New Medicines for Tuberculosis

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Global Health Institute

NM4TB
Sixth Framework Programme

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Outline & overview

- Introduction to TB & therapy
- New TB drugs in trials
- Rationale behind NM4TB
- Criteria for drug discovery
- Examples of target-
  & cpd-based screens

Tuberculosis – does that still exist?

- 2 Million deaths & ~9 Million new cases/yr
- 2 Billion infected – latent disease
- Devastating synergy with HIV: ~15% have TB, >80% in some African regions
- MDR-TB & XDR-TB major concern

Articles

Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa

Neel R Gandhi, Anthony Moll, A Willem Sturm, Robert Pawinski, Thiloshini Govender, Umesh Lalloo, Kimberly Zeller, Jason Andrews, Gerald Friedland

Summary

Background The epidemics of HIV-1 and tuberculosis in South Africa are closely related. High mortality rates in co-infected patients have improved with antiretroviral therapy, but drug-resistant tuberculosis has emerged as a major cause of death. We assessed the prevalence and consequences of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis in a rural area in KwaZulu Natal, South Africa.

Lancet 2006; 368: 1575–80
Published Online October 26, 2006
DOI:10.1016/S0140-6736(06)69573-1

XDR-TB: a growing menace

- 500,000 cases of MDR-TB (70,000 in Europe)
- >50 countries with XDR-TB
- Worst affected countries are next-door!

'We risk converting the largely treatable TB epidemic into a non-treatable one, as it was before antibiotics … An XDR-TB epidemic would threaten all progress made in TB control in recent years'
Senior, K (2007) *Lancet Infectious Diseases 7, 511*

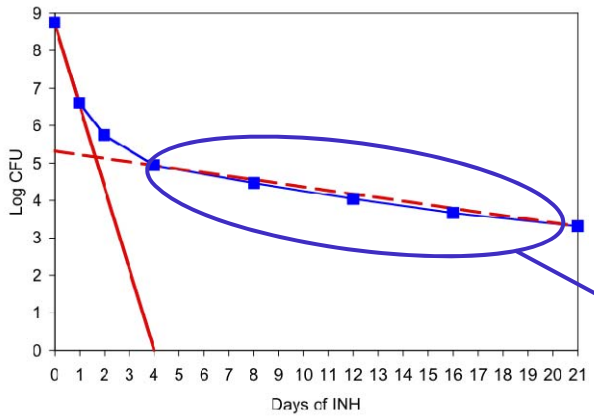
What's available?

- Vaccine - BCG
- Tuberculin skin test
- DOTS
  - Rifampin
  - Isoniazid
  - Ethambutol
  - Pyrazinamide

- 75 years old
- 125 years old
- ~40 years since last new drug

Sad contrast to HIV/AIDS situation!

Common theme - biphasic kill kinetics



In vitro

PERSISTENCE

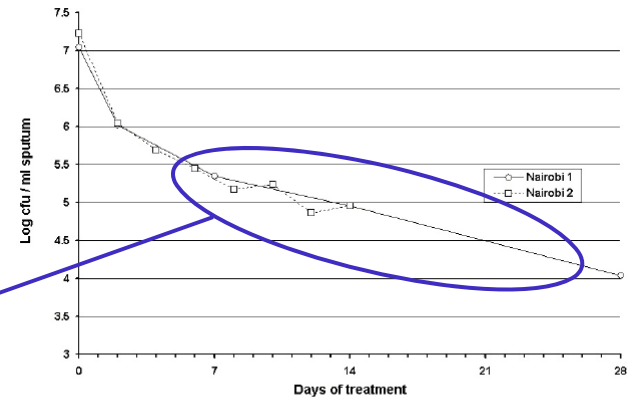
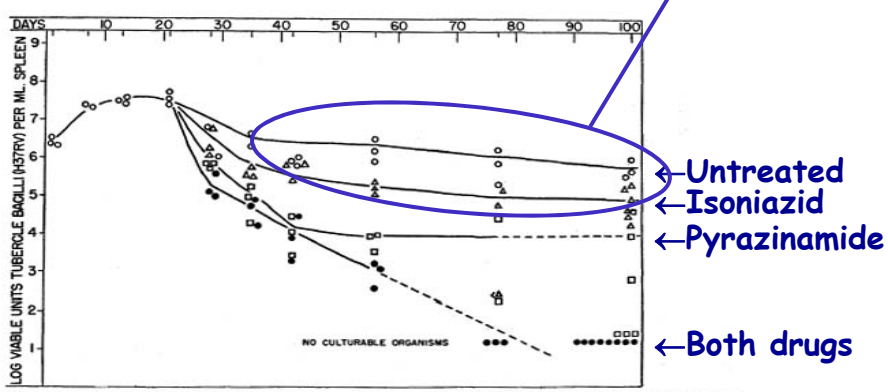


Figure 1. Viable counts of *M. tuberculosis* per ml sputum during the first month of treatment with regimens containing INH. Sputum was collected over-night at fixed intervals from the start of treatment, an aliquot was homogenized with dithiothreitol, which homogenizes by breaking -S-S- bonds but has not antibacterial activity, and plated on selective 7H11 plates. The results in two studies, both on patients in Nairobi, are shown. Data from references 5 and 10.

Humans

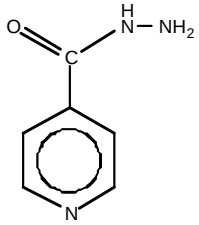


TEXT-FIG. 5. Influence of pyrazinamide and isoniazid used singly and together on populations of tubercle bacilli (H37Rv) in mouse spleens in the presence of lesions.

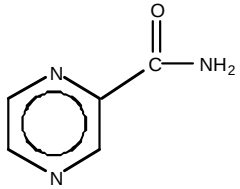
Treatment was started 21 days after initiation of infection. Infecting inoculum 5.7 × 10^8 culturable units tubercle bacilli. ○, control; △, isoniazid; □, pyrazinamide; ●, pyrazinamide-isoniazid.

Animals

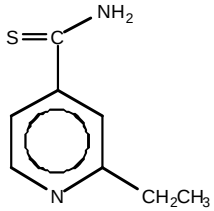
Current TB drugs



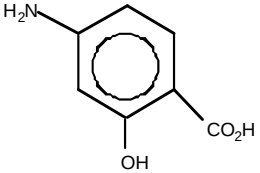
Isoniazid



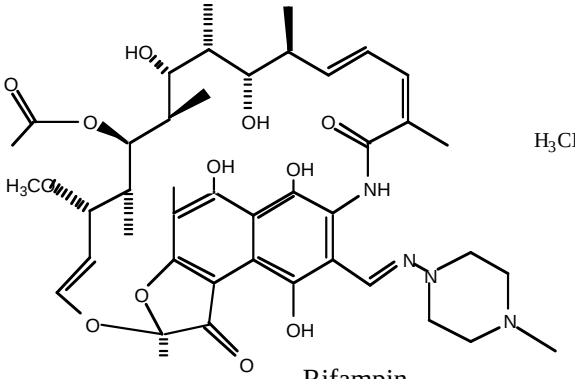
Pyrazinamide



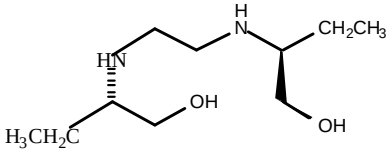
Ethionamide



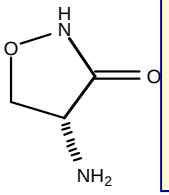
p-Aminosalicylic acid



Rifampin

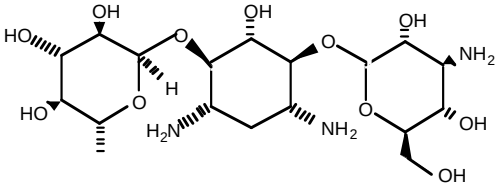


Ethambutol

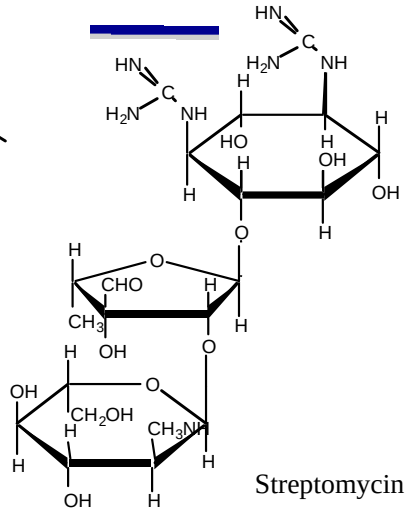


Cycloserine

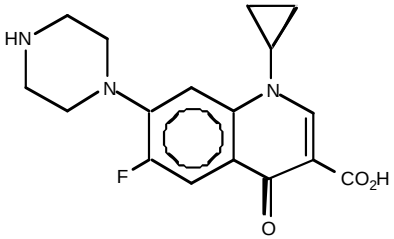
TB drugs are often prodrugs



Kanamycin

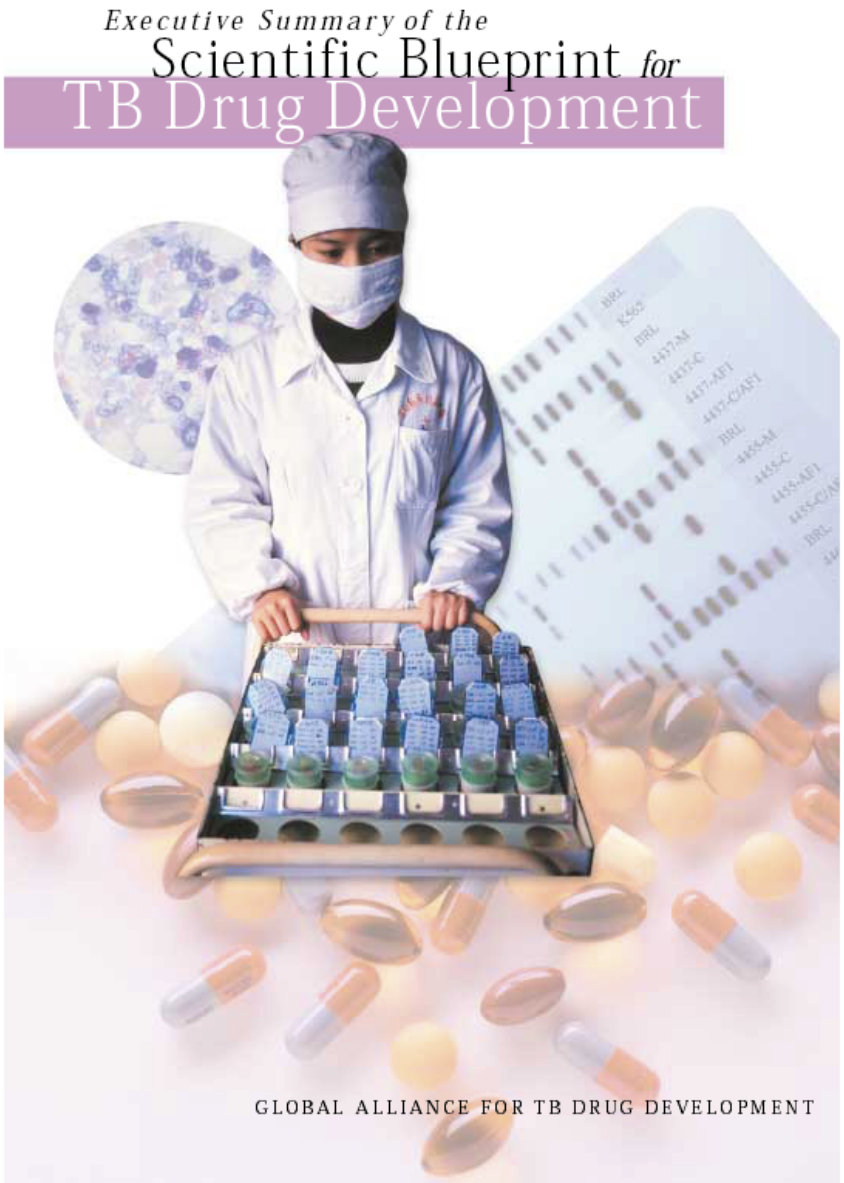


Streptomycin



Ciprofloxacin

What's needed?



- More potent compounds
- Reduce duration of therapy <3m
- Kill persisters
- New MoA to overcome resistance

Global TB drug pipeline

Discovery		Preclinical	Clinical Testing
Bacterial Topoisomerase Inhibitors GlaxoSmithKline,TB Alliance	**Nitrofuranylamides** NIAID, University of Tennessee	**TB Oxazolidinones** Pfizer Inc.	**Diarylquinoline TMC207** Johnson & Johnson
Cell Wall Inhibitors Colorado State University, NIAID	**Nitroimidazole Analogs** TB Alliance, University of Auckland	**Dipiperidines(SQ609)** Sequella Inc.	**Gatifloxacin** OFLOTUB Consortium,Lupin NIAID TBRU Tuberculosis Research Centre, WHO TDR
Dihydrolipoamide Acyltransferase Inhibitors Cornell University, NIAID	**Proteasome Inhibitors** Cornell University, NIAID	**Nitroimidazo oxazole Back-up** Otsuka	**Gatifloxacin** DMID/NIAID/NIH, Case Western Univ.
InhA Inhibitors GlaxoSmithKline TB Alliance	**Protease Inhibitors** Medivir	**ATP SynthaseInhibitor FAS20013** FASgen Inc.	**Moxifloxacin** Bayer Pharm., CDC TBTC, Johns Hopkins University, NIAID TBRU, TB Alliance
Diphenyl ether based inhibitors of InhA Stony Brook, NIH	**Multi-functional molecules** Cumbre, TB Alliance	**TranslocaseI Inhibitors** Sequella Inc., Sankyo	**Moxifloxacin** DMID/NIAID/NIH, Case Western Univ.
Malate Synthase Inhibitors GlaxoSmithKline, Rockfeller University, Texas A&M	**Pleuromutilins** GlaxoSmithKline, TB Alliance	**Non-Fluorinated Quinolones** TaiGen	**NitroimidazolePA-824** Chiron Corporation, TB Alliance
Promazine Analogs Salisbury University	**Quinolones** KRICT/ Yonsei University, TB Alliance		**Nitroimidazooxazole OPC-67683** Otsuka
Riminophenazines Institute of Materia Medica, BTTTRI	**Thiolactomycin Analogs** NIAID, NIH		**Pyrrole LL-3858** Lupin Limited
Natural Products Exploration BIOTEC, California State University, ITR NIAID, TAACF, University of Auckland	**Screening and Target Identification** AstraZeneca		**Diamine SQ-109** Sequella Inc
Natural Products Exploration NERC Center, Univ of Strathclyde, Univ of Illinois	**Focused Screening** GlaxoSmithKline, TB Alliance		**Metroinidizaole for Laten Infection** Imperial College London, Wellcome Trust Gates Foundation
Novartis Portfolio Novartis	**Sanofi-Aventis Portfolio** Sanofi-Aventis		**Linezolid** DMID/NIAID/NIH, Case Western Univ.
Discovery for latent Infection Imperial College London, Wellcome Trust Gates Foundation	**Screening of compounds inhibiting growth of M.tb** NIH, NIAID, TAACF		**Levofloxacin** DMID/NIAID/NIH, Case Western Univ.
New Medicines for TB Portfolio AstraZeneca, European Commission	**Identification of compounds with in vivo activity against M.tb in animal models** NIH, NIAID		**SV07 Immune Modulator** SciClone Pharmaceuticals

NM4TB
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Casenghi *et al.* (2007)
PlosMed 4 e293

New TB drugs

- FQ: moxifloxicin, gatifloxicin
- Nitroimidazole derivatives: PA-824, Otsuka compound OPC67683
- Oxazolidinones: PNU-100480, AZD5847
- JJ cpd, R207910, TMC207

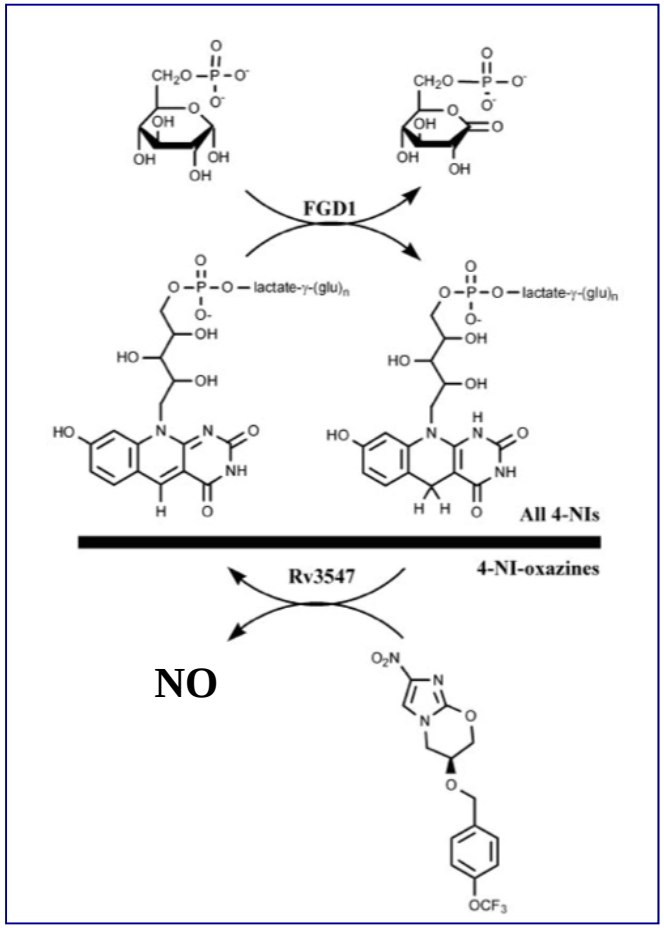
Moxifloxacin reduces treatment duration

Treatment in mice				
	Duration of Treatment (months)			
Treatment	3	4	5	6
2 m RIF, INH, PZA/ 4 m RIF INH	11/12	5/12	1/16	0/12
1 m RIF, MOXI, PZA/ 4 m RIF MOXI	4/12	0/12	0/12	ND
2 m RIF, MOXI, PZA/ 3 m RIF MOXI	2/12	0/12	0/13	ND
5 m RIF, MOXI, PZA	4/12	0/12	0/12	ND
	Nuermberger 2004 AJRCCM			

- On substituting Moxifloxacin for INH, mice cured in only 4m compared to 6m with INH

Activation of PA-824

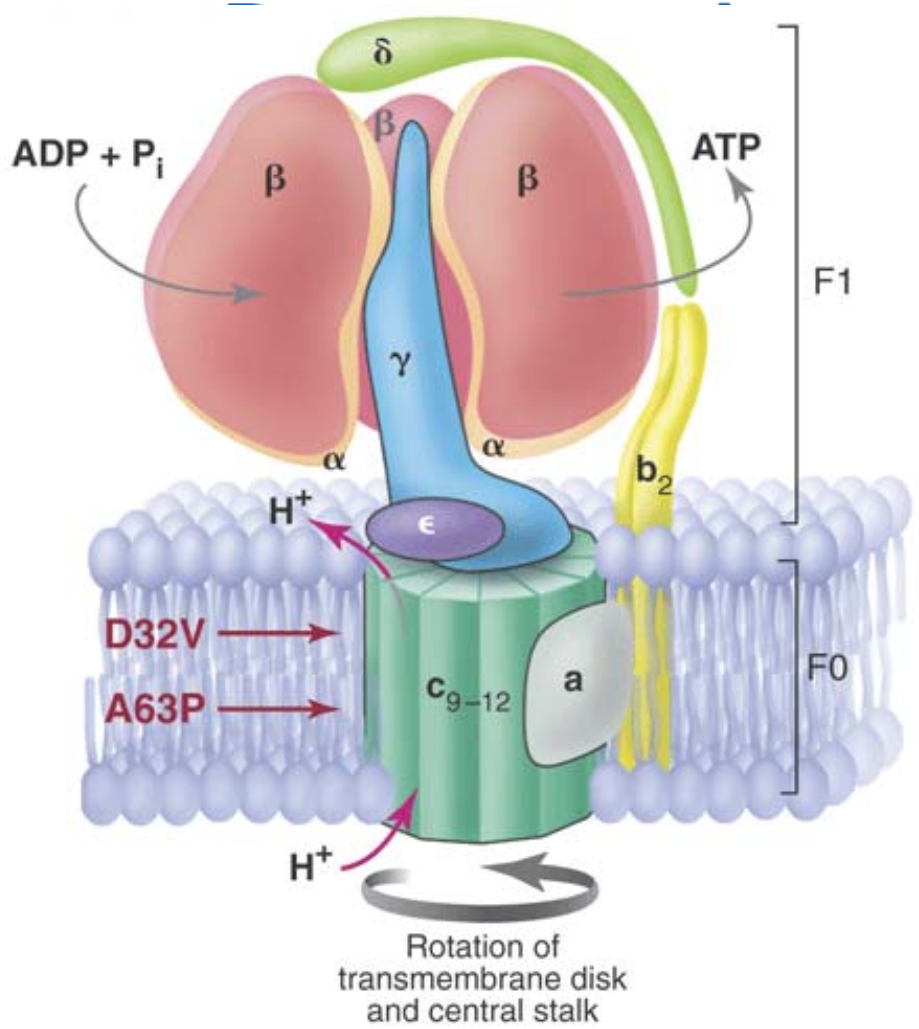
- Nitroimidazoles active microaerophilically
- PA-824 active aerobically & microaerophilically
- Persistence?
- Is a prodrug
- Target(s) unknown but CW likely



Stover et al. (2000) Nature 405:962;
Manjunatha et al. (2006) PNAS 103:431
Singh et al. (2008) Science

A major advance TMC207

- Screening
- Resist mutants
- Whole genome resequencing
- Target ID
- Genetics



Success in MDR-TB clinical trial

Unusual clinical trial

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.

TB Alliance Portfolio

Discovery: TARGET OR CELL-BASED SCREENING	Discovery: LEAD IDENTIFICATION	Discovery: LEAD OPTIMIZATION	Preclinical Development	Clinical Development: CLINICAL PHASE I	Clinical Development: CLINICAL PHASE II	Clinical Development: CLINICAL PHASE III
Natural Products IMCAS	**Whole-Cell Hit to Lead Program** GSK	**Mycobacterial Gyrase Inhibitors** GSK	**Nitroimidazoles** U. of Auckland/ U. Ill Chicago		**PA-824** Novartis	**Moxifloxacin (+ H, R, Z)** Bayer
Protease Inhibitors IDRI	**Malate Synthase Inhibitors** GSK/TAMU	**InhA Inhibitors** GSK	**Preclinical TB Regimen Development** JHU/U. Ill Chicago		**TMC207** Tibotec	**Moxifloxacin (+ R, Z, E)** Bayer
TB Drug Discovery Portfolio NITD		**Diarylquinolines** Tibotec/U. of Auckland			**PA-824/Pyrazinamide**	
Topoisomerase I Inhibitors AZ/NYMC	**Gyrase B Inhibitors** AZ	**Riminophenazines** IMM/BTTTRI			**TMC207/Pyrazinamide**	
	Folate Biosynthesis Inhibitors AZ	**Pyrazinamide Analogs** Yonsei			**PA-824/ Moxifloxacin/ Pyrazinamide**	
	Whole-Cell Hit to Lead Program AZ					
	RNA Polymerase Inhibitors AZ/Rutgers					
	Energy Metabolism Inhibitors AZ/U. Penn					
	Phenotypic Hit to Lead Program U. Ill Chicago					
	Menaquinone Biosynthesis Inhibitors CSU					

Novel TB regimen development

Current first-line TB treatment consists of Isoniazid (H) + rifampicin (R) + pyrazinamide (Z) + ethambutol (E)

OUR R&D PARTNERS

- AstraZeneca (AZ)
- Bayer Healthcare AG (Bayer)
- Beijing Tuberculosis and Thoracic Tumor Research Institute (BTTTRI)
- Colorado State University (CSU)
- GlaxoSmithKline (GSK)
- Infectious Disease Research Institute (IDRI)
- Institute of Materia Medica (IMM)
- Institute of Microbiology, Chinese Academy of Sciences (IMCAS)
- Johns Hopkins University (JHU)
- Johnson & Johnson / Tibotec (Tibotec)
- New York Medical College (NYMC)
- Novartis Institute for Tropical Diseases (NITD)
- Novartis Pharmaceutical (Novartis)
- Rutgers: The State University of New Jersey (Rutgers)
- Texas A&M University (TAMU)
- University of Auckland (U. of Auckland)
- University of Illinois at Chicago (U. Ill Chicago)
- University of Pennsylvania School of Medicine (U. Penn)
- Yonsei University (Yonsei)

New regimen in clinical trial

- Phase II trial, NC001, tests PA-824, MOXI & PZA in South Africa
- EBA trial: 2 w treatment, 3 m of follow-up to evaluate effectiveness, safety, and tolerability.
- NC001 also tests TMC207/PZA and PA-824/PZA

NM4TB - history

NM4TB

SIXTH FRAMEWORK PROGRAMME

- Introduction
- Participants
- Management
- Scientific Meetings
- Links
- Members' Section

Sixth Framework Programme

New Medicines For Tuberculosis
NM4TB

Acronym: NM4TB

Project number: 018923
Requested EC contribution: 10.87 M Euros
Duration: 60 months
Starting date: January, 2006

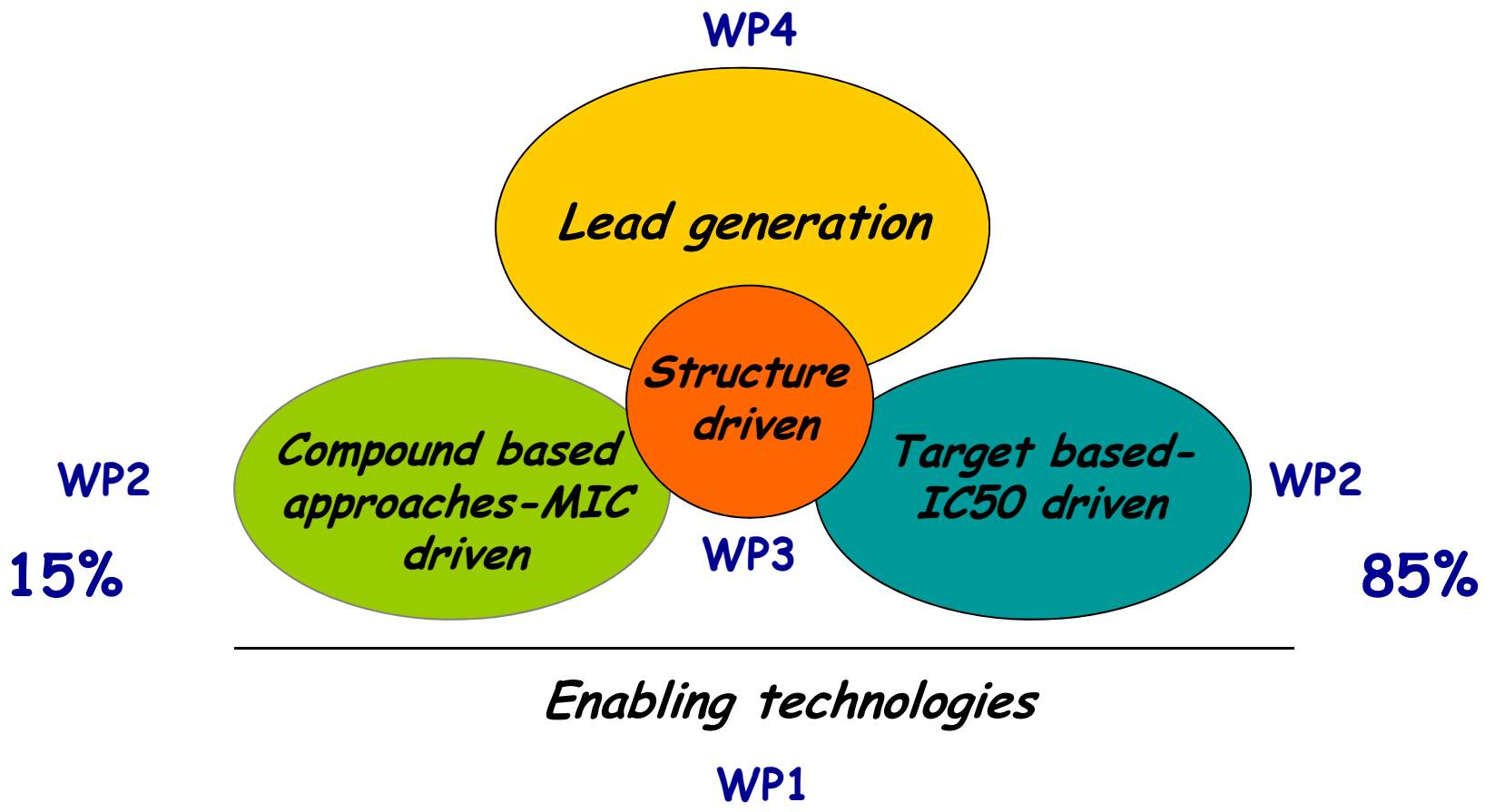
http://WWW/NM4TB.org

Dispersed drug discovery consortium!

New Medicines for Tuberculosis (NM4TB) aims to successfully develop new drugs for the treatment of tuberculosis (TB) through an integrated approach implemented by a team that combines some of Europe's leading academic TB researchers with a major pharmaceutical company and three SMEs, all with a strong commitment to discovering new anti-infective agents. NM4TB has a comprehensive portfolio of potential and validated targets plus several novel, proprietary anti-TB agents in its drug development pipeline. Among the validated targets are several enzymes involved in highly druggable areas such as cell wall biogenesis, nucleic acid synthesis and central metabolic pathways for which assays amenable to high-throughput screening are available. Intensive efforts will focus on rapidly emerging targets that impact upon two as-yet untouched areas of the physiology of *Mycobacterium tuberculosis* signal transduction pathways and persistence.

16 academic partners in EU/CH,
2 academic partners in DEC,
2 SME,
1 big pharma.

NM4TB - Approaches



NM4TB: Drug Profiles

Compounds must have sterilizing, bactericidal activity

Effective X persisters (extra/intracellular)

Novel MoA: active X MDR- & XDR-TB

Selectivity for Mycobacteria

No antagonism with DOTS & compatible with dosing

Compatibility with ART

Toxicologically acceptable for dosing >2 months

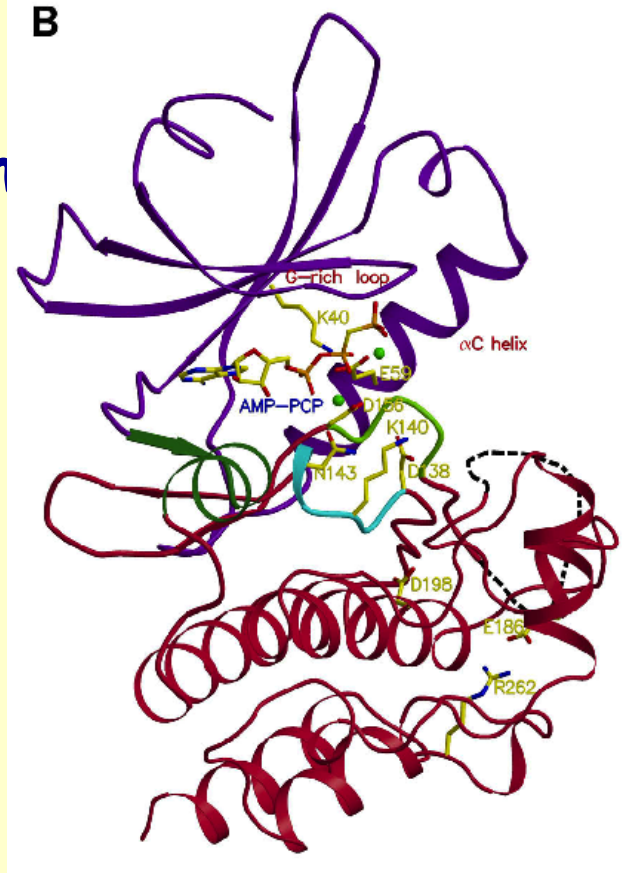
Therapy ideally results in cure within 2 months

Big ask!

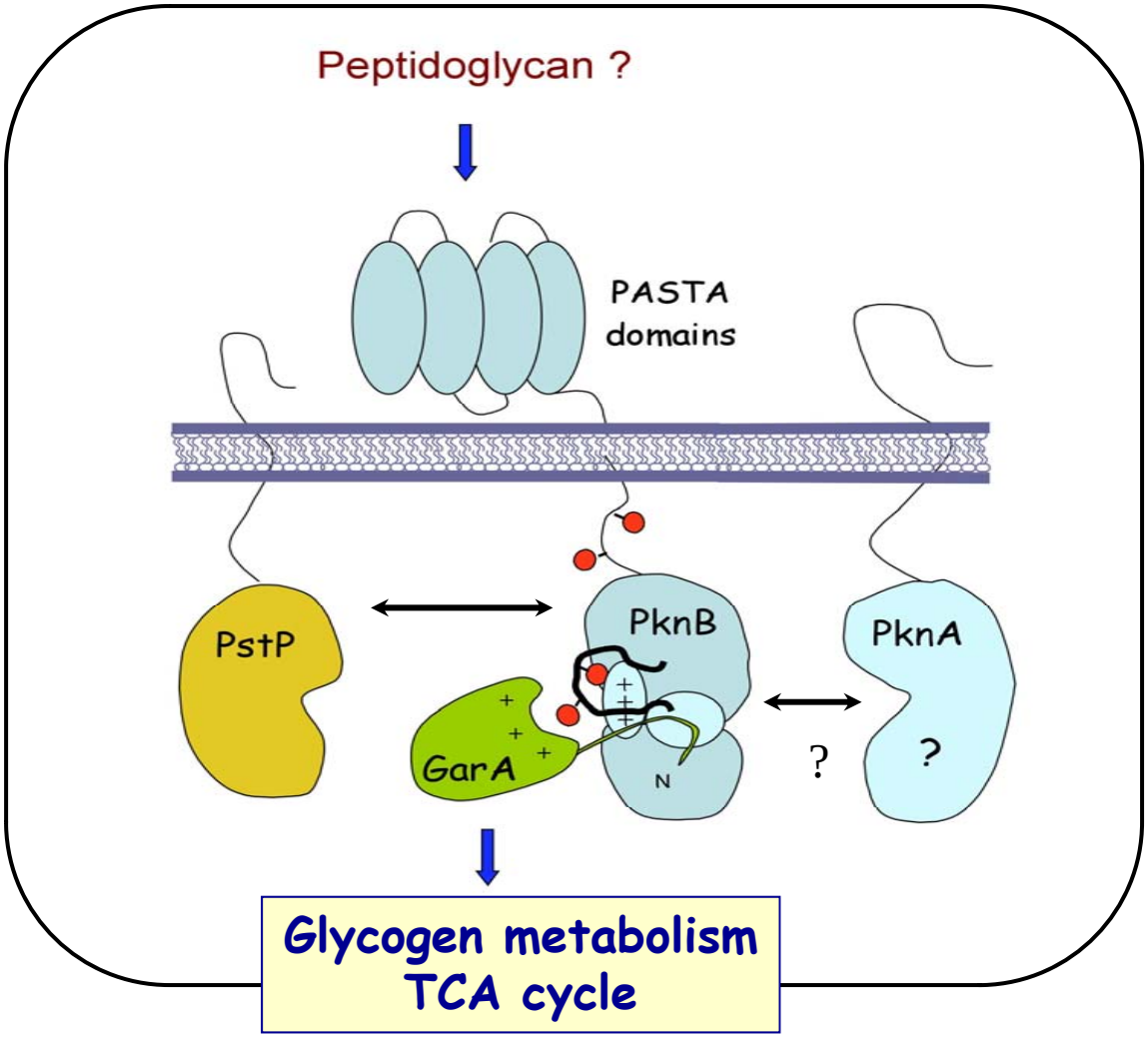
Lead generation
Target-based: IC_{50} driven
Case of PknB

- STPK
- Physiological role: peptidoglycan
- Essential function
- Assay available
- 3D structure known
- Millions of kinase inhibitors

"available"



Signal transduction & drug design



Targeting receptor domain

Targeting kinase activity

Current status

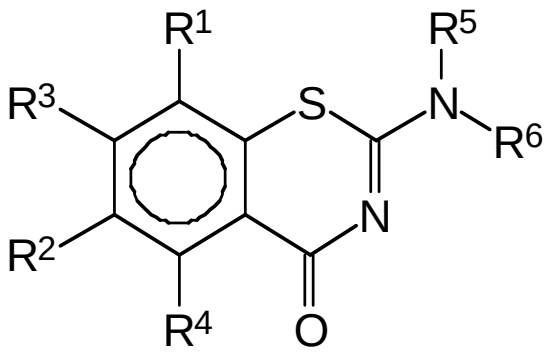
- Finished HTS,
- Finished focused library, virtual & whole cell screens,
- Finished selectivity screens,
- Top hits don't show MIC.

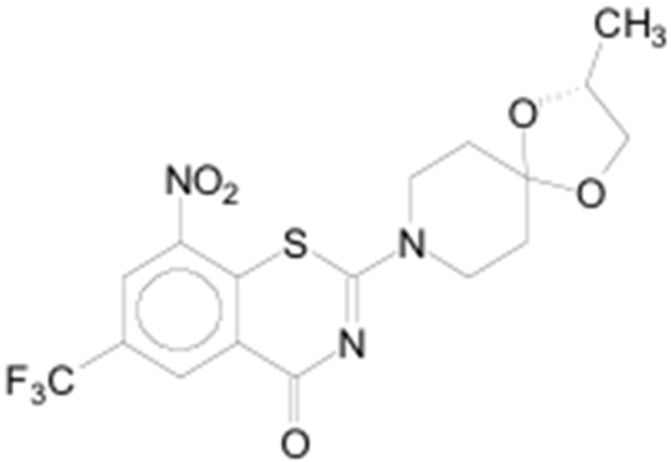
Conclusions to date

- Target-based approaches to drug discovery disappointing
- Extensive attrition
- Target-based hits don't show MIC
- Validation requires more than genetics, chemical validation better

If starting again would include more compound-based screens!

Benzothiazinones (BTZ) as antimycobacterial agents





10526043

2-[(2S)-2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl]-8-nitro-6-trifluoromethyl-4H-1,3-benzothiazin-4-one

PCT/EP2006/004942

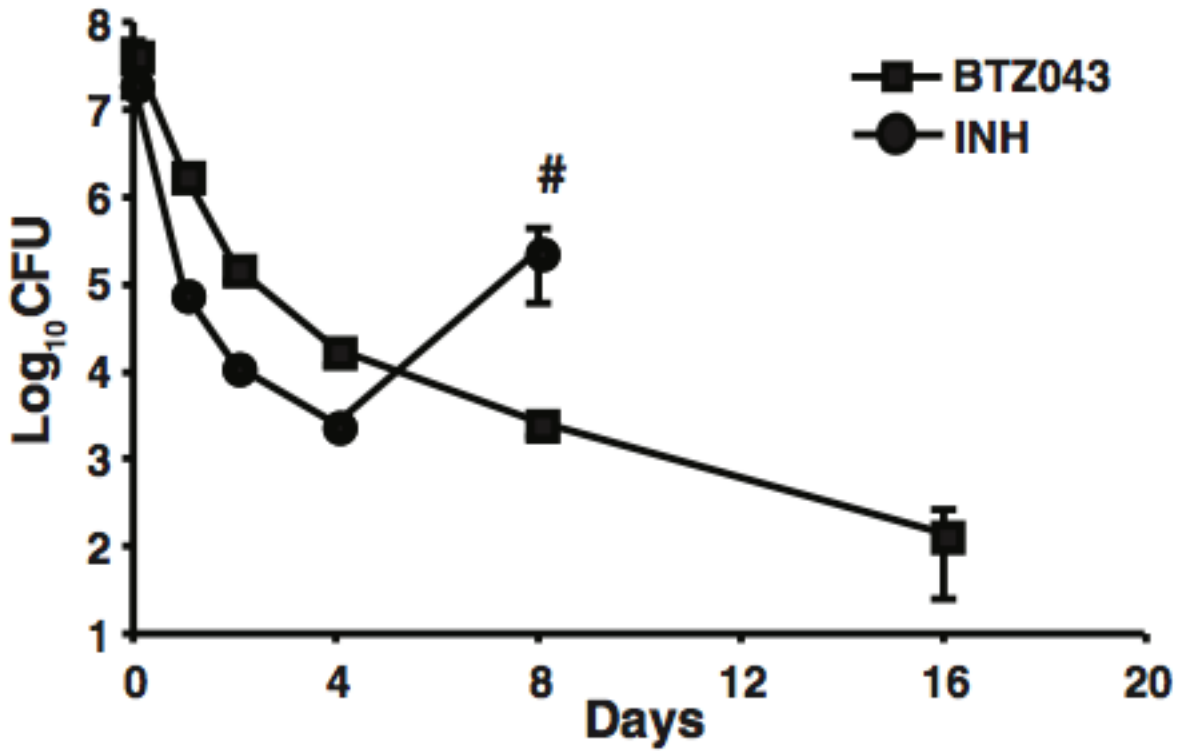
(Makarov, Möllmann, Cole)

Benzothiazinones (BTZ) as antimycobacterial agents

- Highly active against *M. tuberculosis* (MIC 1-10 ng/ml) and other actinobacteria
- Active against MDR- and XDR-TB
- Synthesis involves 7 steps, 36% yield, from commercially available reagents
- Extensive SAR undertaken
- Non-mutagenic/cytotoxic
- Good bioavailability

PCT/EP2006/004942

Comparative in vitro efficacy

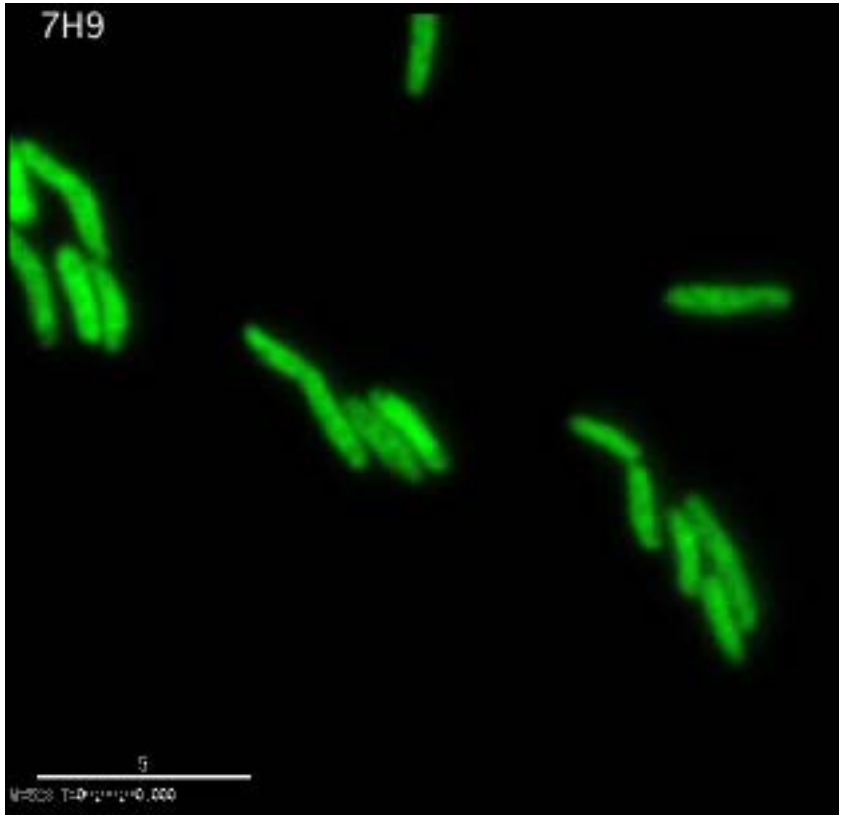


Makarov et al. 2009 Science 324:801

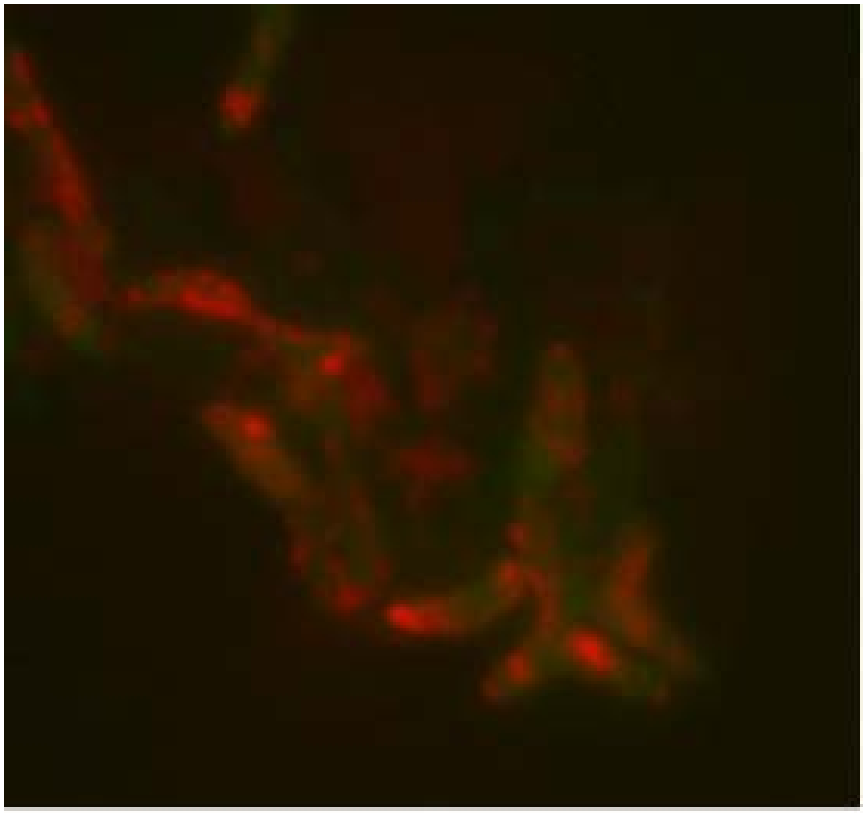
Death in real time!

7H9	10526043 0.2 μg/ml	7H9	Stain
98 h	240 h	265 h	

a

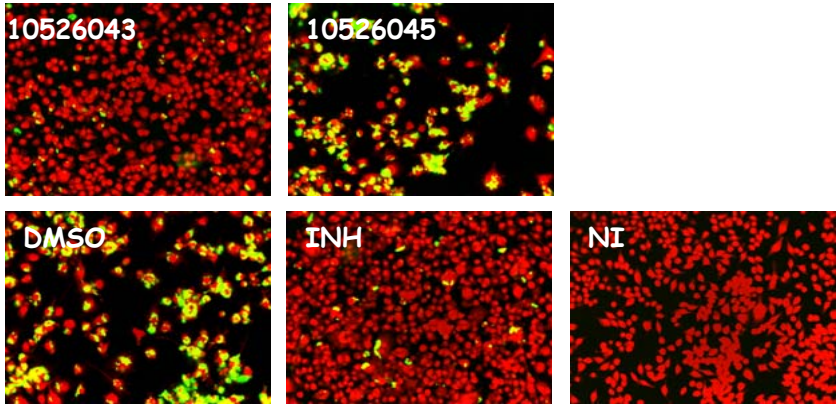


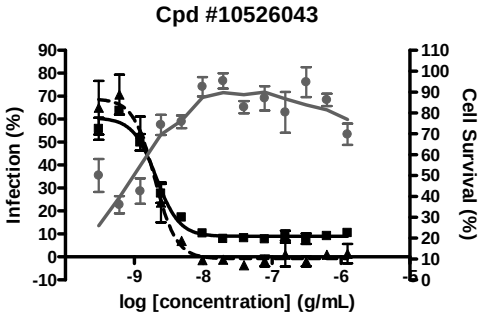
b

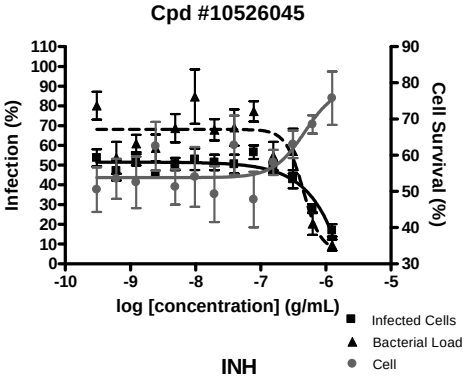


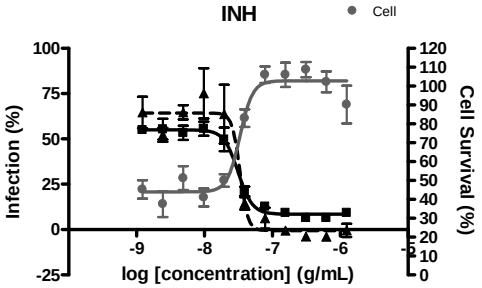
N. Dhar, J. McKinney. EPFL

Comparative ex vivo efficacy







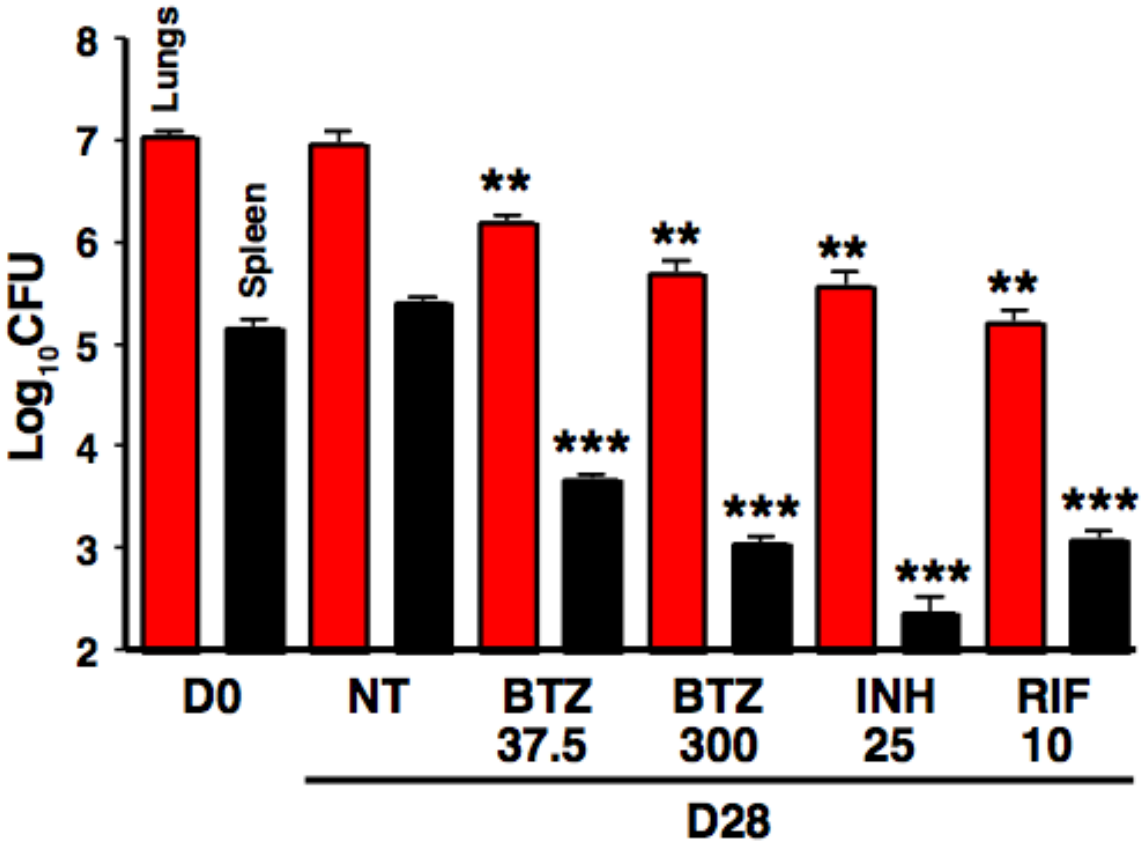


P. Brodin, T. Christophe; IP Korea

Efficacy in mouse model

Similar to INH & RIF in chronic infection model

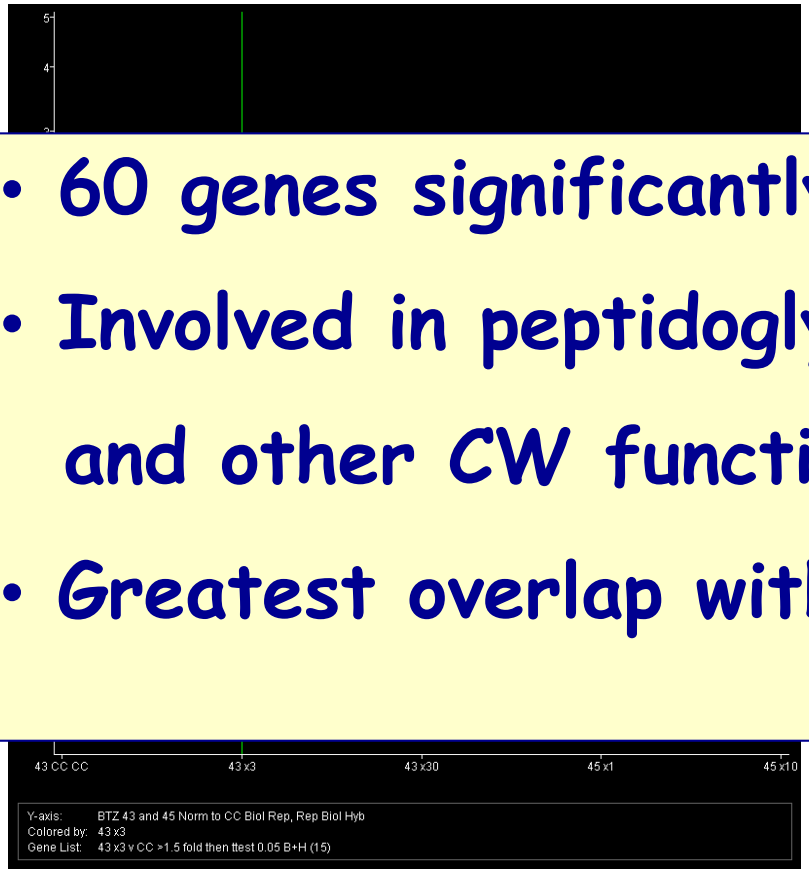
1 log kill in lungs



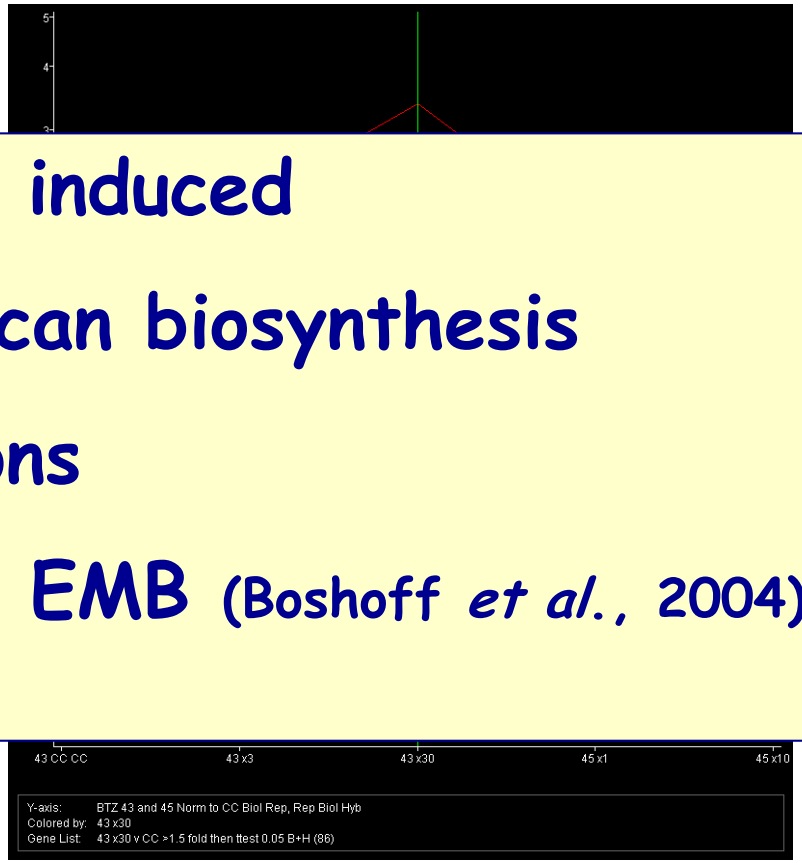
..but how does it act?

AZI, JHU, BI-RAS, EPFL

MOA from transcriptomics



3 ng/mL 4 h



30 ng/mL 4 h

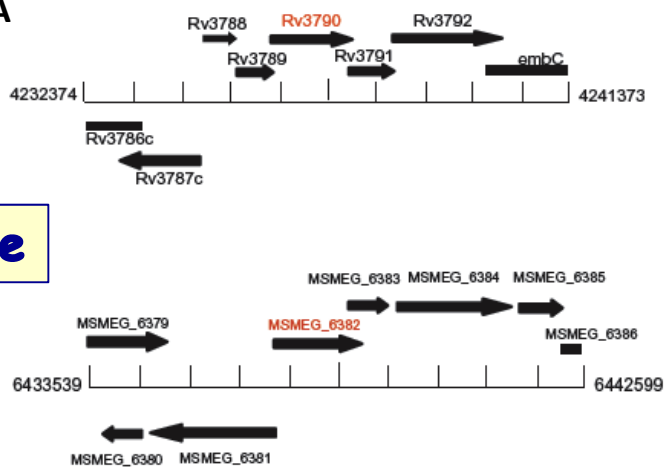
- 60 genes significantly induced
- Involved in peptidoglycan biosynthesis and other CW functions
- Greatest overlap with EMB (Boshoff *et al.*, 2004)

S. Waddell & P. Butcher. SGHMS

Target finding

Dpr epimerase

A



B

Strain	MIC	Codon	Amino acid
M. smegmatis mc^{2}155	4 ng/ml	tgc	Cysteine
M. smegmatis MN47	4 µg/ml	ggc	Glycine
M. smegmatis MN84	>16 µg/ml	tcc	Serine
M. bovis BCG	2 ng/ml	tgc	Cysteine
M. bovis BCG BN2	>16 µg/ml	tcc	Serine
M. tuberculosis H37Rv	0.75 ng/ml	tgc	Cysteine
M. tuberculosis NTB9	250 ng/ml	ggc	Glycine
M. tuberculosis NTB1	10 µg/ml	tcc	Serine

C

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                                   361                 381                 401                 420
                                    |                   |                   |                   |
M. tuberculosis (Rv3790)           SFLNVFKLFGPRNQAPLSFPIPGWNICVDFPIKDGLGKFVSELDRRVLEFGGRLYTAKDS
M. bovis                           SFLNVFKLFGPRNQAPLSFPIPGWNICVDFPIKDGLGKFVSELDRRVLEFGGRLYTAKDS
M. leprae                          SFLNVFKLFGPGNQAPLSFPIPGWNICVDFPIKSGLNEFVNKLDRRVMELGGRLYTAKDS
M. avium                           SALNVFKLFGPGNRAPLSFPMAGWNVAMDFPNKPGVNEFLNELDRRVLQFGGRVYTAKDS
M. avium paratuberculosis          SALNVFKLFGPGNRAPLSFPMAGWNVAMDFPNKPGVNEFLNELDRRVLQFGGRVYTAKDS
M. smegmatis                       SFLNVFKLFGPGNQAPLSFPIPGWNVCVDFPIKAGLHEFVTELDRRVLEFGGRLYTAKDS
M.aurum                            SFLNVFKLFGPRNQAPLSFPIPGWNISVDFPIKDGLGKFVSELDRRVLEFGGRLYTAKDS
M.gilvum                           SFLNVFKLFGPGNQAPLSFPIPGWNVCVDFPITAGLNEFLNGLDKRVLQFGGRLYTAKDS
M.vanbalenii                       SFLNVFKLFGPGNDAPLSFPIPGWNVCVDFQINPGLNEFLNGLDKRVLEFGGRLYTAKDS
M. marinum                         SFLNVFKLFGAGNQAPLSFPIPGWNICVDFPIKAGLNEFVSELDRRVMEFGGRLYTAKDS
Rhodococcus spp.                   SFLNVFKLFGEGNQAPLSFPIPGWNICVDFRIKPGLNEFVTELDKRVLKFGGRLYTAKDS
Nocardia farcinica                 SFLNVFKYFGQGNQAPLSFPMPGWNVCLDFPIKPGLNEFVTELDSRVLEFGGRLYTGKDS
Corynebacterium glutamicum         SALNVFKLFGPGNRAPLSYPMPGWNVCVDFPIRPGLGAFLDDLDKRVMEFGGRLYLAKES
                                   * ***** **  * ****:*:.***:.:*:    *:  *:  ** **:::***:* .*:*
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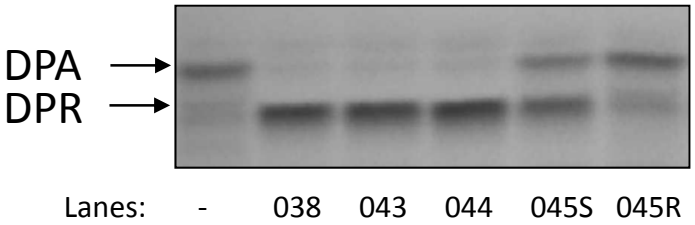
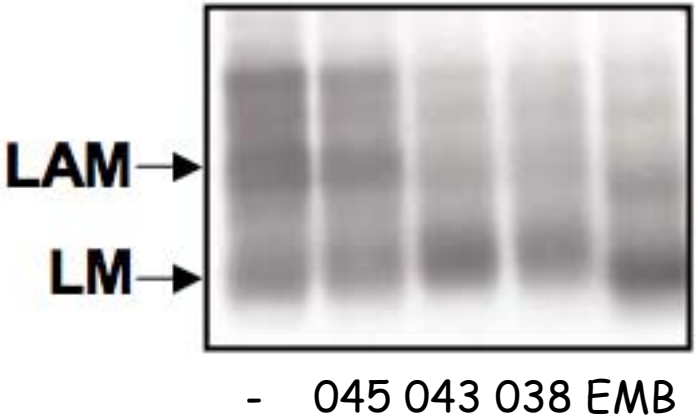
G. Manina & G. Riccardi. U Pavia

Biochemical confirmation

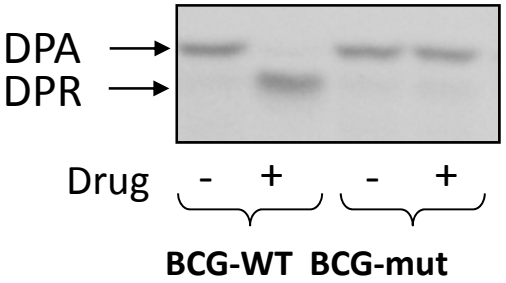
A.



B.

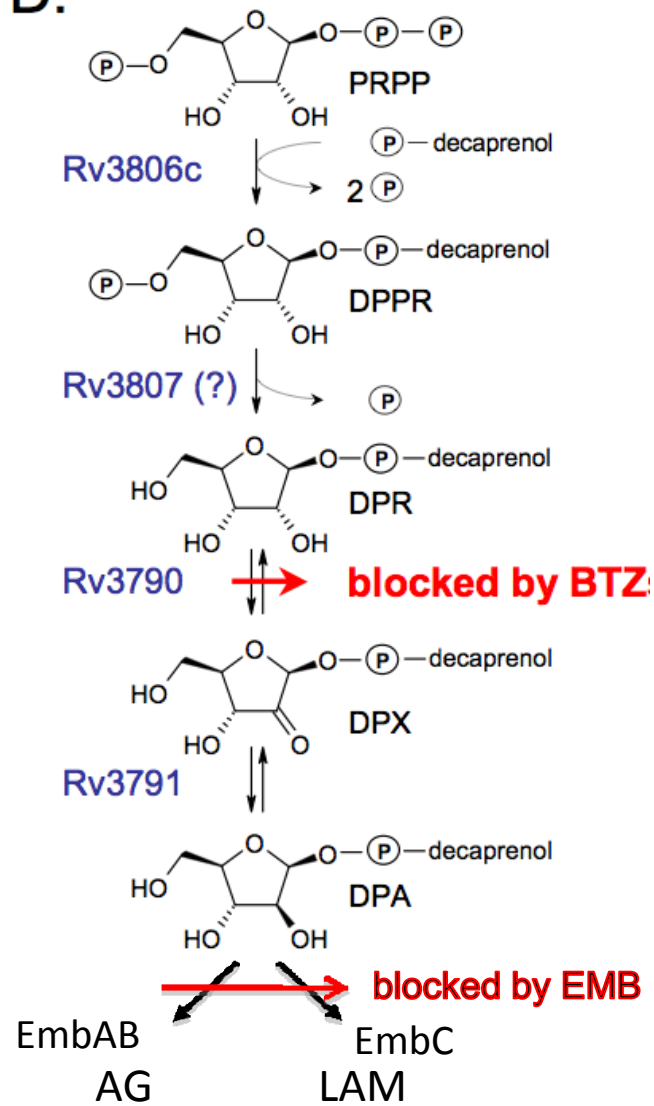


C.



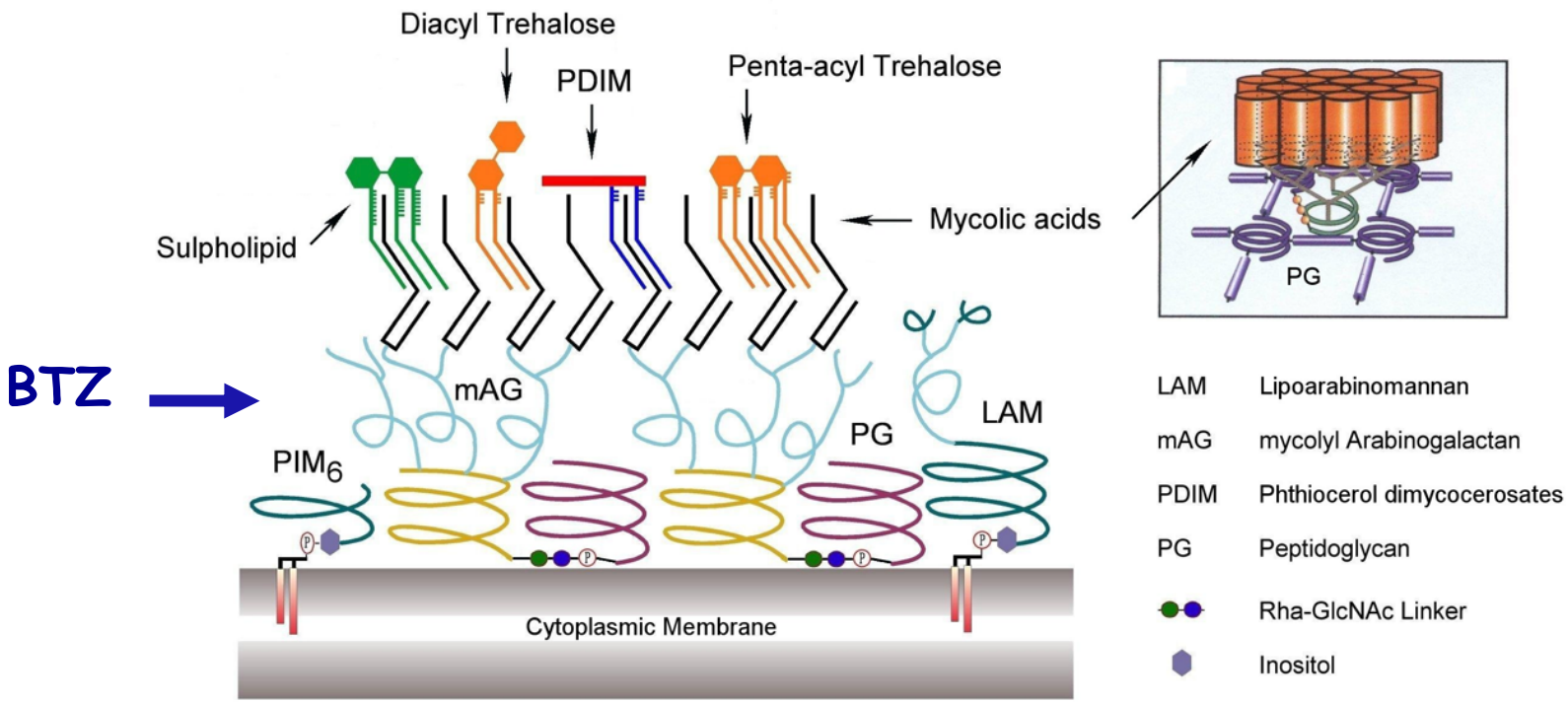
BTZ 1,000-fold more potent than EMB

D.



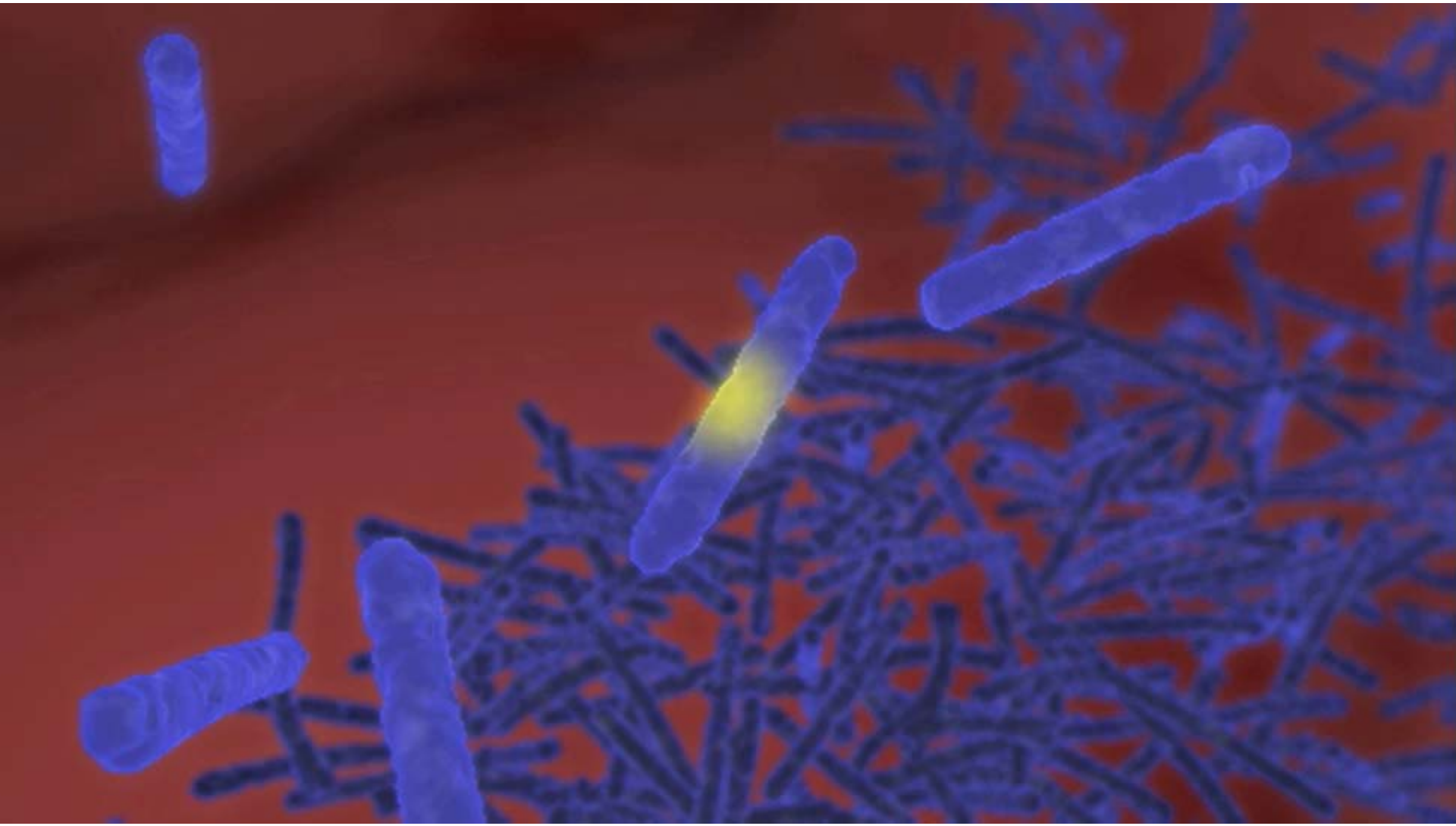
K. Mikusova et al. Comenius U

How BTZ043 works



Kremer et al. (2006) in Tuberculosis and the Tubercle Bacillus. ASM Press

How BTZ043 works



Candidate drug status

- *In vitro* ADME/T properties all favorable.
- Passed acute & chronic toxicity in mice.
- Pharmacological & cardiology profile favorable in mice.
- Additional toxicology & efficacy studies underway in other models.
- Then IND approval.....

Makarov, Bakh Institute; Cole, EPFL

With many thanks to……

NM4TB
Sixth Framework Programme

Neeraj Dhar
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AstraZeneca

EPFL
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE