# Drug Resistance Mechanisms in *M. tuberculosis*

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# Outline

- Current TB Problem
- TB Drugs and Chemotherapy
- Mechanisms of Drug Resistance
- Mechanisms of Persistence
- LTBI
- Pyrazinamide and Clofazimine
- Implications and Future Directions

# TB: A Leading Infectious Killer

- High Burden of Cases and Mortality: 10 million new cases (12% HIV+)(640 million total) and 1.6 million deaths, 2017; 64% treatment coverage

- Latent TB Infection (LTBI): 1/4 world population infected with TB bacillus –1.7 billion people

- Increasing MDR/XDR-TB: 5% MDR globally,

3.3% of new TB cases, 20% of previously treated

558 ,000 cases MDR-TB/190,000 deaths,

50,000 cases XDR-TB/yr

- HIV co-infection worsens TB

# TB Control Measures: Not Effective

- BCG Vaccine: inadequate efficacy
- Diagnostics: not sensitive (smear) or too slow (culture)
- TB Therapy: too long; new drugs for MDR/XDR-TB
- Control Strategy: too simple, not effective
- LTBI not taken into consideration

# 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

# Drugs Used for Treatment of TB

- **First-line drugs:** Isoniazid (INH), Rifampin (RIF), Ethambutol (EMB), Pyrazinamide (PZA);
- Drug susceptible TB:2(INH, RIF, PZA, EMB)+4(INH+RIF) 6 months

Lengthy therapy (persistence) → poor patient compliance → MDR-TB, or direct transmission

- MDR-TB: PZA+EMB + **Second-line TB drugs** fluoroquinolones levofloxacin/moxifloxacin/ofloxacin; amikacin; cycloserine; ethionamide/protionamide; PAS:18-24 months, more toxic/side effects, more expensive, poor cure rates 50-60%;
- Clofazimine; linezolid as core second-line drugs:
- Bangladesh regimen: Clofazimine+other drugs

  cure rate 84.5%, 9-12 months

# Drugs with Activity against TB Persisters

| Drug Name | Target/Mechanism | Activity for Persisters |
|---|---|---|
| Isoniazid (INH) | Mycolic acid synthesis | - |
| Ethambtol (EMB) | Arabinogalactan synthesis | - |
| Pyrazinamide (PZA) | Energy production/Trans-translation | +++ |
| Rifampin/Rifapentine | RNA polymerase/Transcription | ++ |
| Aminoglycosides | Protein synthesis | + |
| Fluoroquinolones | DNA synthesis | + |
| Clofazimine | Energy production? | ++ |
| Bedaquiline/TMC207 | F1F0 ATPase/ATP synthesis | ++ |
| Nitroimidazoles (PA-824, OPC67683) | Reactive nitrogen/DNA damage | + |

# TB Chemotherapy

- **Treatment principle:** Drug combination

for other infections, e.g. HIV-HAART therapy; Hepatitis, H. pylori; cancer MOPP therapy for lymphoma

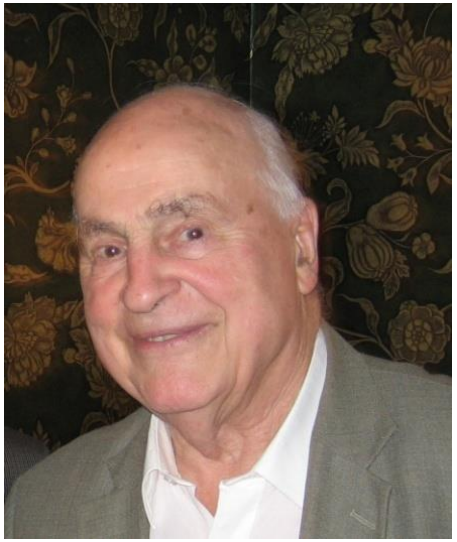
- Why drug combination?

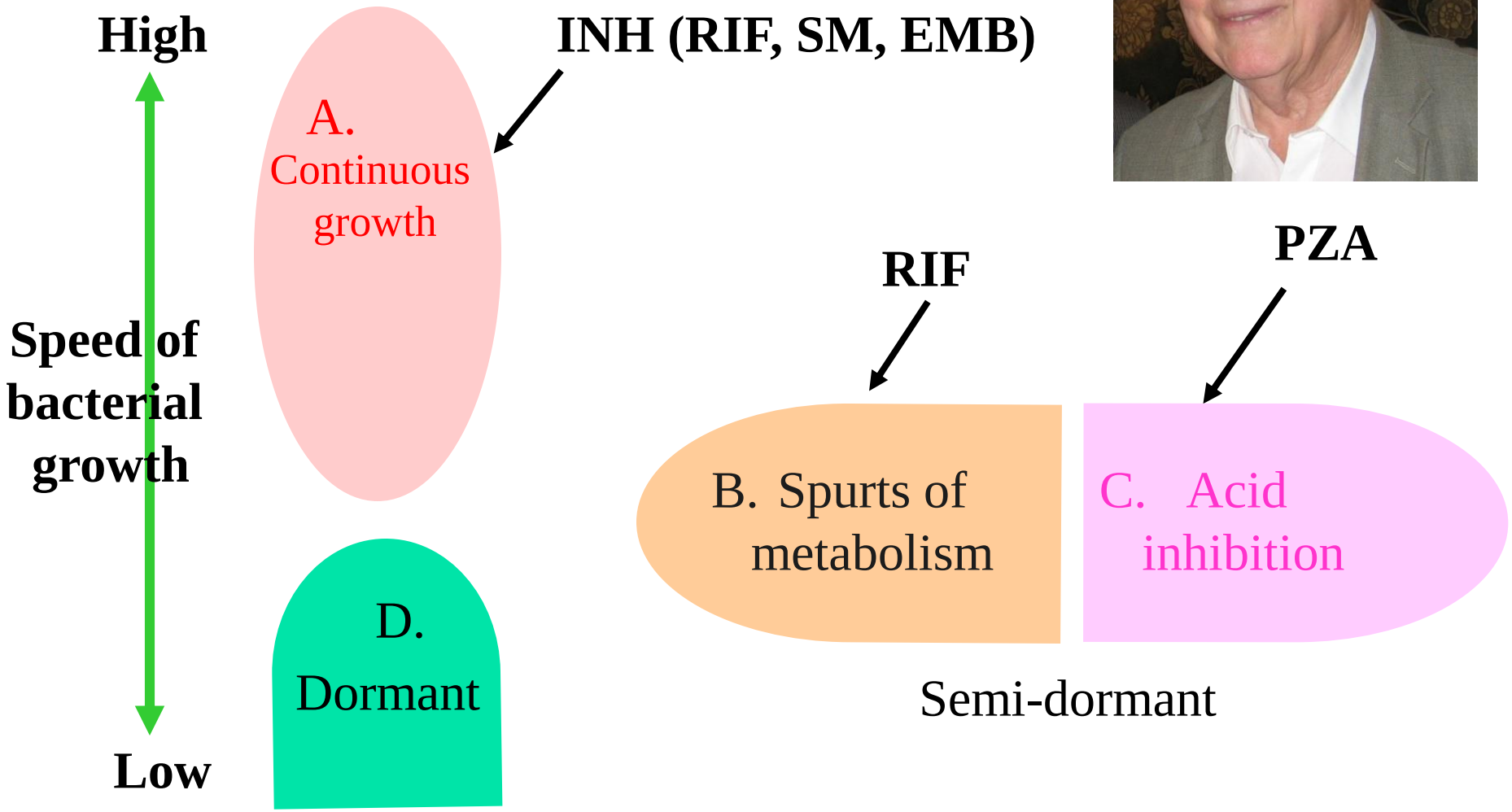
(a) Prevent drug resistance: Spontaneous mutations (e.g., resistance to INH =$10^{-6}$, resistance to RIF = $10^{-8}$, resistance to both INH and RIF = $10^{-14}$)

(b) Enhance efficacy of therapy (Mitchison hypothesis)

# Special Bacterial Populations Theory (Mitchison Hypothesis)

Mitchison DA. 1979, Chest, 76:771-81

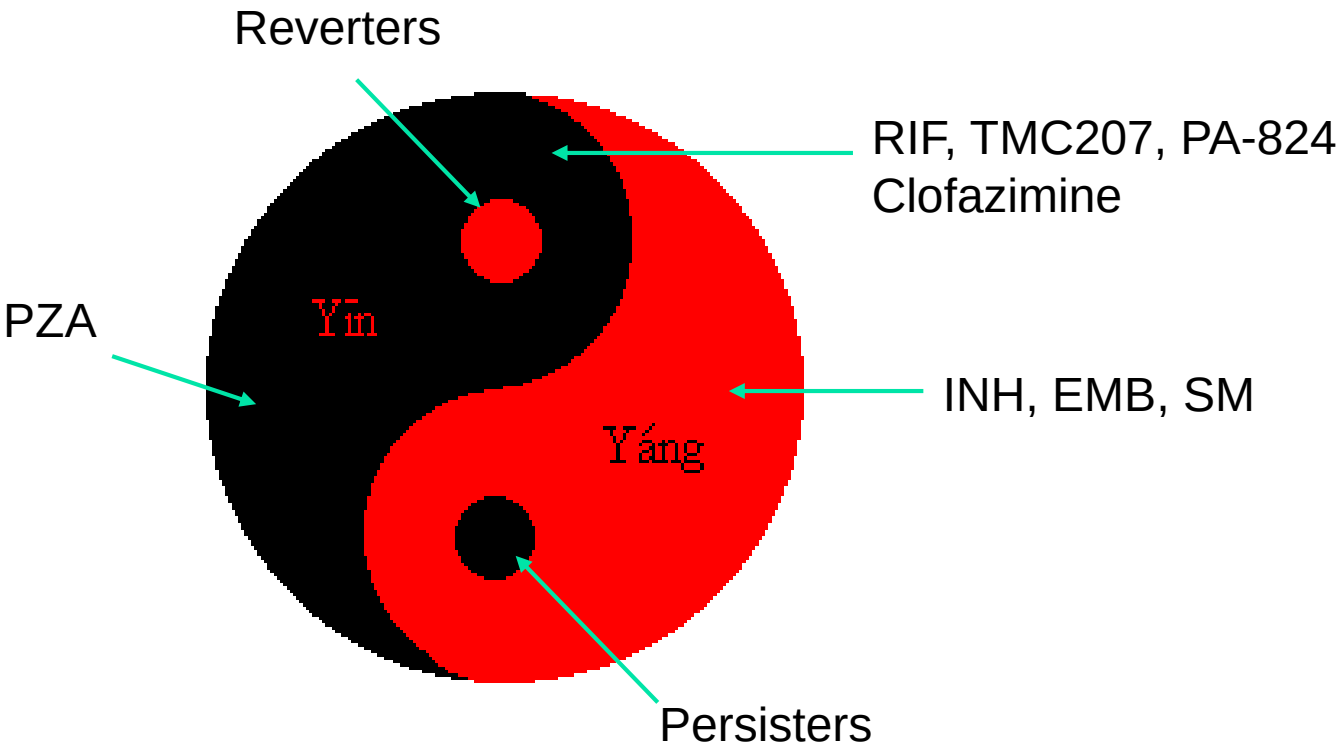




# Yin-Yang Model:

(Y. Zhang, Clin Pharmacol Ther. 2007; 82:595-600;
Zhang et al., 2012, AAC)

- Bacterial populations (Yang / Yin)
- **Genetic resistance** vs **Phenotypic resistance (persistence)**
- Active TB vs LTBI
- Explains current TB therapy
- Explains INH prophylaxis for LTBI
- New TB therapy based on Yin-Yang



# Two Types of Drug Resistance:

**Tolerance/ Phenotypic Resistance**


**Genetic Resistance**

- Genetic drug resistance: chromosomal mutations or plasmids/transposon **in growing bacteria**–

  **Yang Resistance (MIC higher)**

- Phenotypic drug resistance: epigenetic changes in bacterial physiology, stationary phase, persisters, dormant state **in non-growing bacteria**–

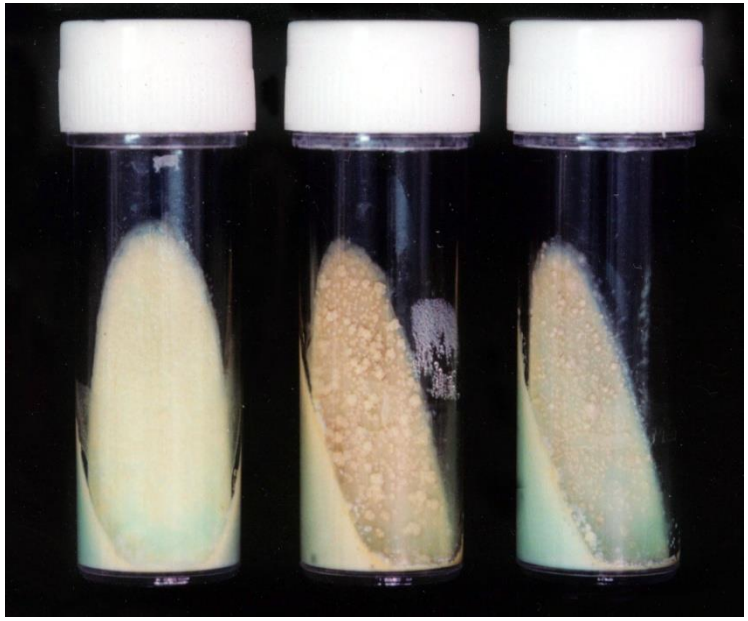
  **Yin Resistance (MIC no change)**

- Overlap/interconversion of Yang and Yin resistances

# Rifampin-dependent/enhanced MDR-TB

## (Zhong et al., 2010. Int J Tuberc Lung Dis. 14: 40-44)

**No RIF  RIF250  RIF50**



***rpoB*** **mutations**

**S531L; F584S**

**35 year-old male treated with WHO recommended thrice wk Regimen 2(HRZE)3, followed by 4(HR)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!**

# Feature of 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 (e.g. strain W in New York: resistant to 7 drugs) → XDR-TB (MDR+resistance to quinolone and injectables) → TDR (totally drug resistant)

# Mechanisms of Drug Resistance:
# Five Major Mechanisms

- 1. Reduced permeability/uptake
- 2. Enhanced efflux: Rv0678 →MmpL5↑ CFZ, BDQ
- 3. Enzymatic inactivation (β-lactamase)
- 4. Alteration or overexpression of drug target:

RpoB, InhA, RpsA, EmbB, GyrA, RpsL, 16S rRNA,

- 5. Loss of enzymes involved in drug activation:
  Isoniazid resistance-KatG (1992)
  Pyrazinamide resistance-PncA (1996)
  Ethionamide-EthA (2000)
  PAS-DHPS/DHFS (2013)

# Mechanisms of Resistance in *M. tuberculosis* to 1st-line and 2nd –line Drugs

| Drug (Year of Discovery) | MIC (μg/ml) | Gene involved in Resistance | Gene Function | Mechanism of Action | Mutation Frequency (%) |
|---|---|---|---|---|---|
| Isoniazid (1952) | 0.02-0.2 | *katG*,<br>*inhA* | Catalase-peroxidase,<br>Enoyl ACP reductase | Inhibition of mycolic acid synthesis and other effects | 50-95<br>8-43 |
| Pyrazinamide (1952) | 16-100 | *pncA*,<br><br>*rpsA*,<br><br>*panD* | Nicotinamidase/ Pyrazinamidase,<br>Ribosomal S1 protein<br><br>Aspartate decarboxylase | Depletion of membrane energy;<br>Inhibition of trans-translation;<br>Inhibition of pantothenate and CoA synthesis | 72-99 |
| Rifampin (1966) | 0.05-1 | *rpoB* | β–subunit of RNA polymerase | Inhibition of RNA synthesis | 95 |
| Ethambutol (1961) | 1-5 | *embB*,<br>*ubiA* | Arabinosyl transferase,<br>*DPPR synthase | Inhibition of arabinogalactan synthesis | 47-65 |
| Streptomycin (1943) | 2-8 | *rpsL*,<br>*rrs*,<br>*gidB* | S12 ribosomal protein<br>16S rRNA<br>16S rRNA methyltransferase | Inhibition of protein synthesis | 52-59<br>8-21 |
| Amikacin/ Kanamycin (1957) | 2-4 | *rrs*,<br>*eis*,<br><br>*whiB7* | 16S rRNA,<br>Aminoglycoside acetyltransferase<br>Transcription regulator | Inhibition of protein synthesis;<br>Inactivation of drug;<br>Control *eis*, *tap* efflux | 76 |
| Capreomycin (1960) | 2-4 | *rrs*<br>*tlyA* | 16S rRNA,<br>2'-O-methyltransferase | Inhibition of protein synthesis | 85 |
| Quinolones (1963) | 0.5-2.5 | *gyrA*<br>*gyrB* | DNA gyrase subunit A<br>DNA gyrase subunit B | Inhibition of DNA synthesis | 75-94 |

# Mechanisms of Resistance in *M. tuberculosis* to 2nd–line Drugs (Continued)

| Drug (Year of Discovery) | MIC (μg/ml) | Gene involved in Resistance | Gene Function | Mechanism of Action | Mutation Frequency (%) |
|---|---|---|---|---|---|
| Ethionamide (1956) | 2.5-10 | *etaA/ethA*<br>*ethR*<br>*inhA* | Flavin monooxygenase,<br>Transcription repressor,<br>Enoyl ACP reductase | Inhibition of mycolic acid synthesis | 37<br>56 |
| PAS (1946) | 1-8 | *thyA*<br>*dfrA*<br>*folC*<br>*ribD* | Thymidylate synthase,<br>Dihydrofolate reductase,<br>Dihydrofolate synthase,<br>Enzyme in riboflavin biosynthesis | Inhibition of folic acid and thymine nucleotide metabolism | 37 |
| Cycloserine (1955) | 10-40 | *alr*<br>*ddl*<br><br>*cycA* | Alanine racemase,<br>D-Alanine-D-alanine ligase,<br>D-Serine proton symporter | Inhibition of cell wall peptidoglycan synthesis | TBD |

# Mechanisms of Resistance to New Drugs and Repurposed Agents in *M. tb*

| **Drug (Year of Discovery)** | **MIC (μg/ml)** | **Gene involved in Resistance** | **Gene Function** | **Mechanism of Action** |
|---|---|---|---|---|
| Pretomanid (PA-824) (2000) | 0.015-0.25 | *ddn*,<br><br>*fgd1*<br><br>*fbiA, B, C* | Deazaflavin-dependent nitroreductase,<br>F420-dependent glucose-6-phosphate dehydrogenase<br>F420 synthesis | Inhibition of mycolic acid synthesis, Production of reactive nitrogen species |
| Delamanid (OPC-67683) (2006) | 0.006-0.012 | *ddn*,<br><br>*fgd1*<br><br>*fbiA, B, C* | Deazaflavin-dependent nitroreductase,<br>F420-dependent glucose-6-phosphate dehydrogenase<br>F420 synthesis | Inhibition of mycolic acid synthesis, Production of reactive nitrogen species |
| Bedaqiline (TMC207) (2005) | 0.06-0.12 | *atpE*,<br>*rv0678* | ATP synthase c chain,<br>Transcription repressor for transporter MmpL5 | Inhibition of ATP production |
| SQ109 (2005) | 0.5 | *mmpL3* | Membrane transporter | Inhibition of mycolic acid synthesis, membrane disruption |
| Clofazimine (1956) | 0.1-0.25 | *rv0678*<br><br>*rv1979c*<br>*rv2535c* | Transcription repressor for transporter MmpL5<br>Possible permease<br>Peptidase | Production of reactive oxygen species, Inhibition of energy production, Membrane disruption |
| Linezolid (1996) | 0.25-0.5 | *rrl*<br>*rplC* | 23S rRNA<br>L3 ribosomal protein | Inhibition of protein synthesis |

# 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

# 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, *rpsA*
- 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**, PCR-SSCP, molecular beacons, hybridization (Line-Probe assay) tests in microarray /macroarray, real-time PCR, Melting curve analysis
- **Line-Probe assays (Hain Lifescience GenoType MTBDRplus)** 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 TB cases and 98% patients with RIF-resistant bacteria in < 2 hours

- Detection of Isoniazid-, Fluoroquinolone-, Amikacin-, and Kanamycin-Resistant Tuberculosis in an Automated, Multiplexed 10-Color Assay Suitable for Point-of-Care Use. J Clin Microbiol. 2016,28;55(1):183-198.

- **Next generation sequencing (NGS):** Illumina, Ion Torrent for Precision Medicine
- RefSeq database: Correlation between mutations and drug resistance

# Treatment of MDR/XDR TB: "Bee Hive"

**MDR-TB: resistant to INH+RIF； XDR: MDR+Q+I**

**Primary MDR vs Acquired/Secondary MDR: Treatment response same???**

- Rapid detection of MDR/XDR TB needed
- Treatment of MDR is longer (18-24 months), more side effects, poor cure rates (50-60%), more costly ($10K-50K vs $100-$500 drug susceptible TB)

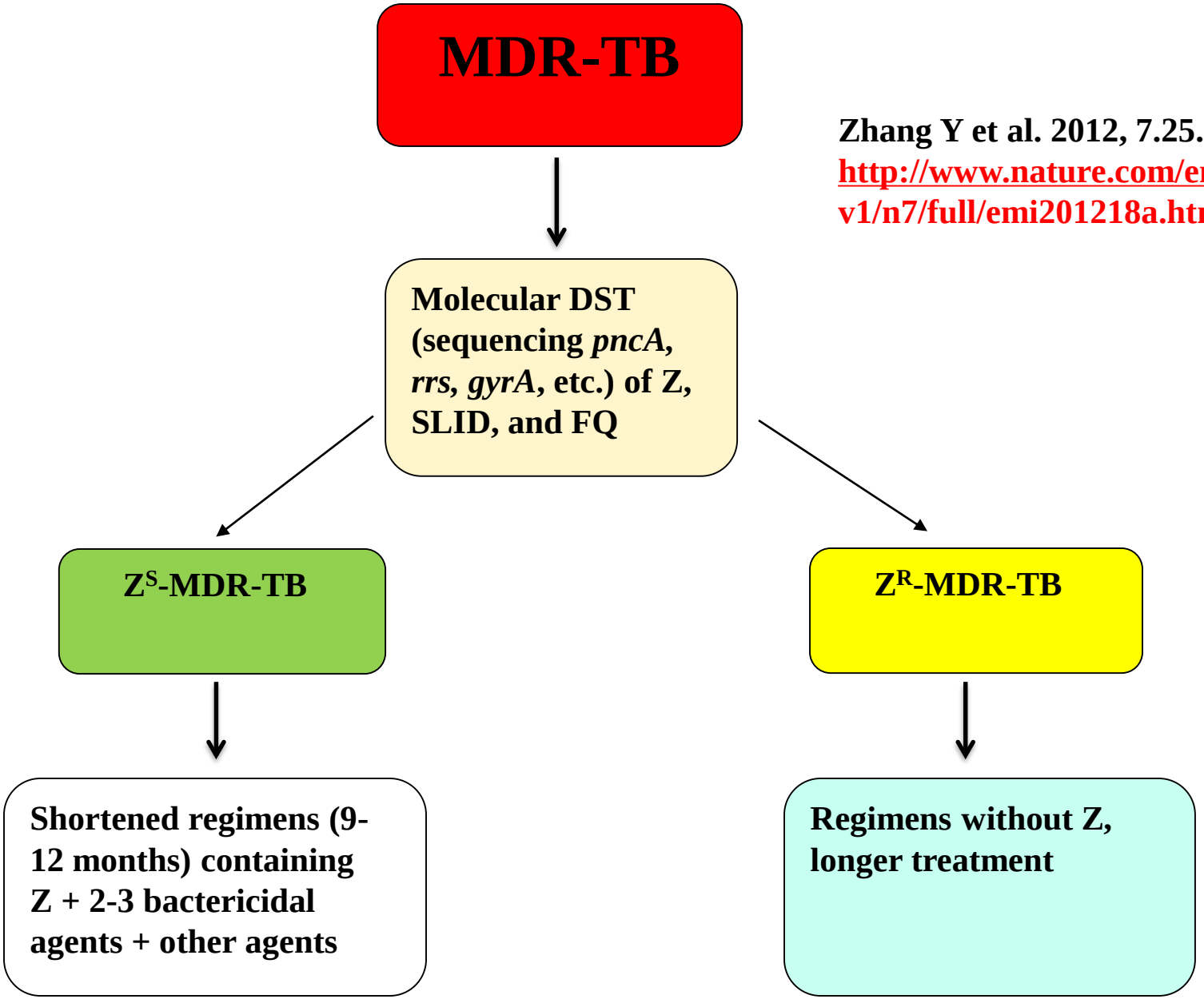
Regimens: PZA+EMB+injectable+quinolone

- Bangladesh regimen (9 months)

standardized, program based, $600

- Based on DST results, individualized

(Precision Medicine)




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

# New Regimens for MDR-TB Treatment

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

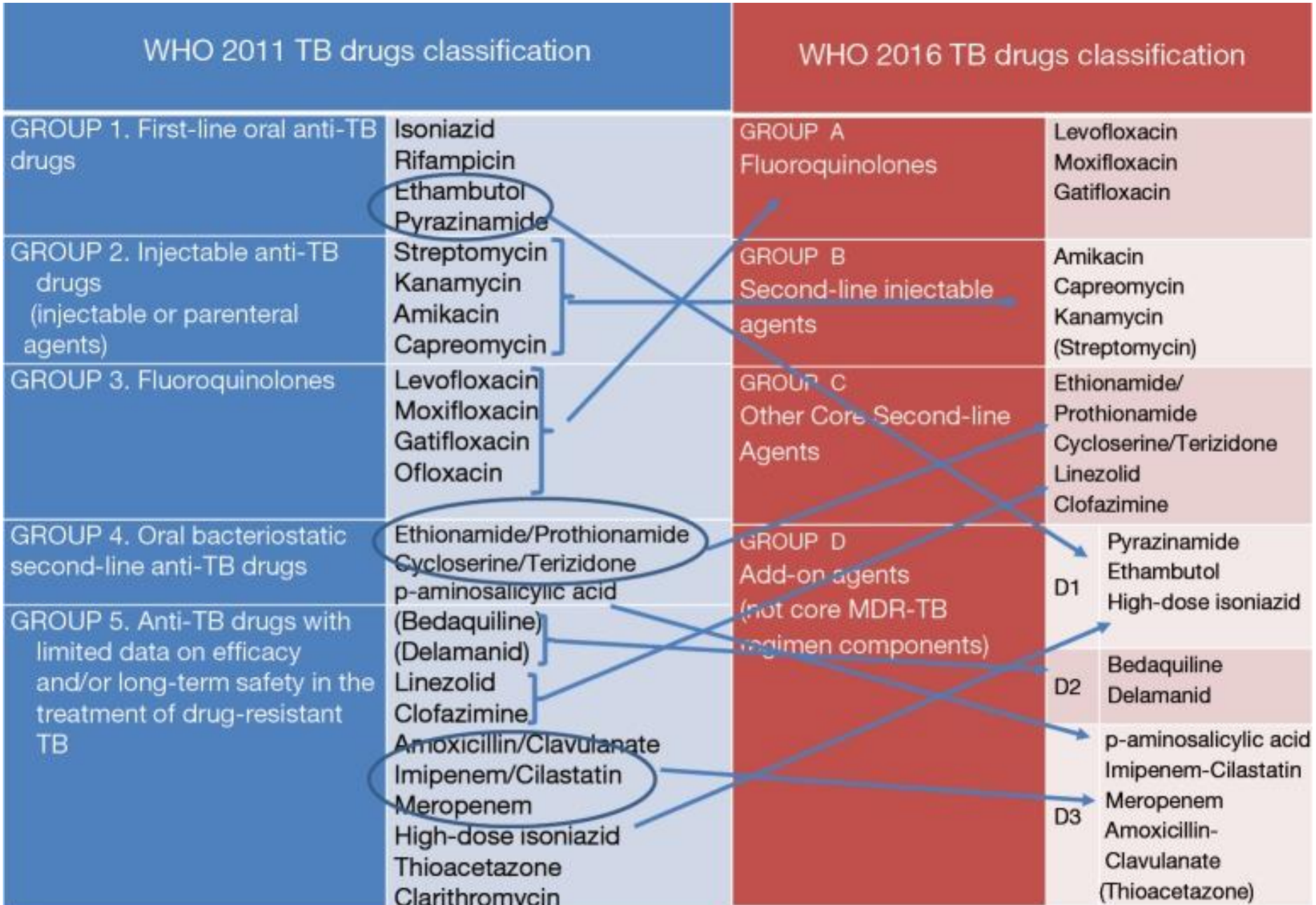
**Higher number of deaths in those given bedaquiline (12/102 subjects, 11.8%) (cardiac arrhythmia due to QT prolongation)compared with placebo (4/105 subjects, 3.8%)**

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

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

# Classification of Drugs Used to Treat MDR-TB

J Thorac Dis. 2016 Oct; 8(10): 2666–2671.



**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**

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.

# Persistence Problem → MDR-TB

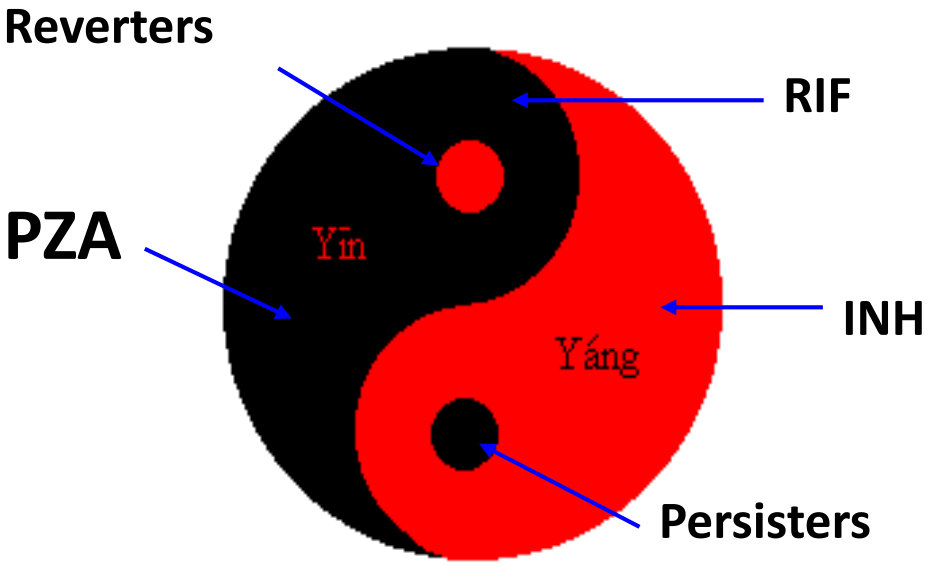
- Underlying lengthy TB therapy (6 month)

-> increasing MDR/XDR-TB

- Post-treatment relapse
- Underlying latent TB infection

# Bacterial Persisters (Yin Resistance) (Phenotypic Resistance/Antibiotic Tolerance)

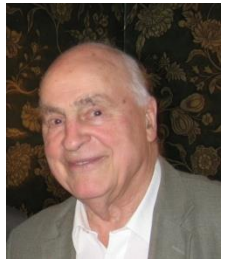
- Persisters first described by Gladys Hobby in 1942;
- "Persister" given by Joseph Bigger in 1944
- Penicillin kills 99% bacteria, residual 1%, not growing, not killed by antibiotic, called "persister"; Revive and still susceptible to antibiotic; Heterogeneous!
- Phenotypic resistance (tolerance), distinct from genetic resistance
- Underlie persistent infections (UTI, Lyme, TB, biofilm, etc.)




(Zhang, Y. (2014). Persisters, Persistent Infections and the Yin-Yang Model. Emerging Microbes and Infections 3, e3; doi:10.1038/emi.2014.3)

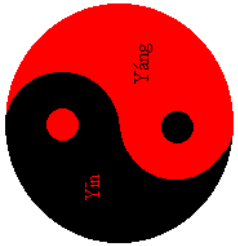
# Evolution of Concept of Persisters

**Dormant non-growing bacteria (all the same): (1942)**



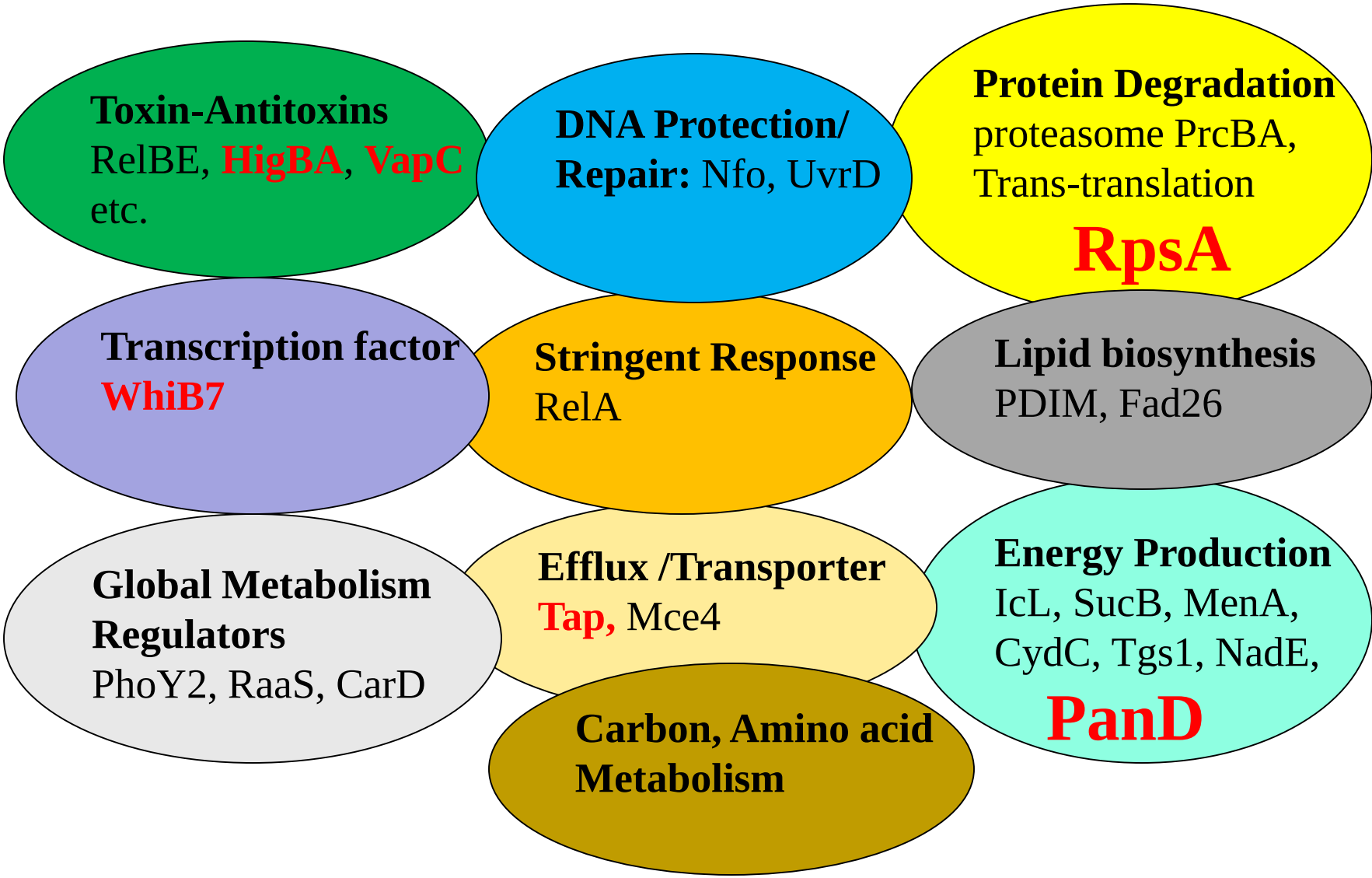
**3 populations of non-growing bacteria – Mitchison Hypothesis (1979)**

**Russian Dolls (2005)**




**Highly heterogeneous non-growing bacteria– Yin-Yang Model (2007)**

# Mechanisms of Persistence in *M. tuberculosis*
## *Similar to other bacteria, biofilm etc.*



# Latent TB Infection (LTBI):

**Definition (WHO): state of persistent immune response (TST, IGRA) to stimulation by M. tuberculosis antigens without evidence of clinically manifested active TB**

**Problem: too vague, very heterogeneous, old infection from no chance to come back to close to ATB**

**Interest: NIH, Gates Foundation, WHO, China**

# Latent Tuberculosis: Reservoir for Active TB

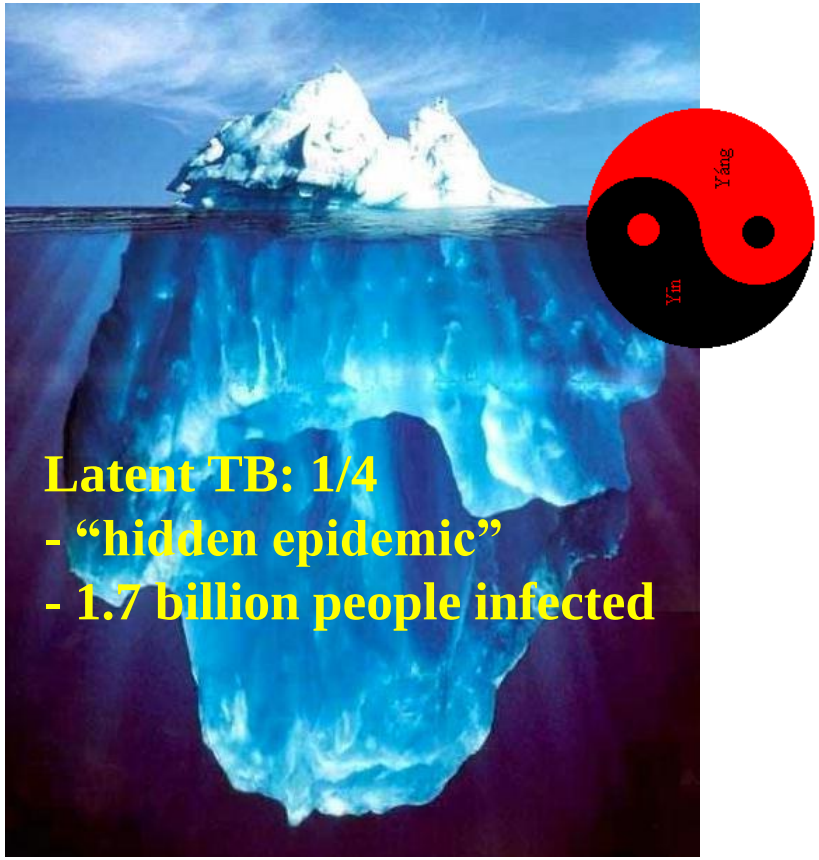
New TB cases are driven by the reservoir of latently infected people

This "hidden epidemic" of people infected with latent TB is enormous

- a time bomb

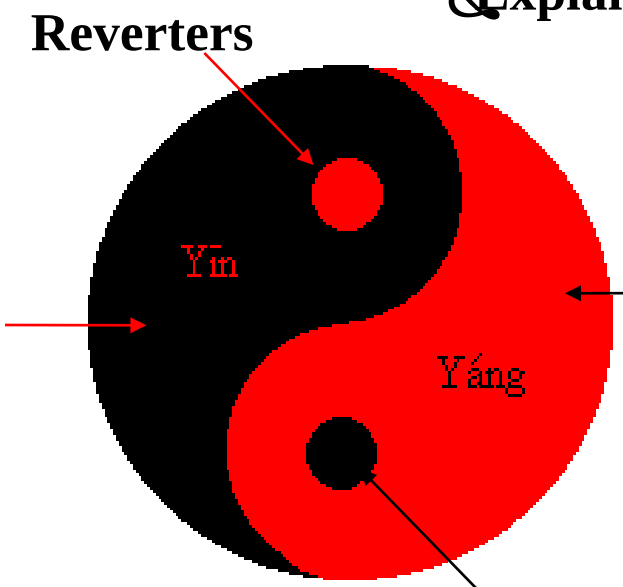
Control the reservoir of infection by chemoprophylaxis or post-exposure vaccine

**Active TB**
**– 10 million new cases a year**
**- Unfortunately just tip of the iceberg**



# Yin and Yang of TB:
# 上医治未病, 下医治已病

- Bacterial populations
- Genetic vs phenotypic resistance
- LTBI vs active TB
- Explains current TB therapy
- Explains INH prophylaxis for LTBI




**Non-growing persisters**
**Phenotypic resistance**
**LTBI**

**Active growing bacteria**
**Genetic resistance**
**Active TB**

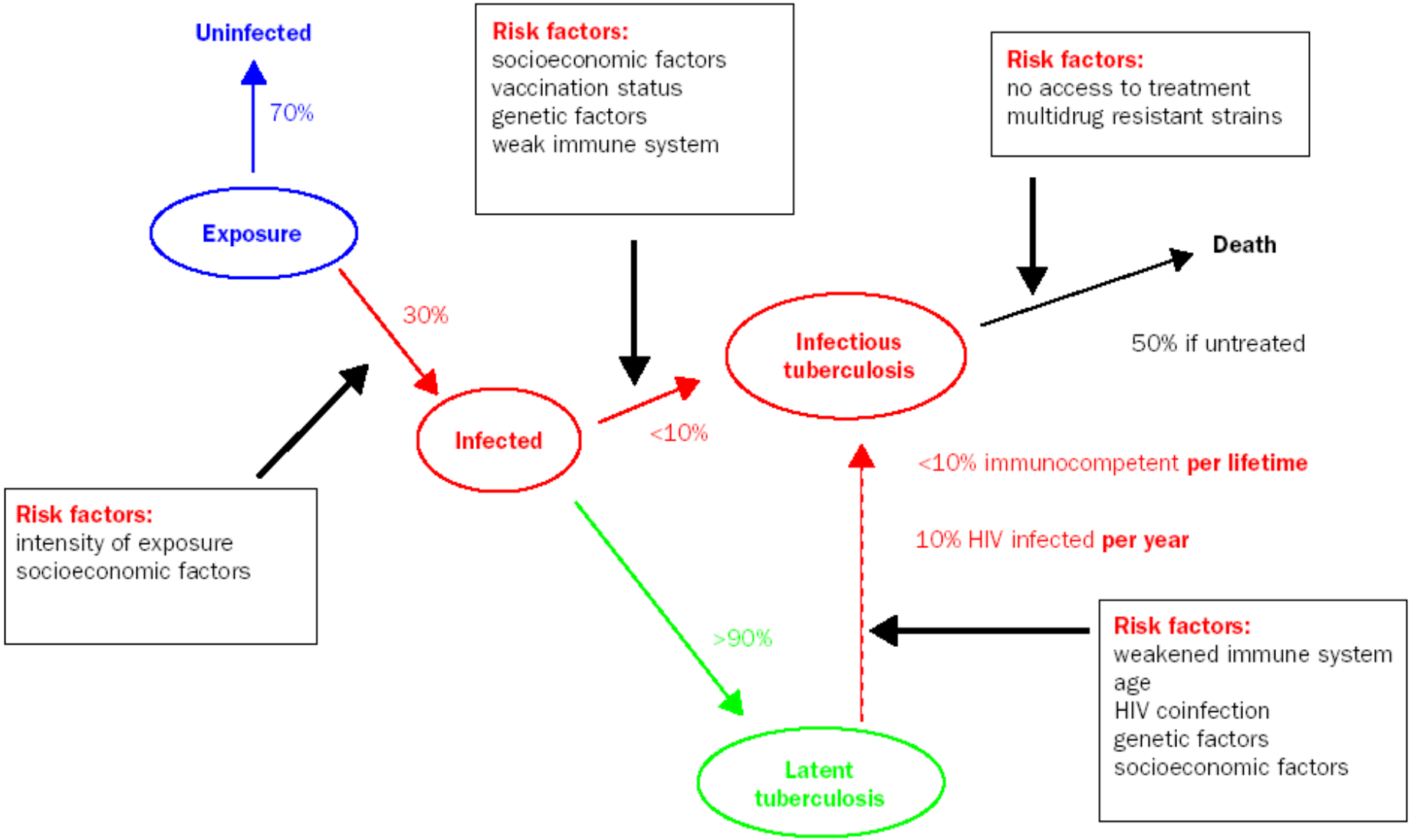
(Y. Zhang, Clin Pharmacol Ther. 2007; 82:595-600; Zhang et al., AAC, 2012)

# Topics of Interest on LTBI:

- Risk factors of LTBI to Active TB
- Host immune factors
- Bacterial factors
- Biomarkers/tests that distinguish LTBI to Active TB
- More effective shorter treatment for LTBI

Targeted chemoprophylaxis

# Risk factors of TB



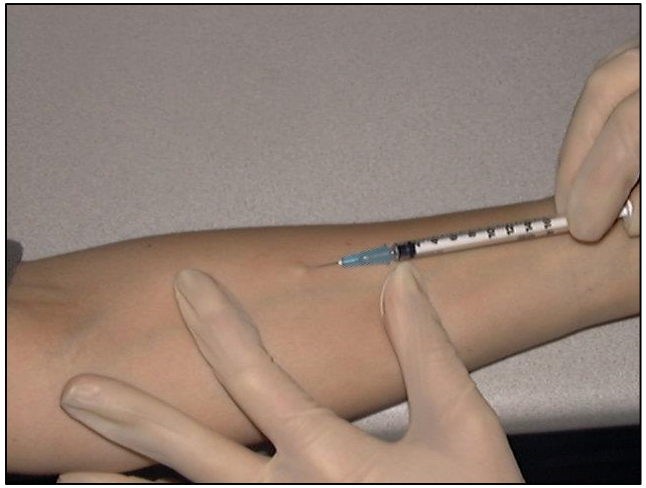
# LTBI Risk Factors

| Condition | OR or RR |
|---|---|
| 1. Immune suppression | |
| a. HIV-positive and tuberculin skin test-positive [71-73] | 50-110 |
| b. AIDS [74, 75] | 110-170 |
| c. Solid organ transplantation related to immunosuppressant therapy [76 77, 78] | 20-74 |
| d. Receiving anti-TNF-alpha treatment [79-81] | 1.5-17 |
| e. Corticosteroids >15mg prednisolone equivalent per day for >2-4 wks* [82, 83] | 4.9 |
| 2. Malignancy | 4-8 |
| a. Haematological malignancy (leukemias, lymphomas) [84] | 16 |
| b. Carcinoma of the head or neck and lung [85] | 2.5-6.3 |
| 3. Gastrectomy [86, 87] | 2.5 |
| 4. Jejunoileal bypass [88, 89] | 27-63 |
| 5. Silicosis [90-92] | 30 |
| 6. Chronic renal failure / haemodialysis [93, 94] | 10-25 |
| 7. Diabetes mellitus [95-98] | 2-3.6 |
| 8. Smoking [99-103] | 2-3 |

Erkens J. ERJ 2010

# LTBI Detection

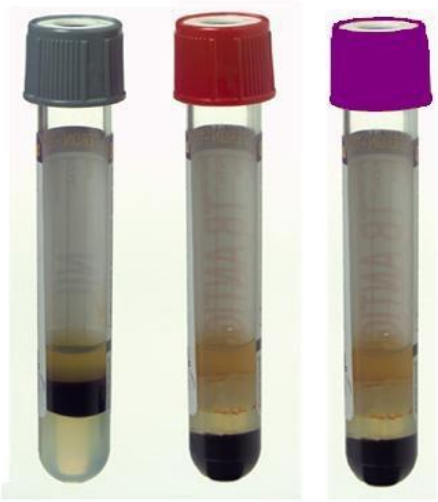
## TST



**False positive (BCG, NTM)**
**Read 48-72h**

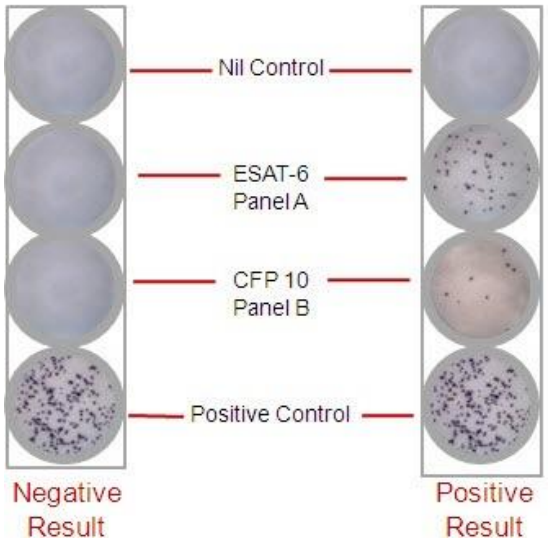
## IGRA

**More specific, fast**



**Quanti-FERON**

**ESAT-6,CFP-10 and TB7.7**




**T-SPOT**

**ESAT-6,CFP-10**

# WHO recommendation

- Either TST or IGRA can be used to test for LTBI in high-income and upper middle-income countries with estimated TB incidence less than 100 per 100 000. *(Strong recommendation, very low quality of evidence)*
- IGRA should not replace TST in low-income and other middle-income countries. *(Strong recommendation, very low quality of evidence) (8)*

**Cannot distinguish LTBI and ATB**

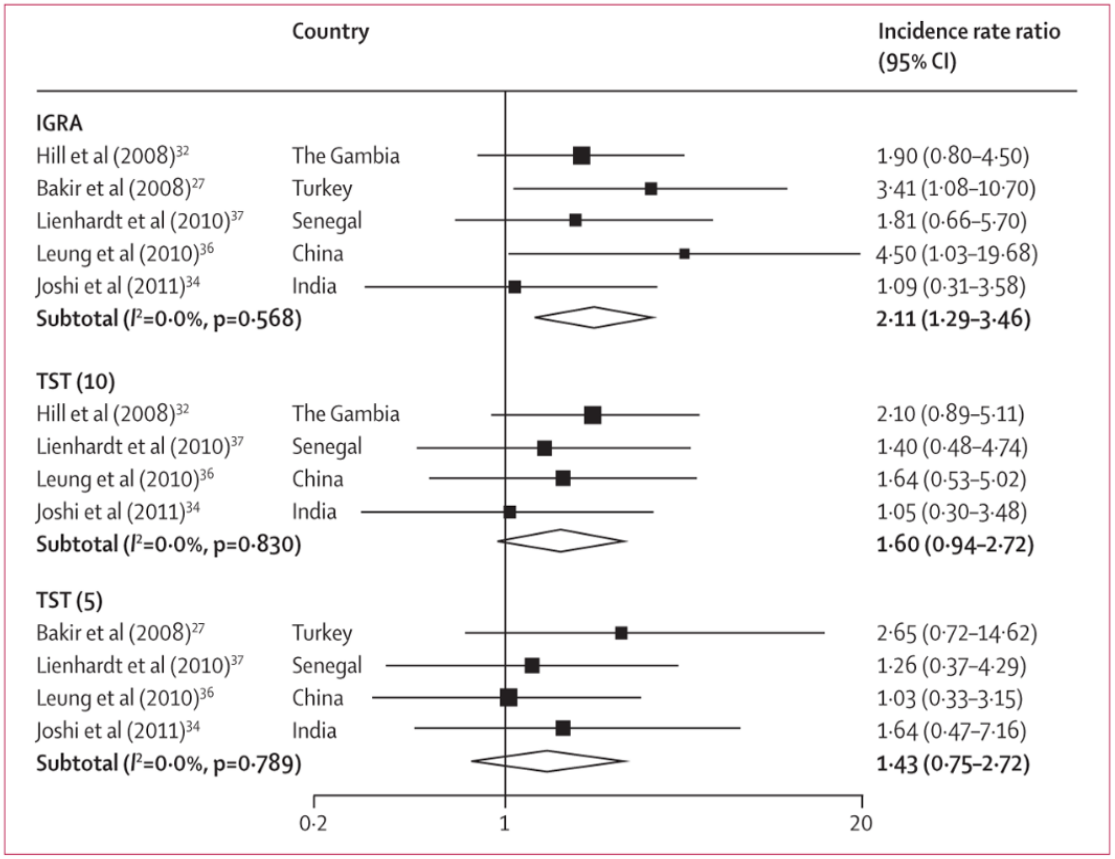
**Biomarkers for ATB and LTBI**

# TST or IGRAs? Predictive power of ATB

*Lancet Infect Dis*. 2012 January ; 12(1): 45–55. doi:10.1016/S1473-3099(11)70210-9.

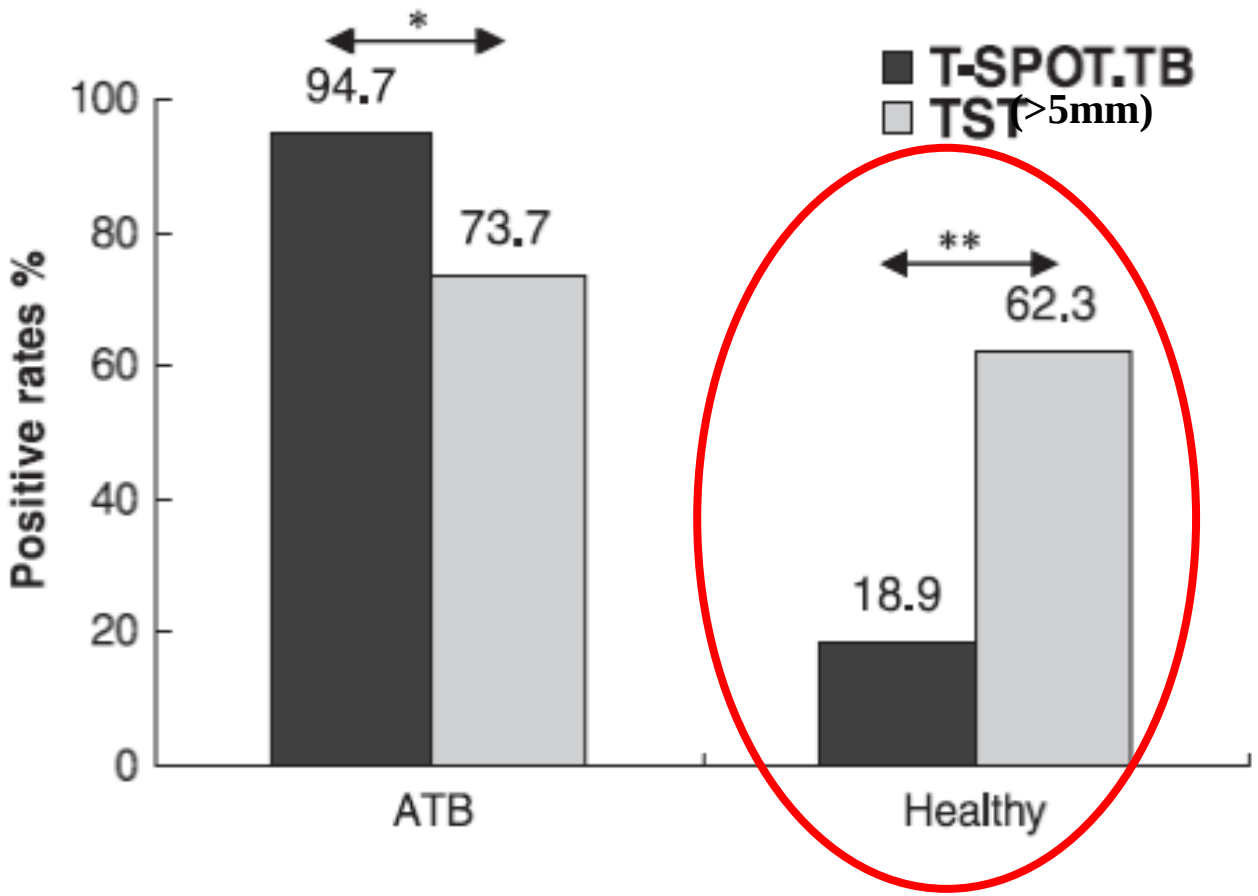
## Predictive value of interferon-γ release assays for incident active tuberculosis: a systematic review and meta-analysis

**Molebogeng X Rangaka, Katalin A Wilkinson, Judith R Glynn, Daphne Ling, Dick Menzies,**




- IGRAs and TSTcannot predict active TB
- Most IGRA and TST positive people remain positive in extended time

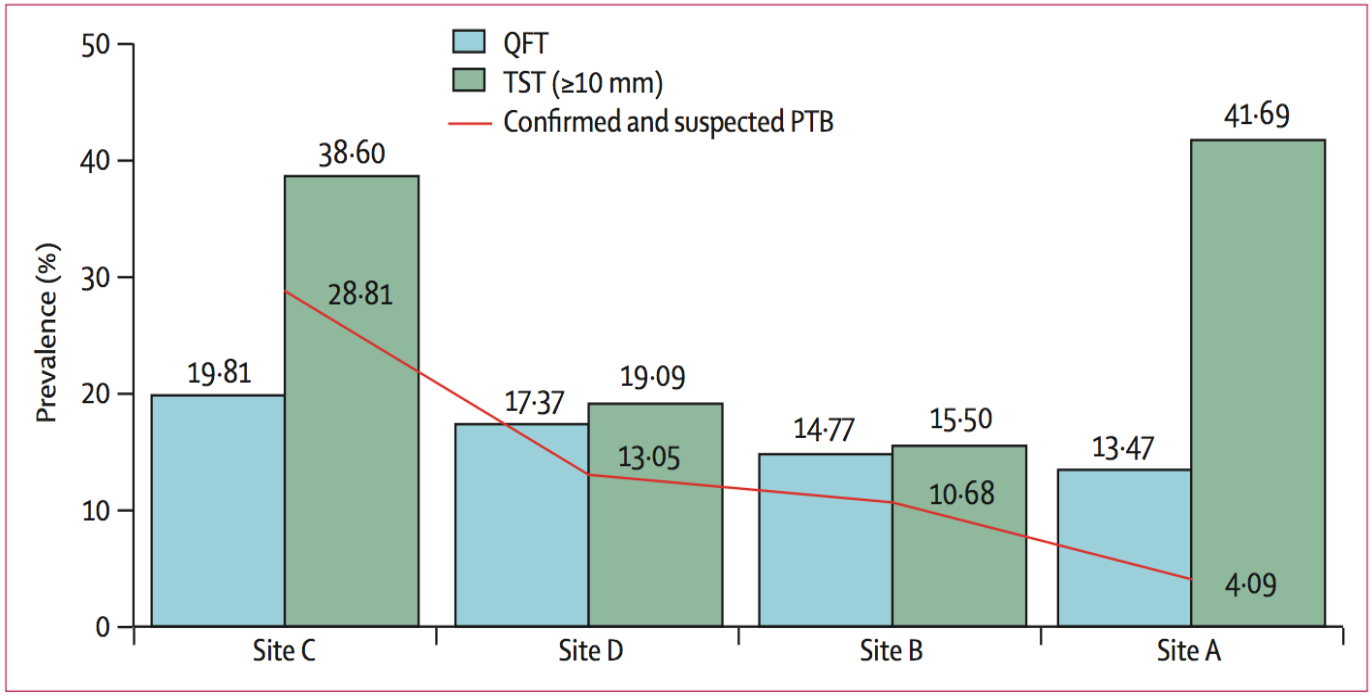
# LTBI in Chinese Population




Zhang S, Shao L, Zhang W, et al. Clin Vaccine Immunol. 2010; (17):12:1985–1990.

# Latent tuberculosis infection in rural China: baseline results of a population-based, multicentre, prospective cohort study

*Lei Gao*, Wei Lu*, Liqiong Bai*, Xinhua Wang*, Jinsheng Xu*, Antonino Catanzaro, Vicky Cárdenas, Xiangwei Li, Yu Yang, Jiang Du, Hongtao Sui, Yinyin Xia, Mufei Li, Boxuan Feng, Zhen Li, Henan Xin, Rong Zhao, Jianmin Liu, Shouguo Pan, Fei Shen, Jian He, Shumin Yang, Hongyan Si, Yi Wang, Zuhui Xu, Yunhong Tan, Tianzhu Chen, Weiguo Xu, Hong Peng, Zhijian Wang, Tao Zhu, Feng Zhou, Haiying Liu, Yanlin Zhao*, Shiming Cheng*,Qi Jin*, for the LATENTTB-NSTM study team†*




- **China BCG vaccination**
- **TST overestimate LTBI**

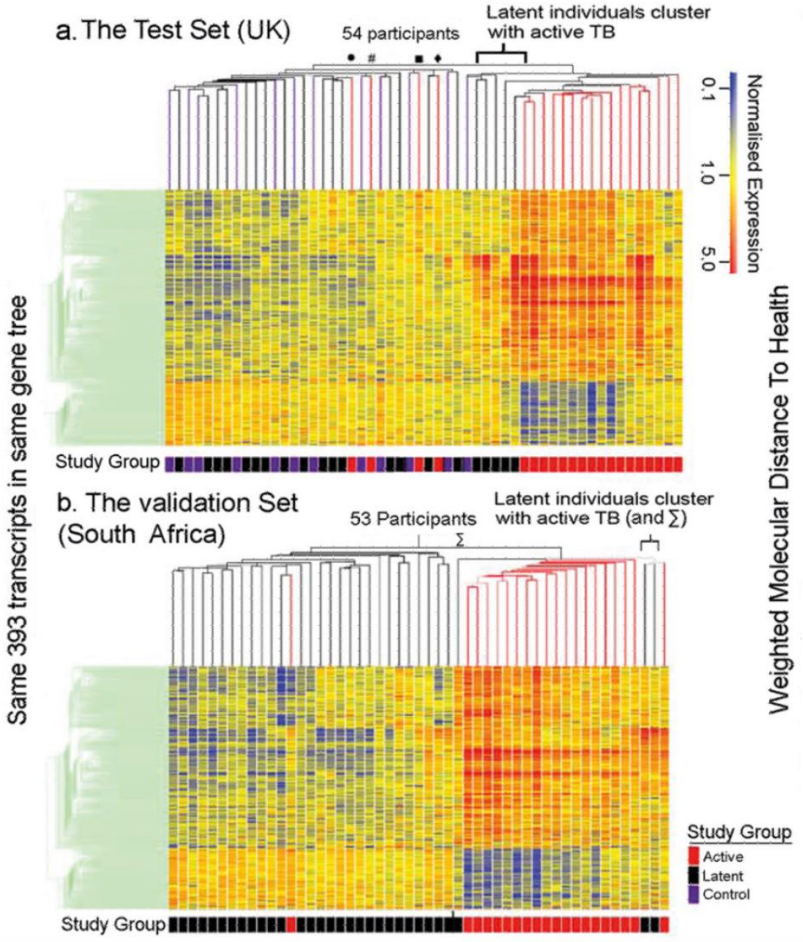
**Gao et al. Lancet Infect Dis 2015.**

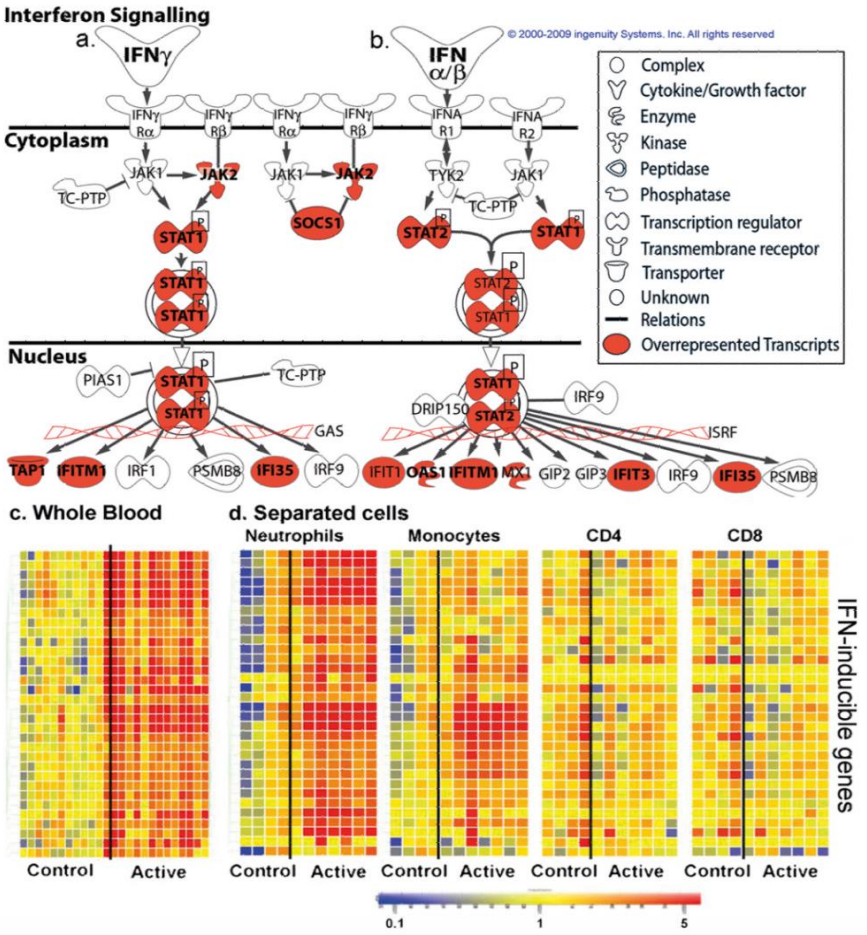
Published in final edited form as:
*Nature*. 2010 August 19; 466(7309): 973–977. doi:10.1038/nature09247.

# An Interferon-Inducible Neutrophil-Driven Blood Transcriptional Signature in Human Tuberculosis

**Matthew P. R. Berry[1], Christine M. Graham[1,*], Finlay W. McNab[1,*], Zhaohui Xu[6], Susannah**

全血提取RNA → Microarray

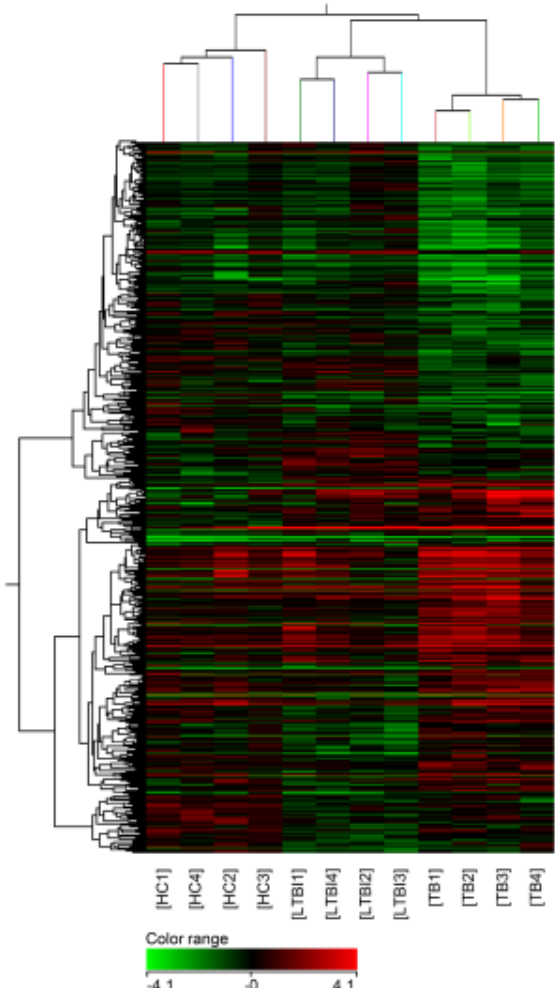


OPEN ACCESS Freely available online PLOS one

# Novel Biomarkers Distinguishing Active Tuberculosis from Latent Infection Identified by Gene Expression Profile of Peripheral Blood Mononuclear Cells

Chanyi Lu[1,2], Jing Wu[1,2], Honghai Wang[2], Sen Wang[1], Ni Diao[1], Feifei Wang[1], Yan Gao[1], Jiazhen Chen[1], Lingyun Shao[1], Xinhua Weng[1], Ying Zhang[1,3]*, Wenhong Zhang[1,4]*




- 研究Active TB和Latent TB人群的差异表达基因，寻找鉴别诊断的分子标记
  - QuantiFERON筛选LTBI
  - Microarray寻找差异表达基因
- 大量人群中验证获得4个基因有差异表达:CXCL10、TLR6、IL2RA、ATP10A

  敏感性80%，特异性89%

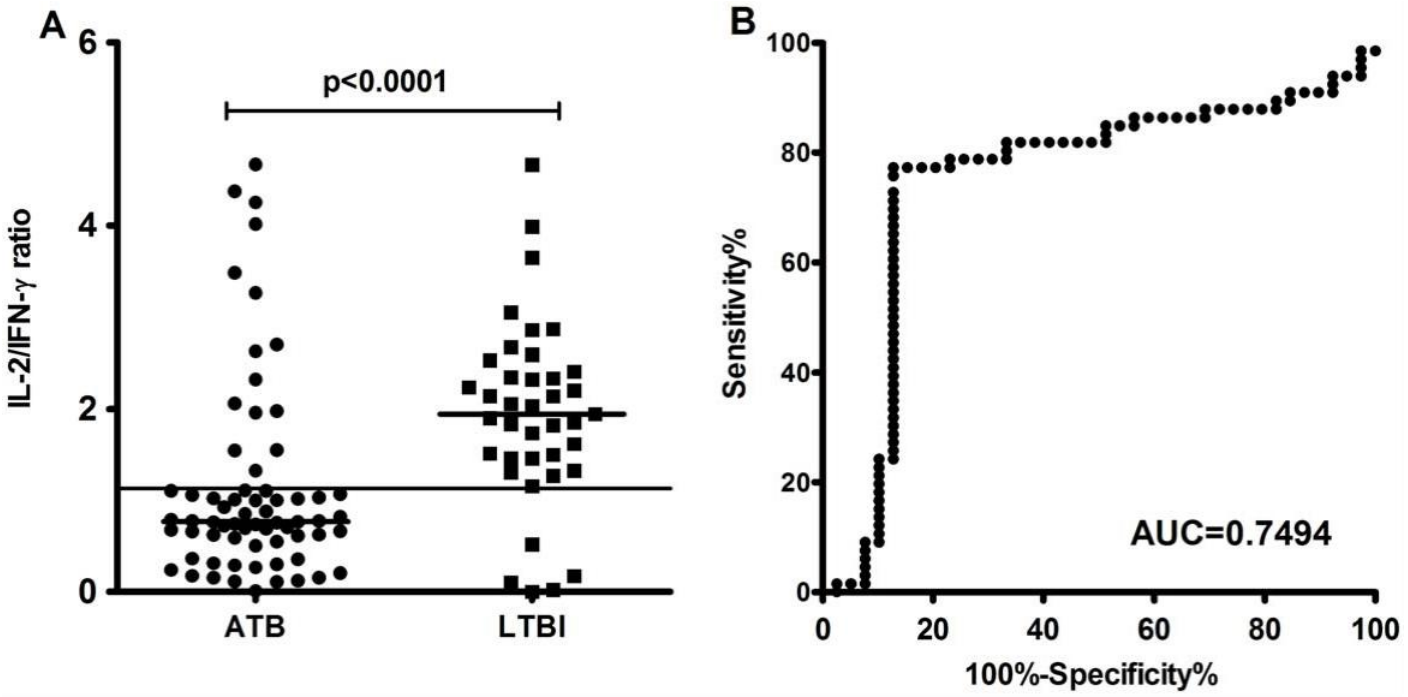
OPEN ACCESS Freely available online

PLOS | ONE

# Evaluation of the Diagnostic Potential of IP-10 and IL-2 as Biomarkers for the Diagnosis of Active and Latent Tuberculosis in a BCG-Vaccinated Population

Sen Wang[1], Ni Diao[1], Chanyi Lu[1], Jing Wu[1], Yan Gao[1], Jiazhen Chen[1], Zumo Zhou[2], Heqing Huang[2], Lingyun Shao[1], Jialin Jin[1], Xinhua Weng[1], Ying Zhang[1,3,4]*, Wenhong Zhang[1,4,5]*

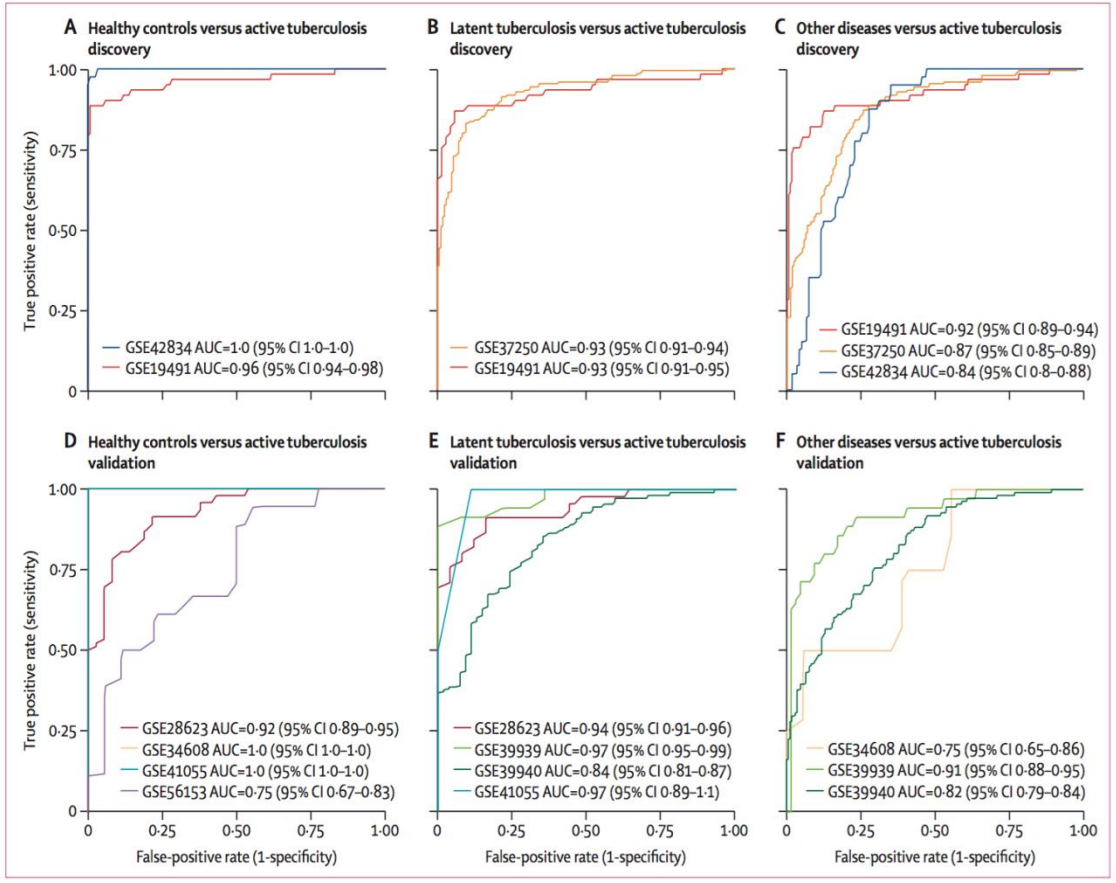
IL-2/IFN-γ ratio distinguish ATB and LTBI;
IFN-γ, IP-10, IL-2 increase sensitivity



# Genome-wide expression for diagnosis of pulmonary tuberculosis: a multicohort analysis

*Timothy E Sweeney, Lindsay Braviak, Cristina M Tato, Purvesh Khatri*

14 database → Data analysis



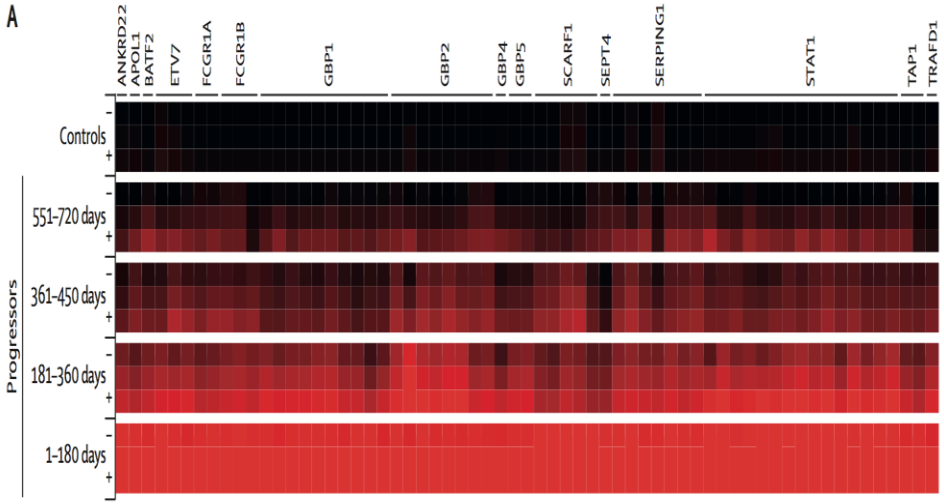

**GBP5, KLF2, DUSP3**
**Distinguish ATB and LTBI**

**No validation yet!**

**Sweeney et al. Lancet Respir Med 2016.**

# A blood RNA signature for tuberculosis disease risk: a prospective cohort study

*Daniel E Zak*, Adam Penn-Nicholson*, Thomas J Scriba*, Ethan Thompson†, Sara Suliman†, Lynn M Amon, Hassan Mahomed, Mzwandile Erasmus, Wendy Whatney, Gregory D Hussey, Deborah Abrahams, Fazlin Kafaar, Tony Hawkridge, Suzanne Verver, E Jane Hughes, Martin Ota, Jayne Sutherland, Rawleigh Howe, Hazel M Dockrell, W Henry Boom, Bonnie Thiel, Tom H M Ottenhoff, Harriet Mayanja-Kizza, Amelia C Crampin, Katrina Downing, Mark Hatherill, Joe Valvo, Smitha Shankar, Shreemanta K Parida, Stefan H E Kaufmann, Gerhard Walzl, Alan Aderem, Willem A Hanekom, for the ACS and GC6-74 cohort study groups‡*




- **Gene set of 16 genes**
- **The signature predicted TB progression with a sensitivity of 66·1% (95% CI 63·2–68·9) and a specificity of 80·6% (79·2–82·0) in the 12 months preceding tuberculosis diagnosis.**

**This risk signature was then adapted to multiplex quantitative real-time PCR, tested in separate cohorts, and found to be predictive of TB in the 12 months preceding the disease with a sensitivity of 53.7% (42.6-64.3) and specificity of 82.8% (76.7-86).**

# Challenges and Problems

- Gene candidates hard to validate in different populations
- Use of PET/CT scans to dynamically monitor people in parallel with their immune activation may provide insights about the host response.

# 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

Chee, Reeves, Zhang, Belknap, 2018, Respirology

- 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

# Pyrazinamide (PZA): A Unique Persister Drug

- Only persister drug among all antibiotics
- Critical for shortening TB therapy by killing persisters not killed by other drugs
- Prototype persister drug: proof of principle
- Recent interest: 3 workshops in 1 yr

# Why Persister Drug PZA Important?
# Dandelion Phenomenon

# PZA: Unconventional and Paradoxical

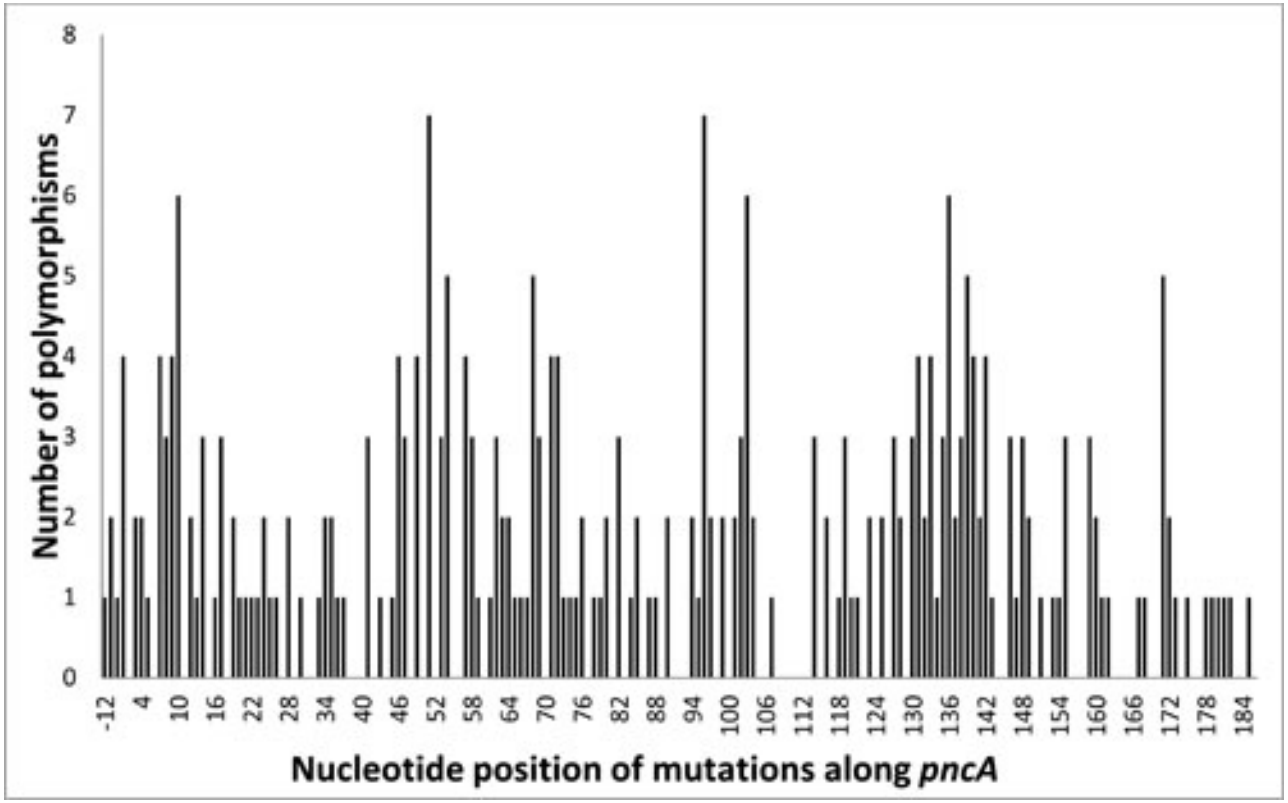
- PZA not active at neutral pH, active at acid pH (McDermott, 1954)
- MIC = 50-100 μg/ml (pH5.5-6.0), poor activity for growing bacilli
- PZA kills non-growing persisters (Zhang et al., 2002), under hypoxic/anaerobic conditions (Wade and Zhang, 2004), more active against RIF-persisters (Hu, Coates and Mitchison, 2006), starvation
- In vivo, impressive sterilizing activity →shortening therapy in mice (McDermott 1956)
- EBA studies in humans and in mice: INH has high EBA in 2 days, PZA low EBA in first 2 weeks (Jindani and Mitchison), BUT in combination PZA kills persisters even during early stage (Grosset et al., 2012, PNAS)

- PZA is opposite to common antibiotics

# *pncA* Mutations: Major Mechanism of PZA Resistance

(Scorpio A and Zhang Y, 1996, Nature Med)

- Mutations in *pncA* : 72-99% (85%), mutations are highly diverse
- A few low level PZA-R no *pncA* mutations




(Zhang et al., 2014, Microbiol Spec, 2: doi:10.1128)

# Detection of *pncA* Mutations as a Rapid Test for PZA Resistance

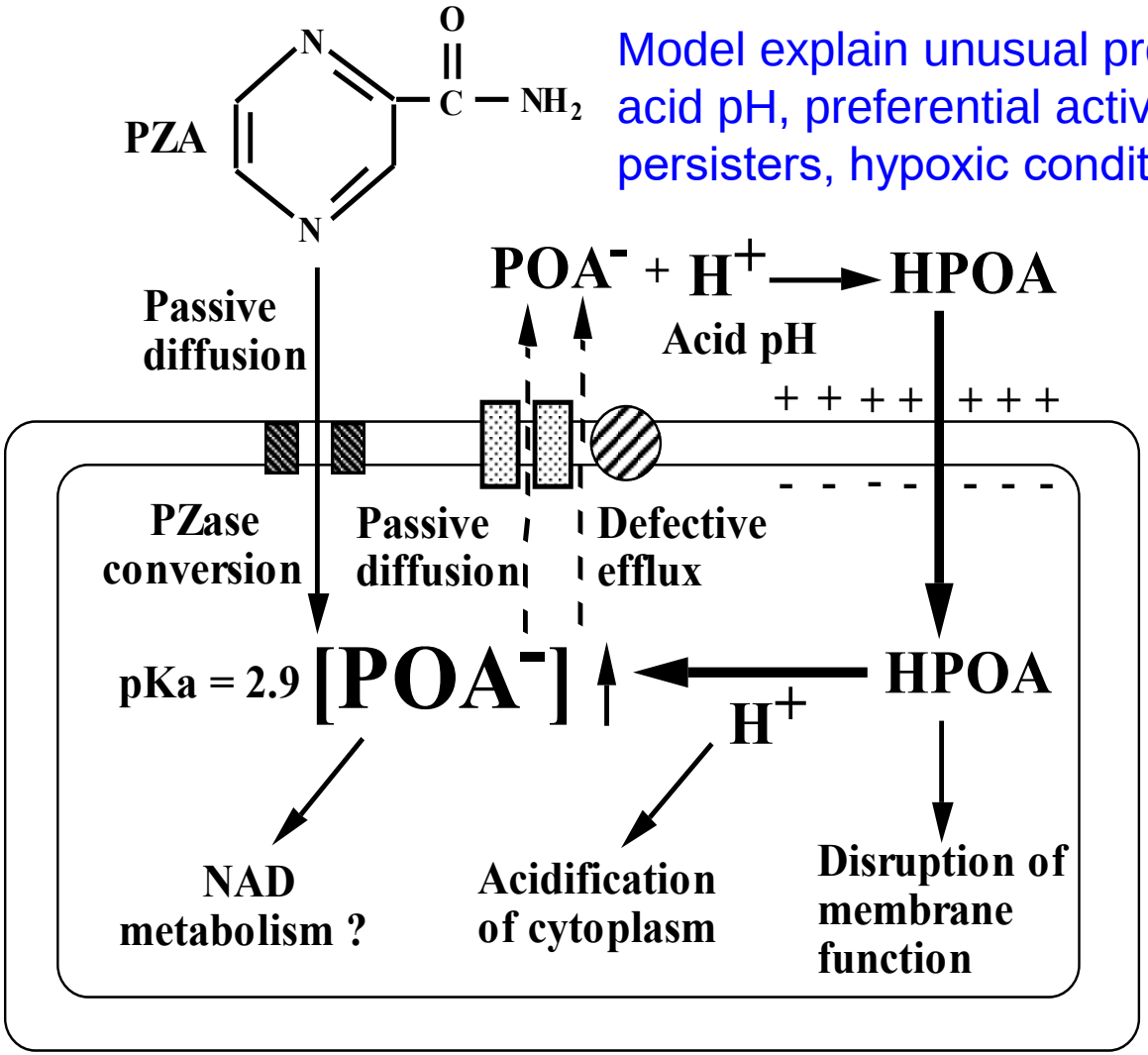
- PZA DST not performed routinely, acid pH, inoculum size, resistance surveys no PZA-R data
- MGIT960 test at pH 6.0 MIC 100 μg/ml prone to false resistance, takes 2 weeks, expensive, not widely used
- Sequencing *pncA* (560 bp): rapid PZA DST, good correlation between *pncA* mutations and PZA-R (85%)
- Mayo Clinic in US

# How Does PZA Work?

- PZA is a prodrug activated by PncA to POA (Scorpio & Zhang 1996)
- Role of acid pH (Zhang et al., 1999)
- Henderson-Hasselbalch equation showing relation pH and PZA activity (Zhang et al., 2002)
- PZA kills old, dormant bacilli (starvation) more effectively than growing bacilli (Zhang et al., 2002), persisters more effectively under hypoxic/anaerobic conditions (Wade and Zhang, 2004)
- POA disrupts membrane potential, inhibits transport (Zhang et al., 2003)

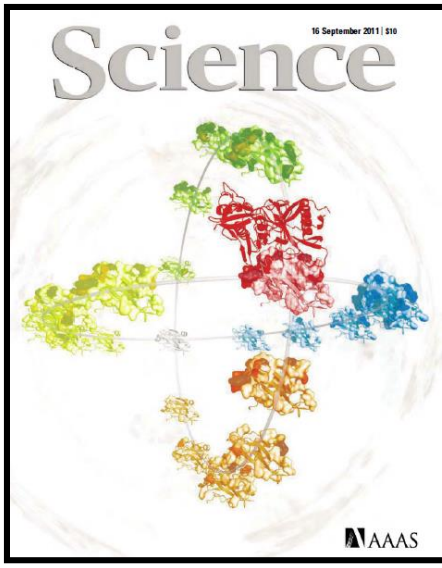
# Mode of Action of PZA

**(Zhang et al., J. Antimicrob. Chemother. 2003, 52:790-5)**



# A New Target of PZA: RpsA

**(Shi et al. Science, 2011, 333: 1630-2)**




REPORTS

## Pyrazinamide Inhibits Trans-Translation in *Mycobacterium tuberculosis*

Wanliang Shi,[1] Xuelian Zhang,[2] Xin Jiang,[3] Haiming Yuan,[2] Jong Seok Lee,[4] Clifton E. Barry 3rd,[5] Honghai Wang,[2] Wenhong Zhang,[6] Ying Zhang[1,6]*

- A new target of PZA: POA binds RpsA (S1 protein)
- RpsA overexpression conferred 5-fold PZA resistance from 100 to 500 μg/ml
- A low level PZA-resistant *M. tuberculosis* DHM444 (MIC 200-300 μg/ml PZA) without *pncA* mutation (Scorpio et al. 1997), contained 3-bp deletion (ΔGCC) Alanine missing in C-terminus of RpsA

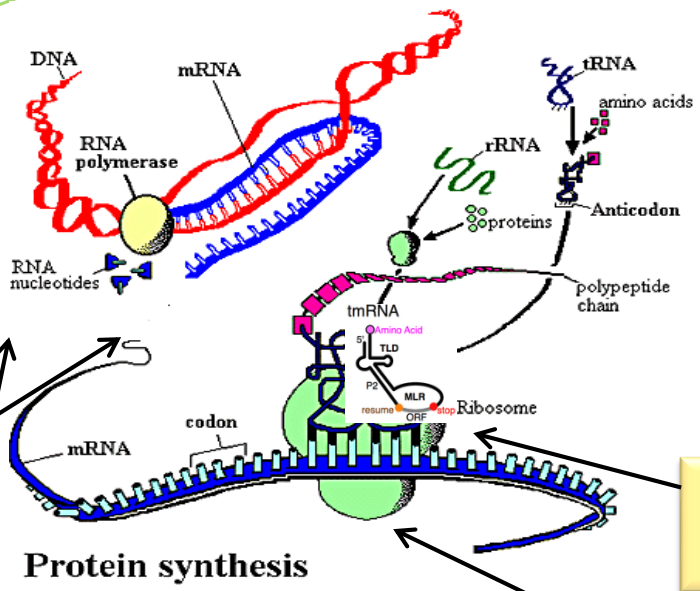
# Persister Drug Pyrazinamide (PZA) Acts Differntly

**Inhibition of cell wall synthesis**
- Beta-lactams
- Glycopeptides

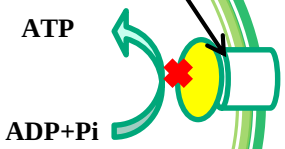
**Disruption of membrane permeability**
- Polymyxin B
- Daptomycin

**Anti-metabolite**
- Sulfa drugs

THFA
DHFA
PABA




**Inhibition of Energy Production**




**Inhibition of trans-translation**

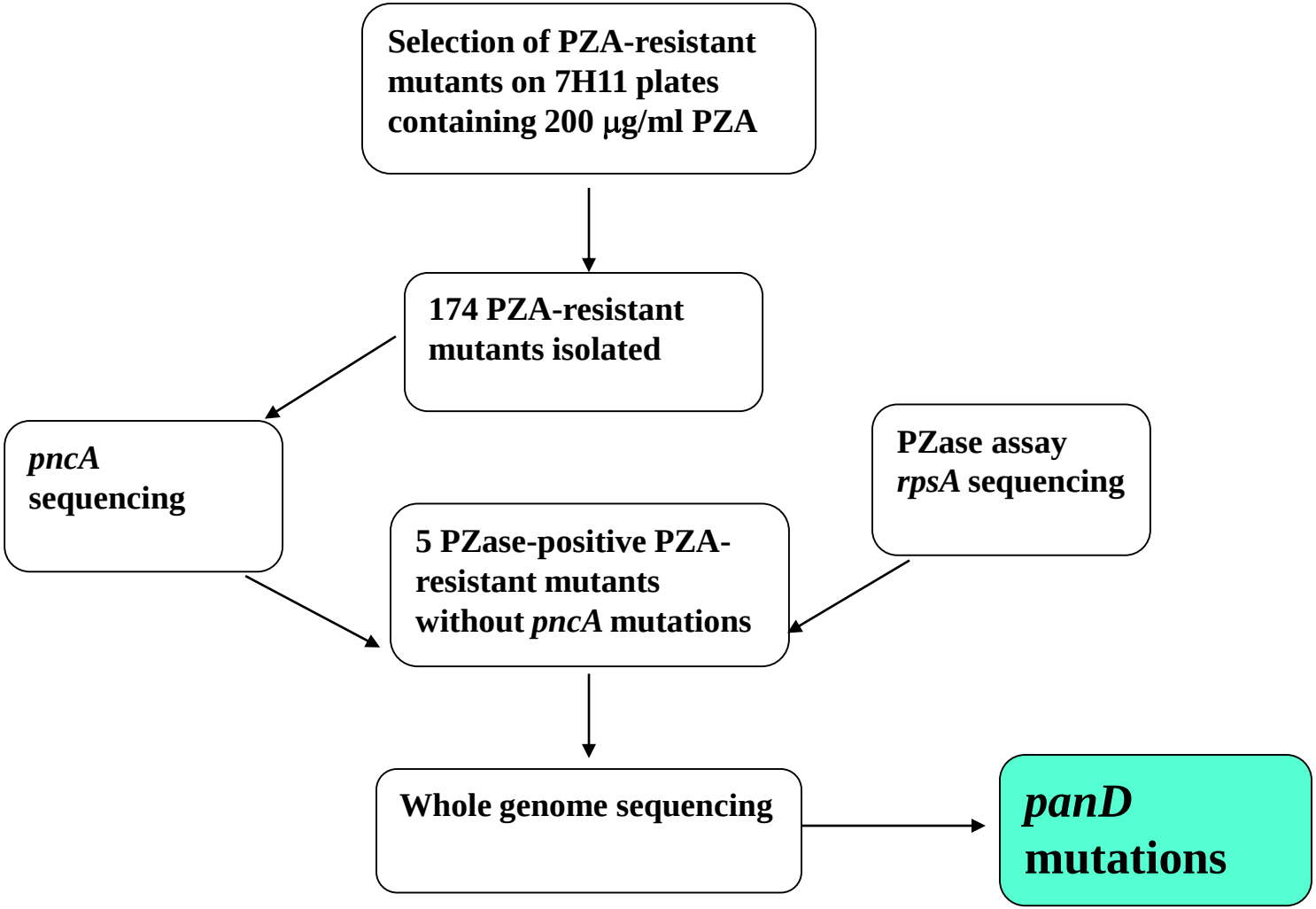
**Inhibition of nucleic acid synthesis**
- Quinolones
- Rifampin

**Inhibition of PanD: Pantothenate (Vit B5) CoA synthesis**

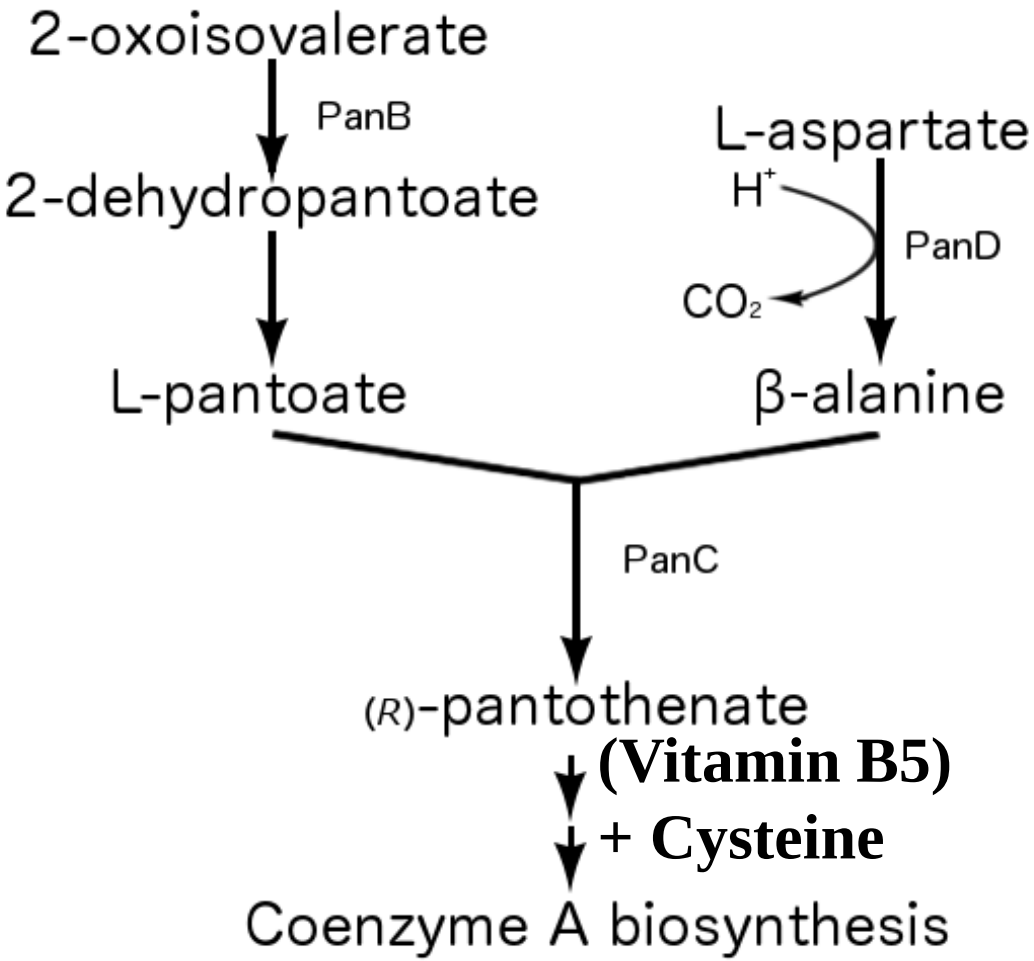
**Inhibition of protein synthesis**
- Aminoglycoside
- Tetracycline
- Macrolides

# *panD*: New Mechanism of PZA Resistance not Mediated by *pncA* or *rpsA* Mutations

**(Emerging Microbes & Infections (2013), June 12, 2: e34)**
**http://www.nature.com/emi/journal/v2/n6/abs/emi201338a.html**

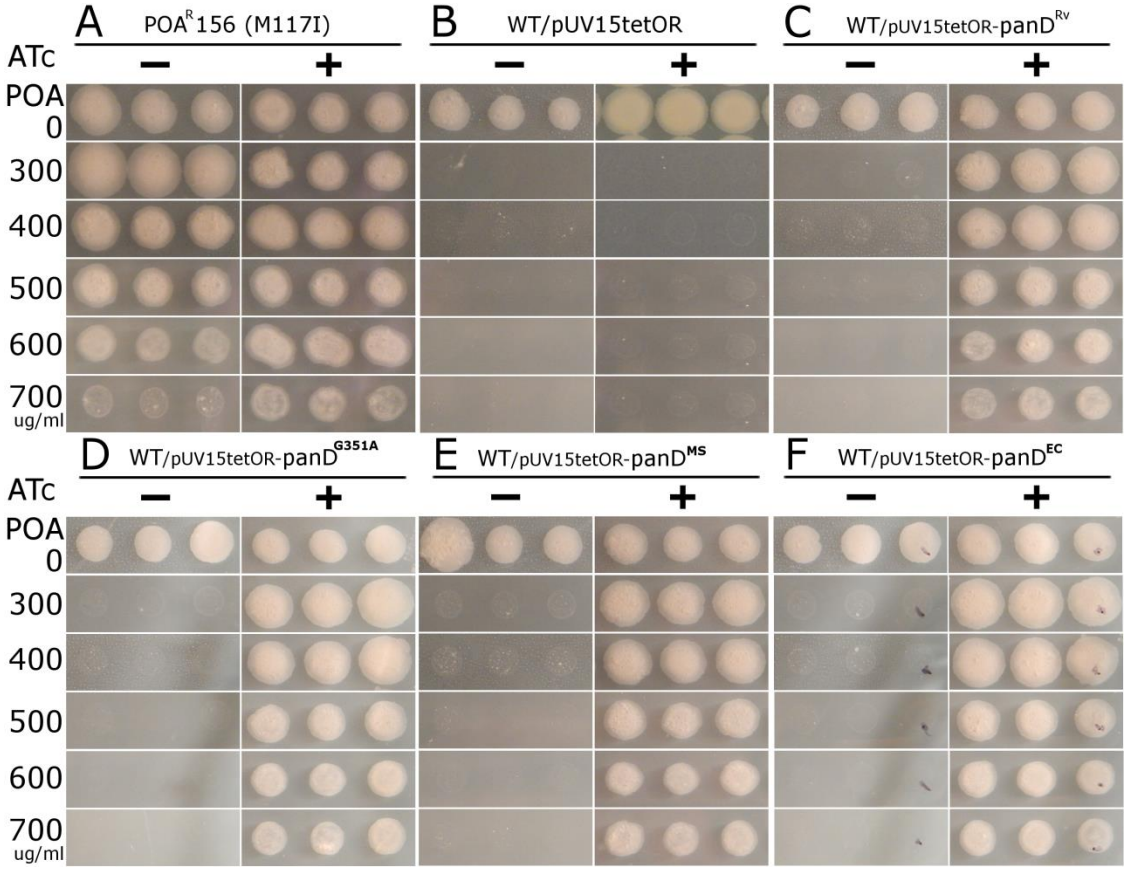


# PanD and Synthesis of β-alanine: Precursor for Pantothenate (Vitamin B5) and Coenzyme A Biosynthesis



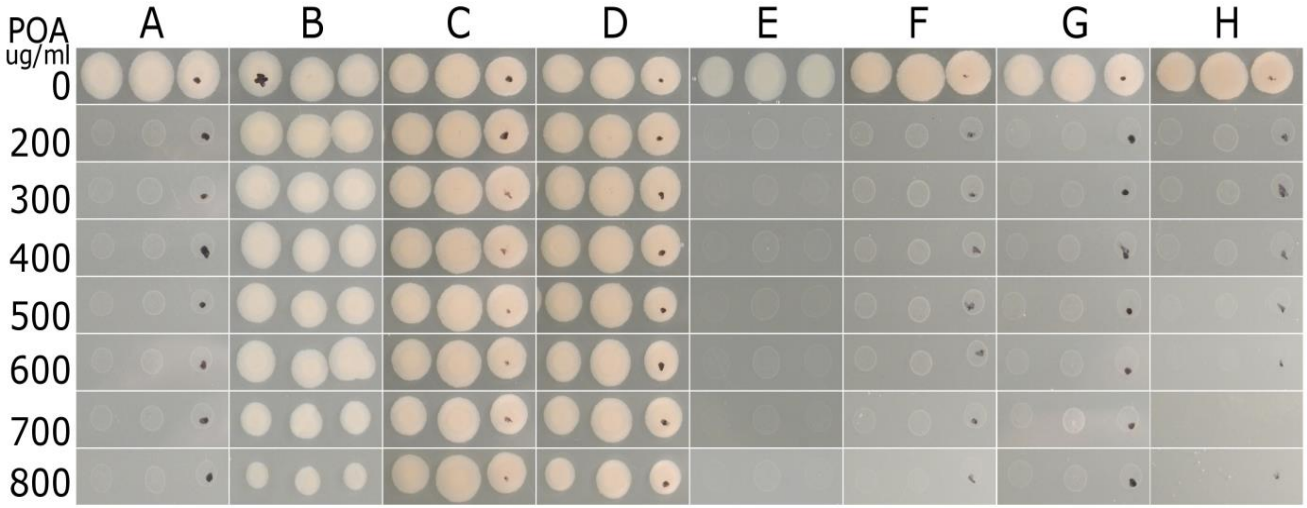

**CoA: central metabolism:**
**TCA cycle, energy production,**
**fatty acid synthesis/oxidation**

# POA Resistance Caused by Overexpression of *panD* from *Mtb*, *Msmeg*, *E. coli* Induced by ATc




**Shi et al., Emerging Microbes & Infections (2014) 3, e58; doi:10.1038/emi.2014.61**
**Published online 13 August 2014**

# Antagonism of POA activity by pantothenate and β-alanine




**(A) *M. tuberculosis* H37Ra, (B) POA resistant mutant with PanD mutation (M117I), (C) Parent strain on 7H11 agar containing 0.1mM pantothenate, (D) Parent strain with 0.1mM β-alanine, (E) Parent strain with 0.1mM L-alanine, (F) Parent strain with 5mM L-aspartate, (G) Parent strain with 10mM L-valine, (H) Parent strain with 10mM glutamate.**

# PZA and New TB Drug Candidates – Indispensable, Synergy

FDA approved drugs:
-Rifapentine:
-Linezolid: Phase I and II trials
Drug candidates under clinical development:
-Moxifloxacin/gatifloxacin, Phase III
-Bedaquiline (TMC207): Phase III trial (MDR-TB)
-Nitroimidazoles: PA-824 and OPC-67683, Phase III
-Ethambutol analog, SQ-109, Phase II

Limitation of current drug candidates: None can replace PZA

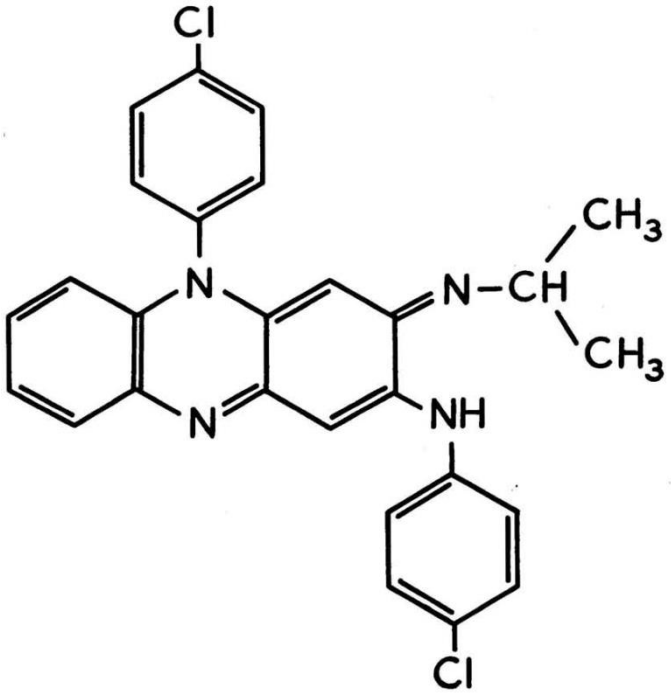
CPTR: Build new regimens: PZA + TMC207 or PA-824 +…

# Summary

- PZA is a paradoxical persister drug, poor in vitro activity, high in vivo activity, shorten therapy
- PZA activity is enhanced by acid pH, low oxygen, starvation, energy inhibitors
- Mutations in *pncA (rpsA, panD, clpC1)* cause PZA resistance
- PZA inhibits multiple targets (energy production, trans-translation, CoA, protein degradation) (unique)
- Renewed interest in PZA, important component for new regimens for shortening treatment

# Clofazimine

- Phenazine, Barry, et al, (1957, Nature, 179:1013-5)
- Treatment for leprosy (1959 YT Chang @NIH), NTM, MDR-TB
- Active against mycobacteria, both slowly and rapidly growing, other Gram-positive bacteria, with MICs of 0.5–2 mg/L (Mtb MIC=0.25 ug/ml)
- Bacteriostatic or bactericidal
- No EBA (A Diacon, AJRCCM, 2015)
- Poorly soluble, brownish skin pigmentation




CLOFAZIMINE (B. 663)

# 9-month Bangladesh Regimen for MDR-TB

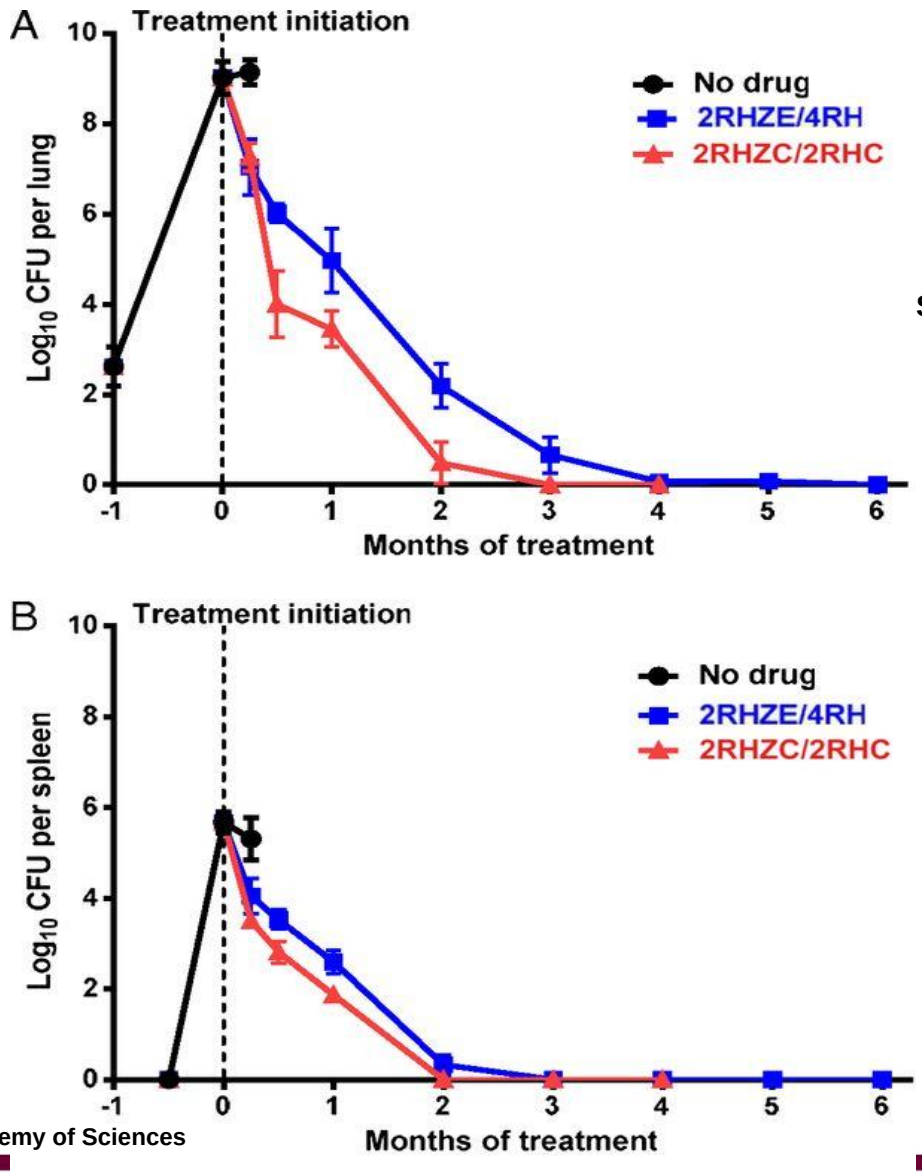
Am J Respir Crit Care Med, 2010. 182(5):684–92.

**May 12, 2016, WHO recommends shortened 9-month treatment regimen for MDR-TB**

- 4 month KCGEHZP + 5 month GEZC → 83% relapse-free cure in MDR-TB vs 48% cure with WHO regimen
- K=kanamycin; C=clofazimine; G=gatifloxacin; E=ethambutol; H=high-dose isoniazid; Z=pyrazinamide; P=prothionamide
- Tang S et al. 2015, CID, 74% vs 54% success, MDR,

Culture conversion/cavity closure sooner

**Effect of CFZ treatment on cfu counts in the lungs and spleens of M. tuberculosis-infected mice: (A) mean cfu counts in mouse lungs and (B) mean cfu counts in the mouse spleens the day after infection (month −1), the day of treatment initiation (day 0)**




Sandeep Tyagi et al. PNAS 2015;112:869-874

# Mode of Action of Clofazimine

- CFZ-mediated Redox Cycling and ROS Production (Yano et al. JBC, 2011;286:10276)
- Membrane destablization/dysfunction (inhibition of K+ transport)(van Rensburg, 1992, AAC, 36: 2729–35), leading to energy depletion
- CFZ may bind DNA (Morrison 1976. *Int. J. Lepr.* 44: 133–135)

# CFZ Resistance

- Mutation in *rv0678* (S68G mutation), encoding a transcription repressor of efflux pump MmpS5-MmpL5, cause overexpression of efflux pump → cross-resistance to CFZ and bedaquiline (BDQ) (Hartkoorn and Cole, AAC; 2014)
- Mutation in *rv0678* in BDQ resistant isolates cross-resistant to CFZ (Andries et al. 2014, PLoS One)

# Mutation analysis of 96 clofazimine-resistant mutants of *M. tb*

- *rv0678: 97% had mutations;* 193 G insertion mutation and A202G (S68G); 28 new mutations
- *rv1979c* T1052C mutation only and two mutants had *rv1979c* T1052C mutation + *rv0678* G193ins
- *rv2535c: stop codon E89**

Zhang S et al., J. Antimicrob. Chemother. (2015) 70 (9): 2507-2510.

| Gene | Nucleotide change | Amino acid change | Mutant count |
|---|---|---|---|
| *rv0678* | G193 deletion | 65 codon shift | 23 |
| *rv0678* | G193 insertion[a] | 65 codon shift | 21 |
| *rv0678* | C466T | R156[b] | 11 |
| *rv0678* | C364 insertion | 122 codon shift | 5 |
| *rv0678* | A202G[a] | S68G | 5 |
| *rv0678* | T2C | start codon mutation | 2 |
| *rv0678* | T29 insertion | 10 codon shift | 1 |
| *rv0678* | G58T | V20F | 1 |
| *rv0678* | C98A | T33N | 1 |
| *rv0678* | C107T | A36V | 1 |
| *rv0678* | G125A | W42[b] | 1 |
| *rv0678* | T128G | L43R | 1 |
| *rv0678* | G137A | C46Y | 2 |
| *rv0678* | A152G | Q51R | 1 |
| *rv0678* | C158T | S53L | 1 |
| *rv0678* | C176T | A59V | 1 |
| *rv0678* | G188A | S63N | 1 |
| *rv0678* | G194A | G65E | 2 |
| *rv0678* | G197T | G66V | 1 |
| *rv0678* | C226T | Q76[b] | 1 |
| *rv0678* | C251A | A84E | 1 |
| *rv0678* | G266T | R89L | 1 |
| *rv0678* | G269C | R90P | 1 |
| *rv0678* | A292 deletion | 98 codon shift | 1 |
| *rv0678* | G304A | A102T | 1 |
| *rv0678* | C305T | A102V | 2 |
| *rv0678* | T341C | L114P | 1 |
| *rv0678* | T365C | L122P | 1 |
| *rv0678* | CGCTGGGC371–378 deletion | 124 codon shift | 1 |
| *rv0678* | CG444–445 deletion | 148 codon shift | 1 |
| *rv1979c* | T1052C | V351A | 3[c] |
| *rv2535c* | G265T | E89[b] | 1 |

# Two New Genes involved in CFZ Resistance

Zhang S et al., J. Antimicrob. Chemother. (2015) 70 (9): 2507-2510.

- Rv1979c (V351A), a possible permease involved in amino acid/CFZ transport

- Rv2535c (stop codon at E89*), a putative peptidase PepQ

  pepQ mutants selected in mice treated with bedaquiline, with or without clofazimine, cross-resistance to bedaquiline and clofazimine MICs 4 times higher than H37Rv (Almeida D et al., 2016, Antimicrob Agents Chemother. 2016 May 16. pii: AAC.00753-16)

# Summary

- CFZ is a promising drug for shortening treatment of both drug susceptible TB and MDR-TB
- Weak activity on its own but in combination with other drugs higher cidal/sterilizing
- Mode of action complex, target unknown
- Mutations in Rv0678 major mechanism of CFZ resistance; Rv1979c and Rv2535c mutations rare; mutations in Rv0678 and Rv1979 cause cross-resistance to CFZ and BDQ
- Future studies to determine frequency of clinical CFZ resistance, assess the relevance of low level resistance clinically, new analogs less side effect/more bioavailable, better understand MOA/drug target

# Why treat TB this long (6 months)?

(a) Persisters

(b) Current TB drugs not good enough!!!
Mainly active against growing bacilli, except PZA, RIF, cannot kill all bacteria (persisters). Drugs that kill persisters shorten therapy.

New drug combos with new agents (Z+ other drugs, PaMZ, etc.)

Challenge: Develop drugs better than PZA, kill persisters and shorten therapy to a few weeks, not 6 months

Model is key: Not easy

# Targeting Persisters for More Effective Cure

- Develop new persister drugs

- Whole cell based: persister screens in vitro; Macrophages

- Target based: Energy production (ATP) inhibitors: Novartis

- Novel drug combos:

- Host immune control (therapeutic vaccines): Host Directed Therapy

# Summary/Future Directions

- TB drugs, chemotherapy, how TB develop drug resistance
- Two types of resistance and mechanisms: Drug Resistance and Persistence
- Shorten MDR-TB treatment: Bangladesh regimen; $Z^S$-MDR vs $Z^R$-MDR; new combinations
- Importance of PZA, CFZ
- LTBI: high risk LTBI detection and targeted prophylaxis
- New TB → Cure！ (Bee Hive!)
- Develop new drugs targeting PERSISTERS to shorten therapy (TB, MDR)


Thank you