New Medicines for Tuberculosis

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Global Health Institute
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
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

Figure 1. Visible growth of M. tuberculosis per ml sputum during the first month of treatment with regimens containing INH. Figure shows the number of bacteria per ml of sputum after treatment with various antibiotics. The results are plotted on a logarithmic scale to show the reduction in bacterial load over time. The graphs display the persistence of bacteria in different conditions: in vitro, in humans, and in animals. The treatment regimens include isoniazid (INH) and pyrazinamide, both alone and in combination.

In vitro
- Untreated
- Isoniazid
- Pyrazinamide
- Both drugs

Humans
Animals

Persistence
Current TB drugs

TB drugs are often prodrugs
What’s needed?

- More potent compounds
- Reduce duration of therapy <3m
- Kill persisters
- New MoA to overcome resistance
# Global TB drug pipeline

<table>
<thead>
<tr>
<th>Discovery</th>
<th>Preclinical</th>
<th>Clinical Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial Topoisomerase Inhibitors</strong>&lt;br&gt;GlaxoSmithKline/TB Alliance</td>
<td>TBC Oxazolidinones&lt;br&gt;Pfizer Inc.</td>
<td>Dihydroquinine TMC207&lt;br&gt;Johnson &amp; Johnson</td>
</tr>
<tr>
<td><strong>Cell Wall Inhibitors</strong>&lt;br&gt;Colorado State University, NIAID</td>
<td>Nitroimidazole Analogs&lt;br&gt;TB Alliance, University of Auckland</td>
<td>Catifloxacin&lt;br&gt;GLUTUB Consortium, Lupin NIAID TDR Tuberculosis Research Centre, WHO TDR</td>
</tr>
<tr>
<td><strong>Dihydrolipoamide Acetyltransferase Inhibitors</strong>&lt;br&gt;Cornell University, NIAID</td>
<td>Pretoxase Inhibitors&lt;br&gt;Cornell University, NIAID</td>
<td>Nitrimidazole oxazoles Back up&lt;br&gt;Osaka</td>
</tr>
<tr>
<td><strong>fhaA Inhibitors</strong>&lt;br&gt;GlaxoSmithKline TB Alliance</td>
<td>Pretux inhibitors&lt;br&gt;Methvl</td>
<td>Catifloxacin&lt;br&gt;DMB/NEAID/NIH, Case Western Univ.</td>
</tr>
<tr>
<td><strong>Diphrenyl ether based inhibitors of fhaA</strong>&lt;br&gt;State Brook, NIH</td>
<td>Multi-functional molecules&lt;br&gt;Cambrex, TB Alliance</td>
<td>Moxfloxacin&lt;br&gt;Bayer Pharma, CDC TBTC, Johns Hopkins University, NIAID TBDR, TB Alliance</td>
</tr>
<tr>
<td><strong>Malate Synthase Inhibitors</strong>&lt;br&gt;GlaxoSmithKline, Rockefeller University, Texas A&amp;M</td>
<td>Pseudosynilis&lt;br&gt;GlaxoSmithKline, TB Alliance</td>
<td>Non-Fluorinated Quinolones&lt;br&gt;Fujifilm</td>
</tr>
<tr>
<td><strong>Pramoxine Analogs</strong>&lt;br&gt;Salisbury University</td>
<td>Quinolones&lt;br&gt;KRRC Tohoku University, TB Alliance</td>
<td>Nitrimidazole PA-864&lt;br&gt;Chiron Corporation, TB Alliance</td>
</tr>
<tr>
<td><strong>Timipisophenezine</strong>&lt;br&gt;Institute of Malaria Medica, BTTRI</td>
<td>Theilactoinecyclic analogs&lt;br&gt;NIAID, NIH</td>
<td>Nitrimidazole OPC-67083&lt;br&gt;Osaka</td>
</tr>
<tr>
<td><strong>Natural Products Exploration</strong>&lt;br&gt;IITB, Caligbria State University, FR NIAID, TTA, University of Auckland</td>
<td>Screening and Target Identification&lt;br&gt;AstraZeneca</td>
<td>Pyrrole-LL-3858&lt;br&gt;LupinLimited</td>
</tr>
<tr>
<td><strong>Natural Products Exploration</strong>&lt;br&gt;NCR Center, Univ of Strathclyde, Univ of Illinois</td>
<td>Focused Screening&lt;br&gt;GlaxoSmithKline, TB Alliance</td>
<td>Diamine SQ-169&lt;br&gt;Sequella Inc</td>
</tr>
<tr>
<td><strong>Novartis Portfolio</strong>&lt;br&gt;Novartis</td>
<td>Sanofi-Aventis Portfolio&lt;br&gt;Sanofi-Aventis</td>
<td>Metrimethene for Later Infection&lt;br&gt;Imperial College London, Wellcome Trust Gates Foundation</td>
</tr>
<tr>
<td><strong>Discovery for Latent Infection</strong>&lt;br&gt;Imperial College London, Wellcome Trust Gates Foundation</td>
<td>Screening of compounds inhibiting growth of M.tb&lt;br&gt;NH, NIAID, TACF</td>
<td>Linezolid&lt;br&gt;DMB/NEAID/NIH, Case Western Univ.</td>
</tr>
<tr>
<td><strong>New Medicines for TB Portfolio</strong>&lt;br&gt;AstraZeneca, European Commission</td>
<td>Identification of compounds with in vivo activity against M.tb in animal models&lt;br&gt;NH, NIAID</td>
<td>Levofloxacin&lt;br&gt;DMB/NEAID/NIH, Case Western Univ.</td>
</tr>
<tr>
<td></td>
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<td>SV97 Immune Modulator&lt;br&gt;SolGene Pharmaceuticals</td>
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**Casenghi et al. (2007)**<br>PlosMed 4 e293
New TB drugs

- **FQ**: moxifloxacin, gatifloxicin
- **Nitroimidazole derivatives**: PA-824, Otsuka compound OPC67683
- **Oxazolidinones**: PNU-100480, AZD5847
- **JJ cpd**: R207910, TMC207
Moxifloxacin reduces treatment duration

<table>
<thead>
<tr>
<th>Treatment in mice</th>
<th>Duration of Treatment (months)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>2 m RIF, INH, PZA/ 4 m RIF INH</td>
<td>11/12</td>
</tr>
<tr>
<td>1 m RIF, MOXI, PZA/ 4 m RIF MOXI</td>
<td>4/12</td>
</tr>
<tr>
<td>2 m RIF, MOXI, PZA/ 3 m RIF MOXI</td>
<td>2/12</td>
</tr>
<tr>
<td>5 m RIF, MOXI, PZA</td>
<td>4/12</td>
</tr>
</tbody>
</table>

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

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 Diarylquinoline TMC207 for Multidrug-Resistant Tuberculosis

## TB Alliance Portfolio

### Discovery

<table>
<thead>
<tr>
<th>TARGET OR CELL-BASED SCREENING</th>
<th>LEAD IDENTIFICATION</th>
<th>LEAD OPTIMIZATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Products IMCAS</td>
<td>Whole-Cell Hit to Lead Program GSK</td>
<td>Mycobacterial Gyrase Inhibitors GSK</td>
</tr>
<tr>
<td>Protease Inhibitors IDRI</td>
<td>Malate Synthase Inhibitors GSK/TAMU</td>
<td>InhA Inhibitors GSK</td>
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<tr>
<td>TB Drug Discovery Portfolio NITD</td>
<td>Diaryquinolines TIBotec/U. of Auckland</td>
<td>Preclinical TB Regimen Development JHU/U. III Chicago</td>
</tr>
<tr>
<td>Topoisomerase I Inhibitors AZ/NYMC</td>
<td>Gyrase B Inhibitors AZ</td>
<td>Riminophenazines IMM/TTTRI</td>
</tr>
<tr>
<td></td>
<td>Folate Biosynthesis Inhibitors AZ</td>
<td>Pyrazinamide Analogs Yonsei</td>
</tr>
<tr>
<td>Whole-Cell Hit to Lead Program AZ</td>
<td>RNA Polymerase Inhibitors AZ/Rugers</td>
<td>Energy Metabolism Inhibitors AZU. Penn</td>
</tr>
<tr>
<td>Phenotypic Hit to Lead Program U. III Chicago</td>
<td>Menaquinone Biosynthesis Inhibitors CSU</td>
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### Preclinical Development

- Nitroimidazoles U. of Auckland/ U. III Chicago
- Preclinical TB Regimen Development JHU/U. III Chicago

### Clinical Development

<table>
<thead>
<tr>
<th>CLINICAL PHASE I</th>
<th>CLINICAL PHASE II</th>
<th>CLINICAL PHASE III</th>
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</thead>
<tbody>
<tr>
<td>PA-824 Novartis</td>
<td>Moxifloxacin (+ H, R, Z)</td>
<td>Bayer</td>
</tr>
<tr>
<td>TMC207 TIBotec</td>
<td>Moxifloxacin (+ R, Z, E)</td>
<td>Bayer</td>
</tr>
<tr>
<td>PA-8241/Pyrizaminide</td>
<td></td>
<td></td>
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<tr>
<td>TMC207/Pyrizaminide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA-8241/Moxifloxacin/Pyrazinamide</td>
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<td></td>
</tr>
</tbody>
</table>

### OUR R&D PARTNERS

- AstraZeneca (AZ)
- Bayer Healthcare AG (Bayer)
- Beijing Tuberculosis and Thoracic Tumor Research Institute (BITTTRI)
- 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. III 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**

**Acronym: NM4TB**

**Project number:** 016923  
**Requested EC contribution:** 10.87 M Euros  
**Duration:** 60 months  
**Starting date:** January, 2006

**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 drugable 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.

http://WWW/NM4TB.org
NM4TB - Approaches

Lead generation

Structure driven

Target based-IC50 driven

Compound based approaches-MIC driven

Enabling technologies

WP1

WP2 15%

WP3

WP4

WP2 85%
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\textsubscript{50} driven
Case of PknB

- STPK
- Physiological role: peptidoglycan metabolism?
- Essential function
- Assay available
- 3D structure known
- Millions of kinase inhibitors “available”
Signal transduction & drug design

Targeting kinase activity

Targeting receptor domain

Glycogen metabolism
TCA cycle

Peptidoglycan ?

PASTA domains

PstP

GarA

PknB

PknA
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

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

(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!

<table>
<thead>
<tr>
<th>7H9</th>
<th>10526043</th>
<th>0.2 µg/ml</th>
<th>7H9</th>
<th>Stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>98 h</td>
<td>240 h</td>
<td></td>
<td>265 h</td>
<td></td>
</tr>
</tbody>
</table>

**N. Dhar, J. McKinney. EPFL**
Comparative ex vivo efficacy

Cpd #10526043

Cpd #10526045

P. Brodin, T. Christophe; IP Korea
Efficacy in mouse model

Similar to INH & RIF in chronic infection model

1 log kill in lungs

AZI, JHU, BI-RAS, EPFL

..but how does it act?
MOA from transcriptomics

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

A

Dpr epimerase

B

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC</th>
<th>Codon</th>
<th>Amino acid</th>
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</thead>
<tbody>
<tr>
<td>M. smegmatis mc2155</td>
<td>4 ng/ml</td>
<td>tgc</td>
<td>Cysteine</td>
</tr>
<tr>
<td>M. smegmatis MN47</td>
<td>4 µg/ml</td>
<td>ggc</td>
<td>Glycine</td>
</tr>
<tr>
<td>M. smegmatis MN84</td>
<td>&gt;16 µg/ml</td>
<td>tcc</td>
<td>Serine</td>
</tr>
<tr>
<td>M. bovis BCG</td>
<td>2 ng/ml</td>
<td>tgc</td>
<td>Cysteine</td>
</tr>
<tr>
<td>M. bovis BCG BN2</td>
<td>&gt;16 µg/ml</td>
<td>tcc</td>
<td>Serine</td>
</tr>
<tr>
<td>M. tuberculosis H37Rv</td>
<td>0.75 ng/ml</td>
<td>tgc</td>
<td>Cysteine</td>
</tr>
<tr>
<td>M. tuberculosis NTB9</td>
<td>250 ng/ml</td>
<td>ggc</td>
<td>Glycine</td>
</tr>
<tr>
<td>M. tuberculosis NTB1</td>
<td>10 µg/ml</td>
<td>tcc</td>
<td>Serine</td>
</tr>
</tbody>
</table>

C

M. tuberculosis (Rv3790)
M. bovis
M. leprae
M. avium
M. avium paratuberculosis
M. smegmatis
M. aurum
M. gilvum
M. vanbaliensis
M. marinum
Rhodococcus spp.
Nocardia farcinica
Corynebacterium glutamicum

G. Manina & G. Riccardi. U Pavia
Biochemical confirmation

A.

DPA → DPR →

Lanes: - 038 043 044 045S 045R

B.

LAM → LM →

- 045 043 038 EMB

C.

DPA → DPR →

Drug - + - +
BCG-WT BCG-mut

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.....
With many thanks to......

Neeraj Dhar
John McKinney
Jacques Grosset
Priscille Brodin

NM4TB
Sixth Framework Programme

AstraZeneca

ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE