Clinical Tuberculosis, Drug Resistant and Latent Tuberculosis

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Historical Perspective

- Old disease: Egyptian mummies, spine TB (Pott’s disease)
- Phthisis (“wasting”, Hippocrates), Consumption, White Plague
- Association with art, literature, unique in human civilization
- Robert Koch discovered *M. tuberculosis*, March 24, 1882
- Calmette and Guerin developed BCG vaccine in 1921
- 20% all deaths in 18th and 19th century due to TB (5% now)
- Improved sanitation, nutrition lowered TB incidence before chemotherapy
- Chemotherapy in 1950s made TB curable -> further decrease
- TB declined in developed countries until 1970s
- In late 1980s incidence in developed countries increased, with emergence of MDR-TB, XDR-TB
TB: A Leading Infectious Killer
Top 10 cause of death

- High Burden of Cases and Mortality: 10 million new cases (12% HIV+) (640 million total) and 1.5 million deaths (0.25 mil HIV), 2018
- Latent TB Infection (LTBI): 1/4 world population infected with TB bacillus – 1.7 billion people
- Increasing MDR/XDR-TB: 3.4% of new TB cases and 18% of previously treated
  500,000 RIF-R TB (RR-TB, 80% MDR) eligible for MDR-TB treatment; 50,000 cases XDR-TB/yr
  India (27%), China (14%) and Russian (9%)
- HIV co-infection worsens TB: 40% AIDS die of TB
Inadequate TB Control

- Diagnostics: not sensitive (smear) or too slow (culture)
- DOTS TB therapy (6 months): too long
- LTBI not addressed
- Increasing MDR-TB
- BCG vaccine: partially protective
- Control strategy: not very effective
The New Tuberculosis

HIV and Drug-resistant TB – A lethal combination and a major threat to TB control

WHO declared TB a global emergency in 1993

TB emergency declared in Africa - August 25, 2005

CDC announced emergence of XDR-TB – Mar 24, 2006 (MMWR, March 24, 2006 / 55:301-5)

TDR-TB: Totally Drug Resistant TB, 2009
Extensively Drug-Resistant TB (XDR-TB)

“Virtually untreatable" form of TB

XDR-TB: MDR-TB (at least INH and RIF) plus resistance to two main second-line drugs: one aminoglycoside (amikacin, kanamycin) or capreomycin and a fluoroquinolone
Causative Agent of TB

- M. tuberculosis complex: M. tuberculosis, M. bovis, M. africanum, M. canetti, M. microti
- Mycobacterium tuberculosis: exclusive human pathogen
- M. bovis: cause TB in both animals (cattle, deer, bison, opossum, badger) and human (zoonosis)
- M. africanum: mainly human TB, a variant between M. tb and M. bovis
- M. microti: TB in rodents, nonpathogenic for humans
Characteristics of \textit{M. tuberculosis}

- Acid-fast (Gram positive) bacilli, rod-shaped bacilli, cell wall mycolic acids responsible for acid-fastness
- Slow growth: doubling time=20 hr, compare E. coli - 20 min, a tough organism to study
- Clumpy in liquid culture due to high lipid content of cell wall, an obligate aerobe
- Genome (H37Rv) contains 4,411,529 bp and 3924 orfs, with a high G+C content of 65.6%
Acid Fast Stain
Mycobacterial Cell Wall
Mycobacteria produce a thick mycolate-rich layer
functions as an efficient barrier
Steps in TB Pathogenesis

1. Inhalation of bacteria
2. Bacteria reach lungs; enter macrophages
3. Bacteria reproduce in macrophages
4. Lesion begins to form (caseous necrosis)
   - Activated macrophages
   - Bacteria cease to grow; lesion calcifies
5. Immune suppression
6. Reactivation
7. Dead phagocytes, necrosis
   - M. tuberculosis
   - Phagocytes, T cells, and B cells trying to kill bacteria
8. Lesion liquifies
9. Bacteria coughed up in sputum
10. Spread to blood, organs
11. Death
Patterns of Infection

- Primary tuberculosis: initial infection, usually in children; initial focus a small subpleural granuloma accompanied by granulomatous hilar lymph node infection - Ghon complex (Ghon focus, 1912). Granulomas resolve, no spread.

- Secondary tuberculosis: in adults as a reactivation of previous infection (or reinfection). Inflammation more severe and widespread. Upper lung lobes are most affected and cavitation can occur.
General Gross Description

- Tuberculous lesions may occur in almost any organ.
- Early lesions are characterized by small, white, soft nodules. Larger lesions, especially in the lung, are characterized by extensive necrosis and cavitation.
- Cavities contain millions of bacilli - intensely infectious.
- Late, healed lesions walled off by fibrosis, white and hard. Calcification can take place → white chalky appearance.
Tuberculous Caseous Lesions (lung)

Ghon Complex

Caseous Necrosis
TB Cavity Formation
Miliary TB of the Lung
TB Granuloma: Macrophages, epitheloid cells, Langhans giant cells, neutrophils, T and B cells
Langhans giant cell (granuloma)
macrophage-activating cytokines IFN-γ in Langhans cell formation
Factors Affecting Granuloma Formation

- Classically activated macrophages (CAMs) activated by Th1 cytokines (interferon-γ and TNF) to produce pro-inflammatory cytokines (TNF and IL-12) and chemokines that kill TB;
- Alternatively activated macrophages (AAMs) activated by Th2 IL4 and IL13, produce anti-inflammatory cytokines (IL-10 and TGF-β);
- Anti-TNF antibody, T cells, HIV, etc affect granuloma formation
Virulence Factors of *M. tuberculosis*

No overt toxins, Multi-factorial

- Lipids, cord factor (Trehalose 6,6'-Dimycolate); phthiocerol dimycocerosate (PDIM)
- PhoP: a transcription factor that controls synthesis of PDIM and sulfalipids and other cell wall lipids
- KatG (catalase-peroxidase)
- Esx-1:EsxAB (ESAT-6 and CFP10) and ESX-1 secretion-associated proteins (Esps), perturb host cell activities, permeabilize phagosomal membrane, allow bacillus to escape into cytoplasm, apoptosis, inhibit IFN-γ
- Signature Tagged Mutagenesis and TraSH screens identified many virulence factors
Protective Immunity to Mycobacterial Infection

- Antibodies not protective
- CD8+ CTL important
- CD4+ Th1 cytokines, IL-2, INF-γ, TNF-α activate macrophages, kill intracellular bacilli, IL-12 also important
- IFN-γ and IL-12 receptor deletions cause increased susceptibility to mycobacterial infections
- TNF-α: Infliximab (Remicade) antibody against TNF-α in treatment of rheumatoid arthritis and Crohn’s disease, cause relapse of latent TB
Innate Immunity to TB Infection

M. tuberculosis

MIR
DC-SIGN
CR3

TLR-2/4/9
MyD88
NOD2

NF-κB
1,25D3/VDR

25D3
1, 25D3

Autophagy
Lysosome

Macrophage/DC

Cathelicidin
Other antimicrobial peptides

PMN

IFN-γ

T cell

γδ T cell

MHC/CD1

IL-1β; IL-18/12/23;
TNF-α; IL-8, Chemokines;
NOS-2/NO

Pathology?
Adaptive Immunity to TB Infection

- **γδ T**
- **CD4**
- **CD8**
- **Treg**
- **Th1**
- **Th2**
- **Th17**

**MHC-Ⅰ Peptide**

- **TGF-β**
- **IL-6**
- **IL-23**
- **IL-12**
- **IL-18**
- **IL-4**
- **IL-5**
- **IL-13**

**MHC-Ⅱ**

- **IFN-γ**
- **IL-2**
- **GM-CSF**
- **IL-4**
- **IL-5**
- **IL-10**
- **IL-13**
- **IL-17**
- **IL-21**
- **IL-22**
- **IL-25**

**Lipid**

- **Phospholipid**
- **CD-1**

**T**

- **Granzymes**
- **Granulysin**
- **Perforin**

**Adaptive Immunity to TB Infection**
Symptoms of TB

- Fever, cough (blood), night sweat, loss of appetite, weight loss
- Local organ involvement (TB can affect any part of body except hair): e.g., hematogenous spread to kidneys can cause kidney symptoms, to meninges causes tuberculous meningitis
Diagnosis

- Symptoms: fever, cough (hemoptysis), sweat, loss of appetite, weight loss
- Chest X-ray: infiltration, cavity, etc
- Microscopy: acid fast staining (Ziehl-Neelsen Stain), sputum smear-positive patients, fast and economic but poor sensitivity (5000-10,000 bacilli)
- **Culture (2-6 weeks):** definitive diagnosis, smear negative (10-100 bacilli), MGIT960, MB/BacT, Lowenstein-Jensen medium
- Molecular tests (1-2 days): PCR-based (16S rRNA): Roche, BD, GenProbe, loop-mediated isothermal amplification test for TB (TB-LAMP), Hain test and Gene Xpert for MDR-TB detection; WGS; fast, more expensive, have not replaced the culture method
- Skin test (PPD): exposure to TB (not necessarily active disease), useful in low incidence area
- QuantiFERON-TB Test: whole blood INF-\(\gamma\) release assay by PPD or TB specific antigens ESAT-6 and CFP-10; ELISPOT-T SPOT
- Interest in POC: point of care test, rapid, reliable
TB Chemotherapy: THE Effective TB Control

- **Pre-antibiotic era:** before 1940s (cod liver oils, bed rest, fresh air)
- **Drugs used to treat TB:** Streptomycin first TB drug (1943), followed by PAS (1946), isoniazid (1952), pyrazinamide (1952), rifampin (1963)
  - (a) Front-line Drugs: *isoniazid* (INH) rifampin (RIF), *pyrazinamide* (PZA), *ethambutol*
  - (b) Second-line Drugs: kanamycin, amikacin, capreomycin, ofloxacin/levofloxacin, *PAS, cycloserine, ethionamide*
- (TB specific drugs are italicized and underlined, and the rest are broad spectrum antibiotics)
History of TB Drugs/Chemotherapy

- Streptomycin (S), 1943
- PAS (P), 1946
- Isoniazid (H), 1952 *(SPH cure TB in 18-24 months)*
- Pyrazinamide (Z), 1952
- Ethambutol (E), 1961
- Rifampin (R), 1966 *(RHSE cure TB in 9 months)*
- **RHZE cure TB in 6 months, 1980s-1990s (WHO, 1995)**
- Pretomanid (PA-824, Pa), 2000 *(PaMZ cure TB in 4 month?)*
- Bedaquiline (TMC207), 2005
- Delamanid (OPC-67683), 2006
DOTS (Directly Observed Treatment, Short-Course) - Best TB Therapy since 1995

- DOTS: 6 month therapy - The best TB therapy (78%-96% cure rate)
- Initial phase (daily, 2 months) with 4 drugs: Isoniazid, Rifampin, Pyrazinamide, Ethambutol
- Continuation phase (4 months) with 2 drugs: Isoniazid, Rifampin
Drugs Used for Treatment of TB

- **First-line drugs:** 6 month (INH, RIF, **PZA**, EMB) cure TB 85-95%
  - Lengthy therapy (persistence) → poor patient compliance → MDR-TB, or direct transmission
- **MDR-TB:** **PZA**+**EMB** + **second-line TB drugs** (injectables kanamycin/amikacin/capreomycin; fluoroquinolones levofloxacin/moxifloxacin/ofloxacin; PAS, cycloserine, ethionamide/protonamide): 18-24 months, more toxic/side effects, more expensive, poor cure rates 50-60% → Shorter, all-oral, bedaquiline-containing regimen for eligible MDR/RR-TB patients
- **WHO:** 9-12 months (Bangladesh regimen) Clofazimine+other drugs cure rate 84.5% (new, uncomplicated MDR)
- **XDR-TB:** 6-9 months with bedaquiline, pretomanid and linezolid
<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Target/Mechanism</th>
<th>Activity for Persisters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid (INH)</td>
<td>Mycolic acid synthesis</td>
<td>-</td>
</tr>
<tr>
<td>Ethambtol (EMB)</td>
<td>Arabinogalactan synthesis</td>
<td>-</td>
</tr>
<tr>
<td>Pyrazinamide (PZA)</td>
<td>Energy production/Trans-translation</td>
<td>+++</td>
</tr>
<tr>
<td>Rifampin/Rifapentine</td>
<td>RNA polymerase/Transcription</td>
<td>++</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Protein synthesis</td>
<td>+</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>DNA synthesis</td>
<td>+</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>Energy production?</td>
<td>++</td>
</tr>
<tr>
<td>Bedaquiline/TMC207</td>
<td>F1F0 ATPase/ATP synthesis</td>
<td>++</td>
</tr>
<tr>
<td>Nitroimidazoles</td>
<td>Reactive nitrogen/DNA damage</td>
<td>+</td>
</tr>
<tr>
<td>(PA-824, OPC67683)</td>
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</table>
Treatment principle: Drug combination for other infections, e.g. ART therapy; H. pylori; cancer

Why drug combination?
- prevent drug resistance: Spontaneous mutations (e.g., R to INH occur at 1 in a million bacilli, R to RMP at 1 in 100 million)
- enhance efficacy of therapy (Mitchison hypothesis)
Special Bacterial Populations Theory (Mitchison Hypothesis)

A. Continuous growth

INH (RIF, SM, EMB)

B. Spurts of metabolism

RIF

C. Acid inhibition

D. Dormant

Semi-dormant

INH (RIF, SM, EMB)
Yin and Yang of Bacterial Life Cycle: Effect of Drugs (Yin-Yang Model)

Drug Resistance: Two Types

- Genetic drug resistance: chromosomal mutations or plasmids/transposon – **Yang Resistance**

- Phenotypic drug resistance: changes in bacterial/cancer physiology, stationary phase, persisters, dormant state – **Yin Resistance**

- Overlap/interconversion of Yang and Yin resistances
Rifampin-dependent/enhanced MDR-TB
(Zhong et al., 2010. Int J Tuberc Lung Dis. 14: 40-44)

35 year-old male treated with WHO recommended thrice wk Regimen 2(HRZE)\textsuperscript{3}, followed by 4(HR)\textsuperscript{3} for 2 weeks, when the symptoms got worse with hemoptysis
- Smear + AFB, cavity in right upper lobe
- INH, RIF, EMB, Levo, Ami, PAS for 2 months, symptoms improved,
  Chest R partial resolution of lesion,
- But after 6 month Rx, patient not cured with hemoptysis again, AFB+, larger cavity
- DST revealed resistance to INH, RIF, SM, and amikacin, PAS, but sensitive to EMB,
  ethionamide, cycloserine, levofloxacin, and RIF-dependency
- Adjust Rx to: add ETH, replace RIF with more powerful Rifapentine, Rx for 3 wk
- Symptoms got even worse: more frequent cough, hemoptysis, chest pain, AFB+
- Rx: (INH+EMB+levofloxacin+ethionamide) without RFP or RIF -> Cure after 12 months!
Drug-Resistant TB

- Drug resistance in M. tuberculosis is NOT mediated by plasmids or transposons, but due to mutations in chromosomal genes.

- MDR-TB (resistant to at least INH, RIF) is caused by sequential accumulation of mutations in different genes (strain W in New York City: resistant to 7 drugs) → XDR-TB (MDR+resistance to quinolone and injectables) → TDR (totally drug resistant)
## Mechanisms of Drug Resistance in *M. tuberculosis*

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Physiologic effect/inhibition</th>
<th>Molecular target</th>
<th>Genes associated with resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH</td>
<td>Cell wall mycolic acid synthesis; oxygen radical-associated damage;</td>
<td>enoyl acyl carrier protein reductase (InhA)</td>
<td>catalase-peroxidase ((katG); inhA)</td>
</tr>
<tr>
<td>RIF</td>
<td>RNA synthesis</td>
<td>RNA polymerase</td>
<td>(rpoB)</td>
</tr>
<tr>
<td>PZA</td>
<td>Membrane function trans-translation Energy production</td>
<td>membrane energy/ trans-translation (RpsA) PanD</td>
<td>pyrazinamidase/ nicotinamidase ((pncA), rpsA) (panD)</td>
</tr>
<tr>
<td>EMB</td>
<td>Cell wall component arabinogalactan synthesis</td>
<td>arabinosyl transferase</td>
<td>(embB)</td>
</tr>
<tr>
<td>SM</td>
<td>Protein synthesis</td>
<td>Ribosome S12 protein; 16S rRNA; methyltransferase</td>
<td>(rpsL; rrs; gidB)</td>
</tr>
<tr>
<td>Quinolone</td>
<td>DNA synthesis</td>
<td>DNA gyrase</td>
<td>(gyrA,B)</td>
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</table>
Virulence and Fitness of Drug-resistant TB

- INH-resistant strains attenuated for virulence in guinea pigs
- KatG-negative INH-resistant strains with high level resistance may be attenuated or less transmissible in humans
- Resistance to other drugs is not associated with attenuation of virulence: e.g., PZA-mono-resistant strain is still fully virulent and cause active transmission
- Fitness of MDR/XDR-TB may not be affected and may still cause active transmission and disease in HIV+ and HIV- individuals with or without compromised immune system
The Problem-Unique

- Lengthy therapy (6 months) and poor compliance readily select drug-resistant TB bacteria
- Unlike detection of drug resistance in other bacteria, TB drug resistance detection is time-consuming due to slow growth of TB bacteria
- Interest in molecular detection of drug resistance:
Correlation between Mutations and Drug Resistance

- INH resistance: KatG315 (80-95%), *inhA* (10-30%), KatG315 and *inhA* –15 C-to-T (95%)
- RIF resistance: *rpoB* (95%), 81 bp, 531, 526, 516
- PZA resistance: *pncA* (85%), scattered
- EMB resistance: EmbB306 (50%)
- Fluoroquinolone resistance: *gyrA* (95%)
- SM resistance: RpsL43/88(60%), *rrs* (20%) amikacin, kanamycin, capreomycin: *rrs* 1400A->G
Molecular Detection of Drug Resistance Mutations

- PCR, followed by DNA sequencing, SSCP, molecular beacons, hybridization (Line-Probe assay) in microarray /macroarray, real-time PCR; High-Resolution Melting Curve analysis
- Line-Probe assays (Hain Lifescience GenoType MTBDRplus) being evaluated in the field with promising results
- **Xpert MTB/RIF TB test- Cepheid** (New England J Med, 2010) 1,730 patients with suspected drug-sensitive TB or MDR TB, identified 98% of all confirmed TB cases and 98% patients with RIF-resistant bacteria in < 2 hours ($17,000 Instrument; $17/test)
  - **Xpert Ultra, Omni** (POC)
Treatment of MDR/XDR TB: “Bee Hive”

- Only 20% MDR-TB receives treatment
- MDR-TB therapy is longer (20 months), more side effects, poor cure rates (50-60%), more costly ($10K-90K vs $100-$500 drug susceptible TB)
- Regimens: Quinolone (A)+injectables (B)+Cycloserine or PTO or CFZ or LNZ (C) + PZA or EMB or H\textsuperscript{h} (D1) or D2 (BDQ or DLM) or D3 (PAS, beta-lactams, Thz)(standardized, program); 20 months; 50-60% cure
- Bangladesh regimen (9-12 months): 85% cure ($1000)
  Four drugs (CFZ, Moxi, PZA, EMB) for 9–12 months,
  followed by three drugs (Kan, high-INH and PTO) in the initial 4–6 mos
- Based on DST/molecular DST results, Individualized (Precision Medicine) vs Program
Group A drugs: Levofloxacin/moxifloxacin, bedaquiline, and linezolid.

Group B drugs: Clofazimine, cycloserine/terizidone

Group C drugs: Ethambutol, delamanid, pyrazinamide, imipenem-cilastatin, meropenem, amikacin, ethionamide/prothionamide, and p-aminosalicylic acid, can be included to complete the regimens when drugs from groups A and B cannot be used.

Importantly, kanamycin and capreomycin are no longer recommended because their use is associated with increased risk of treatment failure and relapse.

On August 17, 2018, WHO announced major changes in MDR-TB treatment regimens. 12,000 patient data from 50 studies in 26 countries supported evidence-based revisions of the priority ranking of anti-tuberculous drug
MDR-TB

Molecular DST (sequencing pncA, rrs, gyrA, etc.) of Z, SLID, and FQ

Z^s-MDR-TB
- Shortened regimens (9-12 months) containing Z + 2-3 bactericidal agents + other agents
  - 82-83% success

Z^r-MDR-TB
- Regimens without Z, longer treatment
  - 62% success

Zhang Y et al. 2012, 7.25. EMI
http://www.nature.com/emi/journal/v1/n7/full/emi201218a.html

Latent TB Infection: Risk factors

Collins HL. Lancet Infectious Diseases, 2001; 1: 21
New TB cases are driven by the reservoir of latently infected people.

This “hidden epidemic” of people infected with latent TB is enormous - a time bomb.

Control the reservoir of infection by chemoprophylaxis or post-exposure vaccine.

Active TB
- 9 million new cases a year
- Unfortunately just the tip of the iceberg

Latent TB
- “hidden epidemic”
- 2 billion people infected
Diagnosis of LTBI

Stimulate immune cells → Allow time for response by immune cells → Measure response

TST

QFT-GIT shake

T-SPOT.TB

LAB

Courtesy of Susan Dorman
LTBI Treatment: Targeted Prophylaxis
contact, HIV+, anti-TNF, transplant, dialysis, silicosis

- 6 or 9 month INH;
- 3 - 4 month RIF or RIF+INH;
- 3 month INH+Rifapentine (weekly)
- 1 month INH+Rifapentine (daily)
- Ultra short (<1 month) with new drugs (BDQ, DLM) or drug combo?

- 2 month RIF+PZA (not recommended)
LTBI Future Directions

- Better understand complexity of LTBI immunopathogenesis
- Evaluate known biomarkers in disease prediction
- Develop new immune-based diagnostics (Ag; Biomarkers) that predict high risk to active TB
- Develop new shorter/less side effect LTBI treatment
- Develop immune-based therapeutic vaccines
BCG Vaccine

- BCG (Bacillus Calmette-Guerin): single most widely used vaccine (76% newborn), derived from M. bovis through 231 subcultures over 13 years from 1908-1921
- Given to newborns, protect from hematogenous spread (against serious TB, i.e. miliary and tuberculous meningitis)
- Reduce risk of severe pediatric TB disease (70-80%)
- Unreliable protection for adult pulmonary TB (0-80%), poor efficacy in certain parts of world (e.g., India, no protection)
- Despite wide use, no apparent impact on growing global TB
- Current activities in developing new TB vaccines
New TB Vaccine Development

- Pre-exposure (prophylactic) Vaccine
  Strategy: BCG replacement vaccine or BCG prime + booster
  - Recombinant BCG, expressing Ag85B, listeriolysin (endosome escape)
  - Live attenuated vaccines (mutants of Mtb, RD-1, phoP)
  - Subunit vaccines (fusion proteins 72f, Ag85/ESAT6, Ag85/TB10.4, plus novel adjuvants)
  - Non-replicating viral vector vaccines (poxvirus and adenovirus) expressing Ag85A (MVA85A)
  - DNA vaccine (hsp60, Ag85)

- Post-exposure Vaccine (Latency)/ Therapeutic Vaccine
  - RUTI:
  - M. vaccae
14 vaccine candidates in clinical trials

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phase IIa</th>
<th>Phase IIb</th>
<th>Phase III</th>
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<tbody>
<tr>
<td>AEC/BC02 Anhui Zhifei Longcom</td>
<td>RUTI® Archivel Farma, SL</td>
<td>DAR-901 Dartmouth</td>
<td>Vaccae™ Anhui Zhifei Longcom</td>
</tr>
<tr>
<td>Ad5Ag85A McMaster University, Can Sino</td>
<td>MTBVC Biofabri, TBVI, Zaragosa</td>
<td>M72 + AS01E GSK Aeras</td>
<td>VPM1002 SII, Max Planck, VPM, TBVI</td>
</tr>
<tr>
<td>ChAdOx1.85A MVA85A University of Oxford</td>
<td>ID93+GLA-SE IDRI, Wellcome Trust</td>
<td>H56:IC31 SSI, Valneva, Aeras</td>
<td>Immuvac ICMR, Cadila Pharmaceuticals</td>
</tr>
<tr>
<td>TB/FLU-04L RIBSP</td>
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- **Protein / adjuvant**
- **Viral Vector**
- **Mycobacterial – whole cell or extract**
- **Mycobacterial – live**
Persister Problem in TB

- Underlying prolonged TB therapy for 6 months -> increasing MDR/XDR-TB
- Post-treatment relapse
- Underlying latent TB infection
- Interest to understand persister mechanisms and develop persister drugs (PZA story)
Bacterial Persisters: Phenotypic Resistance

- The phenomenon first described by Gladys Hobby in 1942
- Joseph Bigger coined “persister” in 1944
- Penicillin killed 99%, residual 1% called “persisters”
- Genius: 1% inspiration and 99% perspiration; Less is more
Mechanisms of Persistence in *M. tuberculosis*

- **Energy Production**
  - IcL, SucB, MenA, CydC, Tgs1, NadE, PanD
- **Efflux /Transporter**
  - Tap, Mce4
- **Stringent Response**
  - RelA
- **Protein Degradation**
  - proteasome PrcBA, Trans-translation
  - RpsA
- **DNA Protection/Repair**
  - Nfo, UvrD
- **Global Metabolism Regulators**
  - PhoY2, RaaS, CarD
- **Transcription factor**
  - WhiB7
- **Toxin-Antitoxins**
  - RelBE, HigBA, VapC etc.
- **Lipid biosynthesis**
  - PDIM, Fad26
- **Carbon, Amino acid Metabolism**
- **DNA Protection/Repair**
  - Nfo, UvrD
- **Efflux /Transporter**
  - Tap, Mce4
- **Protein Degradation**
  - proteasome PrcBA, Trans-translation
  - RpsA
- **Global Metabolism Regulators**
  - PhoY2, RaaS, CarD
- **Transcription factor**
  - WhiB7
- **Toxin-Antitoxins**
  - RelBE, HigBA, VapC etc.
Why Persister Cells Important?
Dandelion Phenomenon
Pyrazinamide (PZA): Unconventional, Paradoxical, Persister Drug

- PZA: 1st line drug critical in shortening therapy, but no activity against growing TB bacteria at pH7 but active at pH5.5-6.0 (MIC=50-100 ug/ml).
- Kills non-growing persisters, hypoxic/anaerobic
- PZA is opposite to common antibiotics
- Only *bona fide* persister drug among all antibiotics
- Prototype persister drug: proof of principle
- Recent interest: 3 workshops in 1 yr
Why Persister Drug Matters?  
**Pyrazinamide: A Remarkable Persister drug**  

PZA - important front-line TB drug, plays a key role in DOTS by shortening therapy, because PZA kills persister TB bacilli not killed by other TB drugs

From McCune R M, et al.  
How Does PZA Work?

- PZA is a prodrug activated by PncA to POA (Scorpio & Zhang 1996)
- Role of acid pH (Zhang et al., 1999)
- PZA kills old, dormant bacilli more effectively than actively growing bacilli (Zhang et al., 2002), persisters more effectively under hypoxic/anaerobic conds. (Wade and Zhang, 2004)
- POA disrupts membrane potential (Zhang et al., JAC, 2003)
- Inhibition of trans-translation (Shi et al., Science, 2011)
- Inhibition of PanD (pantothenate, CoA synthesis) (Zhang S, et al., 2013; Shi et al., 2014)
Common Mechanisms of Action of Antibiotics (in green)
In contrast to PZA (in red)

1. Inhibition of cell wall synthesis
   • Beta-lactams
   • Glycopeptides

2. Disruption of membrane permeability
   • Polymyxin B
   • Daptomycin

3. Inhibition of protein synthesis
   • Aminoglycoside
   • Tetracycline
   • Macrolides

4. Inhibition of nucleic acid synthesis
   • Quinolones
   • Rifampin

5. Anti-metabolite
   • Sulfa drugs

Inhibition of Energy Production
PZA, TMC207

Inhibition of trans-translation

Inhibition of Pantothenate/Coenzyme A synthesis
2019 Global New TB Drug Pipeline

Preclinical Development

Early Stage

- Caprazene nucleoside
  CPZEN-45*

- Spectinamide 1810*

- Pyrazolopyridine carboxamide TB-47*

- Sanfetrinem
  S-004992*

GMP/GLP Tox.

- TBAJ-587
- TBAJ-876
- GSK-286*
- SPR720*
- TBI-223
- BTZ-043*
- TBI-166
- Macozinone*
  (PBTZ-169)
- GSK-656* (070)
- TBA-7371*
- Contezolid

Clinical Development

Phase 1

- OPC-167832*
- Telacebec
  (Q203*)
- Delpazolid
  (LCB01-0371)
- Sutezolid
  (PNU100480)
- SQ-109*

Phase 2

- Bedaquiline*
- Delamanid*
- Pretomanid*

Phase 3

- Underline = new to Phase since October 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

www.newtbdrugs.org
Updated: March 2019
2019 Global TB Drug and Regimen Clinical Research

Ongoing Clinical Development Research: Strategy / Optimization / Regimen Development

**Phase 2**
- OPC-167832*
- **Bedaquiline-Delamanid** (ACTG 5343)
- **Bedaquiline-Pretomanid-Moxifloxacin-PZA** (SimpliciTb Trial, NC-008)
- **Bedaquiline-Pretomanid-Moxifloxacin**-PyrAzinamide (BPaMZ) (NC-005)
- **Levofloxacin** with OBR for MDR-TB (OPTI-Q)
- **Linezolid** Dose-Ranging
- **Beta-Lactams; Nitazoxanide**
- **High Dose Rifampicin** (PANACEA)
- **TB PRACTECAL** - regimens with **Bedaquiline-Pretomanid-Linezolid**

**Phase 3 Regimens**
- **Bedaquiline-STREAM MDR-TB**
  - Trial Stage 2 with oral OBR (9 mo) or OBR with injectables (6 mo)
- **Bedaquiline-Pretomanid-Linezolid** (NiX-TB)
- **Delamanid** with OBR for MDR-TB
- **High Dose Rifampicin** for DS-TB (RIFASHORT)
- **Rifapentine - Moxifloxacin** for DS-TB (CDC TBTC 31, ACTG 5349)
- **Pretomanid-Moxifloxacin-PyrAzinamide** (STAND)

**Optimization/Post Market**
- **Bedaquiline-Linezolid** with OBR for MDR-TB (NExT Trial)
- endTB 5-Regimen Trial for MDR TB
- PredictTB – PET/CT, biomarkers DS-TB, 4 mo
- TRUNCATE-TB Trial, 2 mo

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](http://www.newtbdrugs.org/pipeline/clinical)

2 OBR = Optimized Background Regimen

www.newtbdrugs.org

Updated: March 2019
New Regimens for MDR-TB Treatment

Bedaquiline added: 76%-80% culture conversion at 6 months; 70% at 18 month follow-up

Higher number of deaths in bedaquiline (12/102 subjects, 11.8%) (cardiac arrhythmia due to QT prolongation) compared with placebo (4/105 subjects, 3.8%)

FQ resistance, add linezolid while still use high-dose FQ

‘Precision Medicine’ for MDR/XDR-TB treatment: personalized versus standardized program based treatments
Targeting Persisters

- Develop persister drugs
  - Whole cell based: persister screens in vitro; Macrophages
  - Target based: Energy production (ATP) inhibitors:
- Novel drug combos:
- Host immune control (therapeutic vaccines): Host Directed Therapy
Systems Approach to TB Control

TB control measures

- Diagnostics
- BCG
- DOTS

Factors making TB worse

- Healthcare System
- Political, Socioeconomic
- Immune Control Nutrition
- Latent TB/Persisters
- HIV
- Drug-Resistant TB