

- Develop enzyme assays for high throughput screening against targets (SDH2, NDH-2 and cytochrome bd)
- Identify low µM/nM inhibitors – target *pathogen* in appropriate models *(in vitro* and animal) (lab strains *versus* clinical)
- Need to explain essentiality and understand how inhibitors kill cells

N

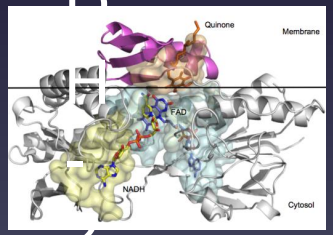
D

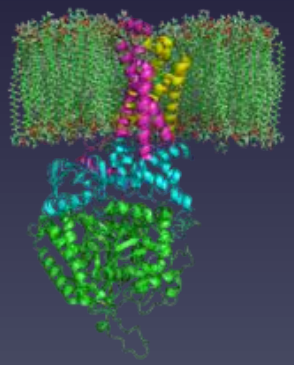
H-2

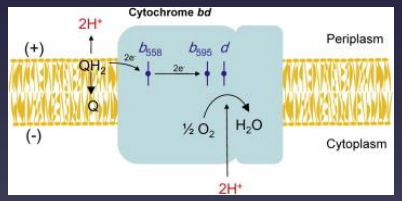
SDH

cytochrome bd

ATP Synthase

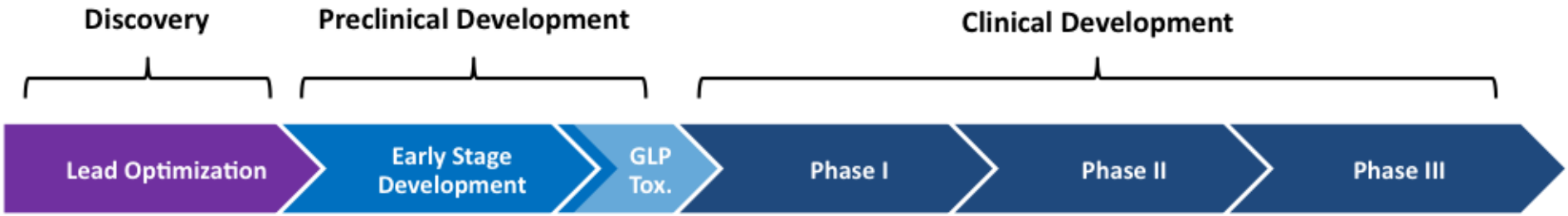






New MDR-TB regimen

Global TB Drug Pipeline [1]



Lead Optimization

- Cyclopeptides
- Diarylquinolines
- DprE Inhibitors
- InhA Inhibitor, Indazoles
- LeuRS Inhibitors, Ureas
- Macrolides, Azaindoles
- Mycobacterial Gyrase Inhibitors
- Pyrazinamide Analogs
- Ruthenium(II) Complexes
- Spectinamides
- SPR-10199
- Translocase-1 Inhibitors

Early Stage Development

- CPZEN-45
- BTZ043
- DC-159a
- SQ609
- SQ641
- TBI-166

GLP Tox.

- PBTZ169
- TBA-354
- Q203

Goal - 21 new or repurposed anti-TB drugs in Phase 1 clinical trials by 2015

Phase II

- AZD5847
- Bedaquiline (TMC-207)
- Linezolid
- Novel Regimens[2]
- PA-824
- Rifapentine
- SQ-109
- Sutezolid (PNU-100480)

Phase III

- Delamanid (OPC-67683)
- Gatifloxacin
- Moxifloxacin
- Rifapentine

Chemical classes: fluoroquinolone, rifamycin, oxazolidinone, nitroimidazole, diarylquinoline, benzothiazinone

[1] Details for projects listed can be found at http://www.newtbdrugs.org/pipeline.php and ongoing projects without a lead compound series identified can be viewed at http://www.newtbdrugs.org/pipeline-discovery.php

[2] Combination regimens: NC-001 -(J-M-Pa-Z), phase 2a, NCT01215851; NC-002-(M-Pa-Z), phase 2b, NCT01498419; NC-003-(C-J-Pa-Z), phase 2a, NCT01691534; PanACEA-MAMS-TB-01-(H-R-Z-E-Q-M), phase 2b, NCT01785186

WORKING GROUP ON NEW TB DRUGS

www.newtbdrugs.org

Updated: July 2014

Australian Synchrotron and Royal Society
Department of Microbiology and Immunology

Collaborators:
Koen Andries, J&J Research

Michael Berney and Bill Jacobs, AECOM

Clif Barry and Helena Boshoff, Tuberculosis Research Section, NIH

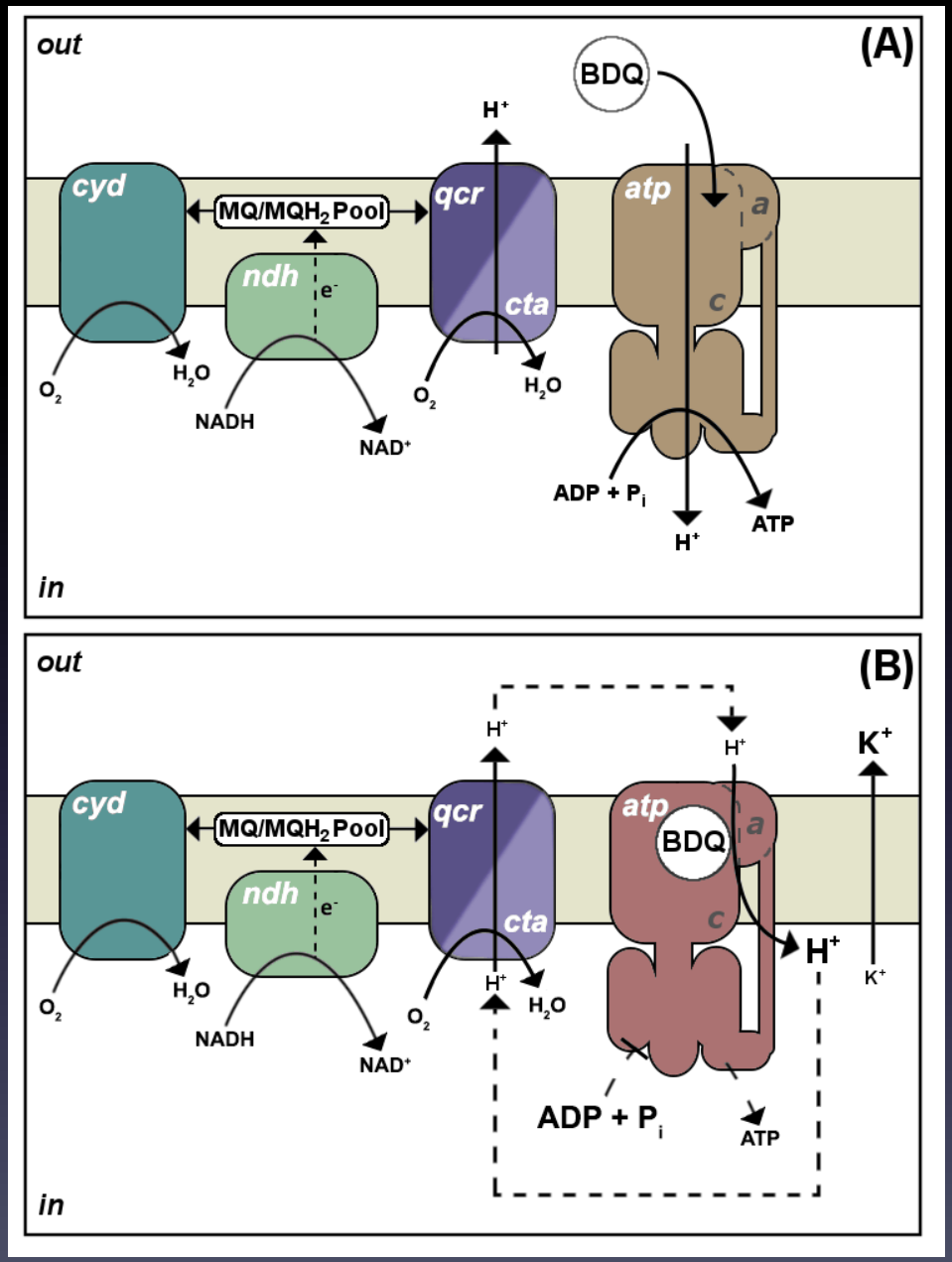
John Walker and Leo Sazanov, MRC Cambridge

Ted Baker, Auckland SBS
Shaun Lott

Joel Tyndall, Pharmacy

HRC, Marsden, Royal Society, Lottery, OMRF, MWC

MAURICE WILKINS CENTRE
FOR MOLECULAR BIODISCOVERY


out
(A)
BDQ
H+
cyd
MQ/MQH2 Pool
qcr
atp
a
ndh
e-
cta
c
O2
H2O
NADH
NAD+
ADP + Pi
ATP
in
out
(B)
K+
BDQ
in

TB in New Zealand

- 9.2% between 2007 (282 cases) and 2011 (308 cases)
- Incidence New Zealand born 2.5/100,000 (low), 8/100,000 (overall)
- Asia (62/100,000), Pacific Islands (27), Sub-Saharan Africa (39) - 75% TB cases people born overseas (Australia 87%)

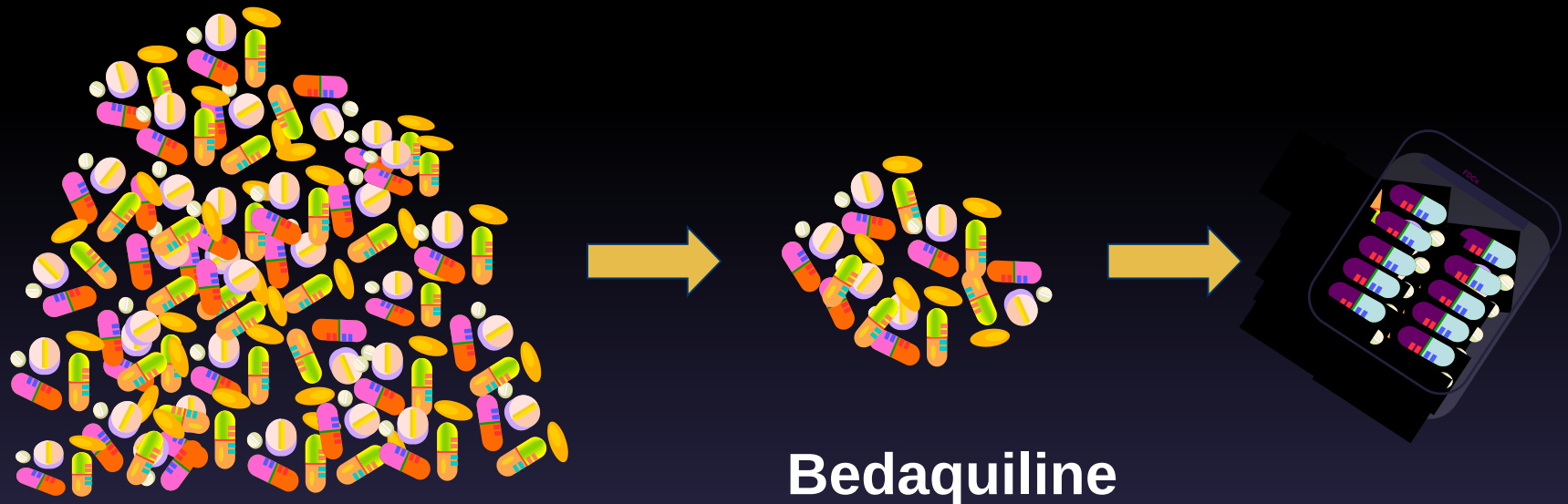
Table 7: New Zealand-born and overseas-born tuberculosis disease cases by ethnicity, 2011

Ethnicity[1]	Born in New Zealand		Born overseas	
	No.	%	No.	%
Māori	37	50.0	0	-
Pacific Peoples	13	17.6	33	14.5
Asian	5	6.8	160	70.5
MELAA	1	1.4	15	6.6
European or other	18	24.3	11	4.8
Unknown	0	-	8	3.5
Total	**74**	**100**	**227**	**100**

[1] Ethnic groups were prioritised in the following order: Māori, Pacific Peoples, Asian, Middle Eastern/Latin American/African (MELAA), European or Other Ethnicity (including New Zealander)

Bissielo, A., E. Lim, and H. Heffernan. 2011. Tuberculosis in New Zealand: annual report Institute of Environmental Science and Research Limited.

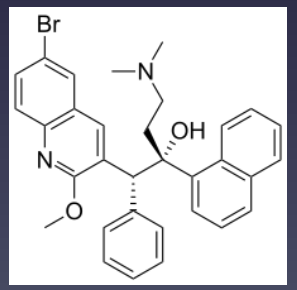
New fast-acting drugs – deeper scientific understanding of how drugs work

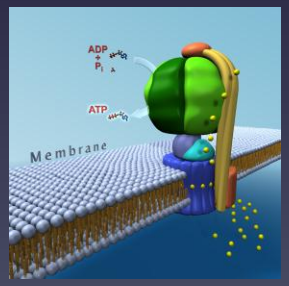


6-30 months
PZA
INH
Ethambutol
Rifampicin
Streptomycin

Bedaquiline

2-4 months

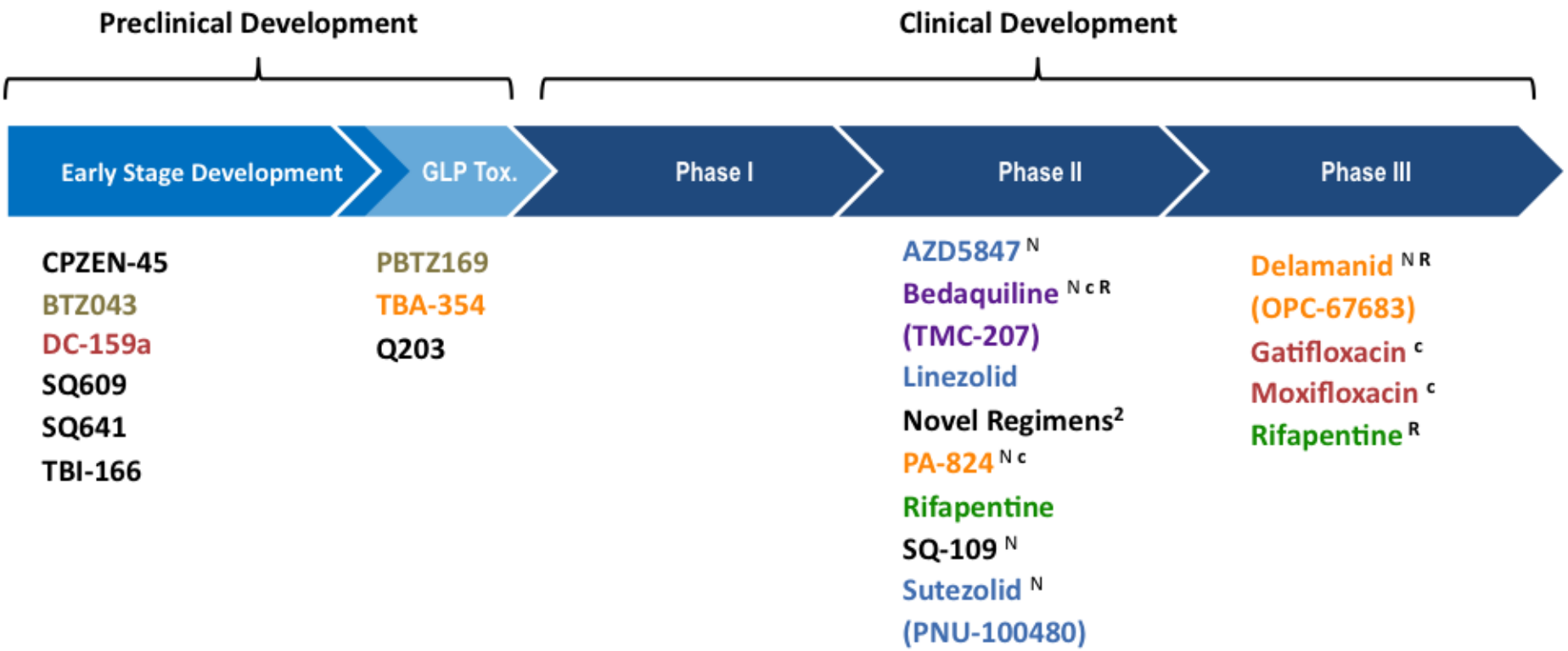




2 weeks

?

Global TB Drug Pipeline [1]



Chemical classes: fluoroquinolone, rifamycin, oxazolidinone, nitroimidazole, diarylquinoline, benzothiazinone

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[C] Drug candidate currently in combination regimen in clinical testing

[R] Submitted for approval or approved by stringent regulatory authority (i.e., FDA, EMA, WHO Prequalification)

[N] New chemical entity

WORKING GROUP ON NEW TB DRUGS

www.newtbdrugs.org

Updated: July 2014

Global TB Drug Discovery Pipeline [1]

Hit-to-Lead

Actinomycete Metabolites (U ILL Chicago, Myongii U)
ATP Synthesis Inhibitors (GATB, Calibr)
Fungal Metabolites (Mycosynthetix, U ILL Chicago)
Indigoids (U ILL Chicago)
Isoprenoid Biosynthesis Inhibitors (Lilly Ddi)
M. tb Energy Metabolism Inhibitors (UPenn, GATB)
M. tb Protein Kinase Inhibitors (Vertex Pharmaceuticals)
Malate Synthase Inhibitors (GSK, TAMU, GATB)
Menaquinone Synthase Inhibitors (CSU)
Novel Hit-to-Lead Programs (Lilly DDi) GATB
Phenotype Hit-to-Lead (Lilly DDi)
RNA Polymerase Inhibitors (GATB, Rutgers U)
Whole-Cell Hit-to-Lead (AZ, GSK, GATB, Sanofi)

Lead Optimization

Cyclopeptides (GATB, Sanofi)
Diarylquinolines (GATB, U Auckland, Janssen)
DprE Inhibitors (GATB, Calibr)
InhA Inhibitors (GSK, GATB)
LeuRS Inhibitors (Anacor Pharmaceuticals)
Macrolides (GATB, Sanofi)
Mycobacterial Gyrase Inhibitors (GATB, GSK)
Pyrazinamide Analogs (GATB, Yonsei U)
Ruthenium(II)phosphine/picolinate Complexes (FAPESP/Brazil)
Spectinamides (St. Jude, U Tenn, CSU, UZ, Microbiotix)
Translocase-1 Inhibitors (Sequella)
Azaindoles (GATB)
Ureas (Sanofi, CRO-Wuxi)
Indazoles (GATB, GSK)
SPR-10199 (Gates Foundation)

[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: **AZ**-AstraZeneca; **BRI**-Beijing Tuberculosis and Thoracic Tumor Research Institute; **CSU**-Colorado State University; **FAPESP**-São Paulo Research Foundation; **GATB**-Global Alliance for TB Drug Development (TB Alliance); **GSK**-GlaxoSmithKline; **IMM**-Institute of Materia Medica; **Lilly DDi**-Lilly TB Drug Discovery Initiative; **RI**-Research Institute; **St. Jude**-St. Jude Children's Research Hospital; **TAMU**-Texas A&M University; **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: July 2014

The Maurice Wilkins Centre for Molecular Biodiscovery (CORE)

Tuberculosis Flagship

- New TB drug TBA-354: 2nd-generation analogue of PA-824. 10-fold more potent, with a three-fold longer half-life than PA-824 (clinical trial in March 2014)
- Improved 2nd-generation analogue of Bedaquiline: 50-fold more potent and 1000-fold less lipophilic than bedaquiline. Clinical candidate selected for development by the end of 2014
- Development of new OXPHOS inhibitors (new targets)
- WGS: Professor Kyi Kyi Thinn and Dr Thanda Tun (University of Medicine 1, and National Health Laboratory, Myanmar) focusing on the characterization of MDR and XDR *M. tuberculosis* strains from smear positive sputum samples in Myanmar

Table 6. Antimicrobial resistance among tuberculosis isolates by mycobacterial species, 2012

Antimicrobial	Resistant[a]					
	M. tuberculosis (*n* = 227)		*M. bovis* (*n* = 4)		All isolates[b] (*n* = 233)	
	No.	%	No.	%	No.	%
Isoniazid (0.1 mg/L)	17	7.5	1	25.0	18	7.7
Isoniazid (0.4 mg/L)[c]	10	4.4	0	0.0	10	4.3
Rifampicin	4	1.8	0	0.0	4	1.7
Ethambutol	2	0.9	0	0.0	2	0.9
Pyrazinamide	1	0.4	4[d]	100.0	6[e]	2.6
Streptomycin	21	9.3	0	0.0	21	9.0

[a] Includes resistance alone or in combination with other antimicrobials.

[b] Includes two isolates only identified as *M. tuberculosis* complex.

[c] All isolates resistant to isoniazid at the standard breakpoint concentration of 0.1 mg/L were also tested at the higher concentration of 0.4 mg/L.

[d] *M. bovis* is intrinsically resistant to pyrazinamide.

[e] Pyrazinamide susceptibility was not available for one of the two *M. tuberculosis* complex isolates. The other *M. tuberculosis* complex isolate was pyrazinamide resistant.