Genotypic-Phenotypic DST Correlations and Identification of Reinfection in Trials

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TB ALLIANCE

Outline of Discussion

1. Concept of genotypic – phenotypic correlations

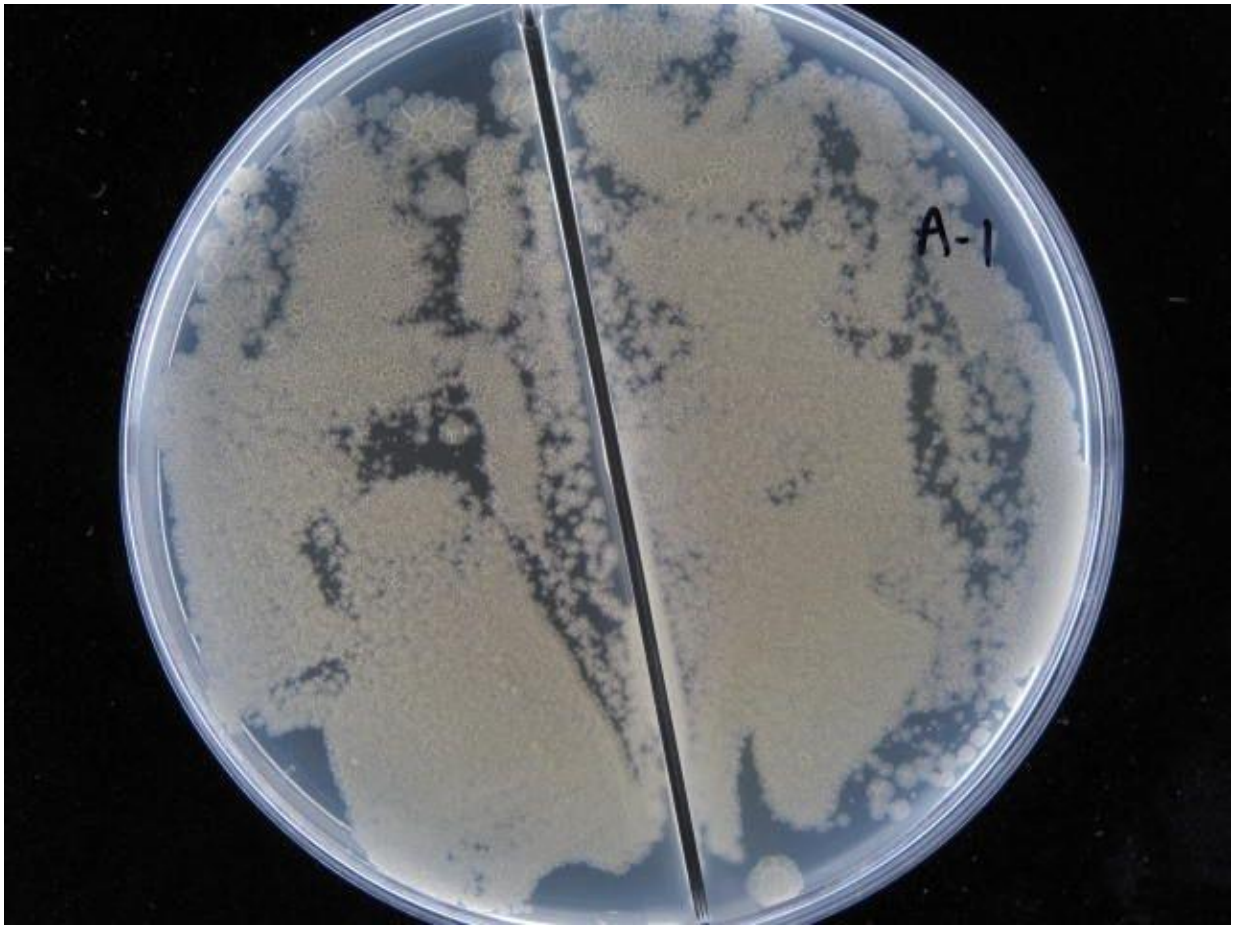
2. Whole Genome Sequencing to Identify Relapse vs Reinfection in Trials

3. Case Study of Relapse in the Nix-TB Trial

Concepts for Drug Susceptibility Testing

- Phenotypic Test
  - Requires cultures to grow
- Genotypic Test
  - “Rapid molecular test”
- Whole Genome Sequencing
  - Typically a research laboratory
  - Not for the rural health post!

The Tuberculosis Bacteria from a Sputum Sample Growing in a Culture



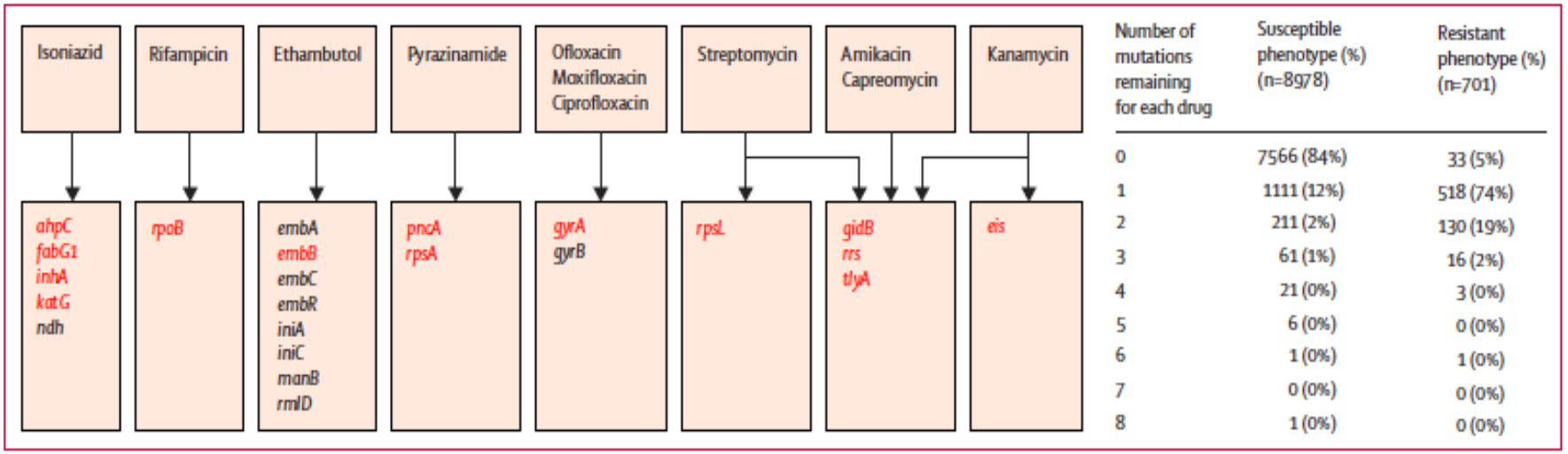
TB Colony Forming Units "CFUs" – Now Countable – from a Diluted Sputum Specimen

Concepts in Phenotypic DST

- Minimum Inhibitory Concentration
  - For an individual isolate
  - MIC90 – for a population of isolates
- Epidemiologic Cut-off Value (ECV), or Critical Concentration
  - Separates wild-type from non-wild type population
  - E.g. ECV 95% may be called “susceptible”
- Clinical Breakpoint
  - Not defined for all drugs
  - US FDA Product Label for Bedaquiline:

    “The actual MIC should be reported. A specialist in drug-resistant TB should be consulted in evaluating therapeutic options.”

Genes, mutations and phenotype



Number of mutations remaining for each drug	Susceptible phenotype (%) (n=8978)	Resistant phenotype (%) (n=701)
0	7566 (84%)	33 (5%)
1	1111 (12%)	518 (74%)
2	211 (2%)	130 (19%)
3	61 (1%)	16 (2%)
4	21 (0%)	3 (0%)
5	6 (0%)	0 (0%)
6	1 (0%)	1 (0%)
7	0 (0%)	0 (0%)
8	1 (0%)	0 (0%)

Figure 1: Candidate genes and mutations

The number of potentially predictive mutations in genes relevant to each drug after lineage-defining and synonymous mutations have been set aside and are shown by susceptible and resistant phenotypes for 2099 training-set isolates. Genes from which one or more of the 120 resistance-determining mutations were algorithmically characterised are coloured red.

Walker TM et al. Lancet Infect Dis ***2015*** *June 24, 2015*
http://dx.doi.org/10.1016/ S1473-3099(15)00062-6

WGS in the REMox Trial to Distinguish Relapse from Re-infection

- REMox TB Trial
  - Trial in patients with DS-TB
    - 6 months dosing of HRZE vs 4 months of a regimen substituting in moxifloxacin
  - Pairs of isolates – one at baseline and one at failed Rx at 17 weeks or later recurrence
    - 50 pairs of isolates (11 HIV+)
    - None showed resistance to study drugs

Bryant JM et al. Lancet Respir Med 2013. 1:786-92

REMox WGS of 50 Pairs of Isolates

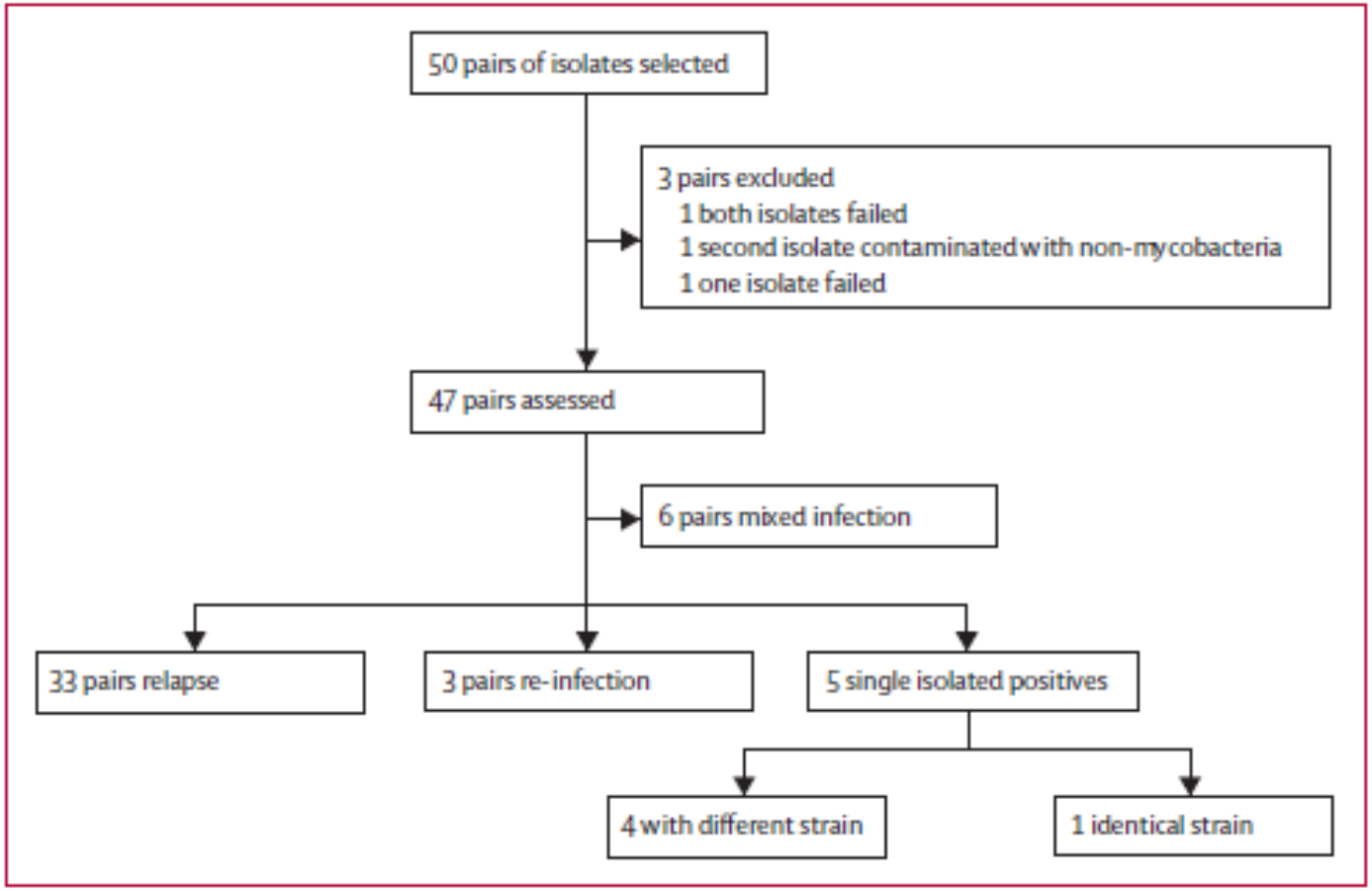


Figure 1: **Distribution of the case outcomes for study patients based on sequencing quality data, sequence comparison, and clinical evaluation**

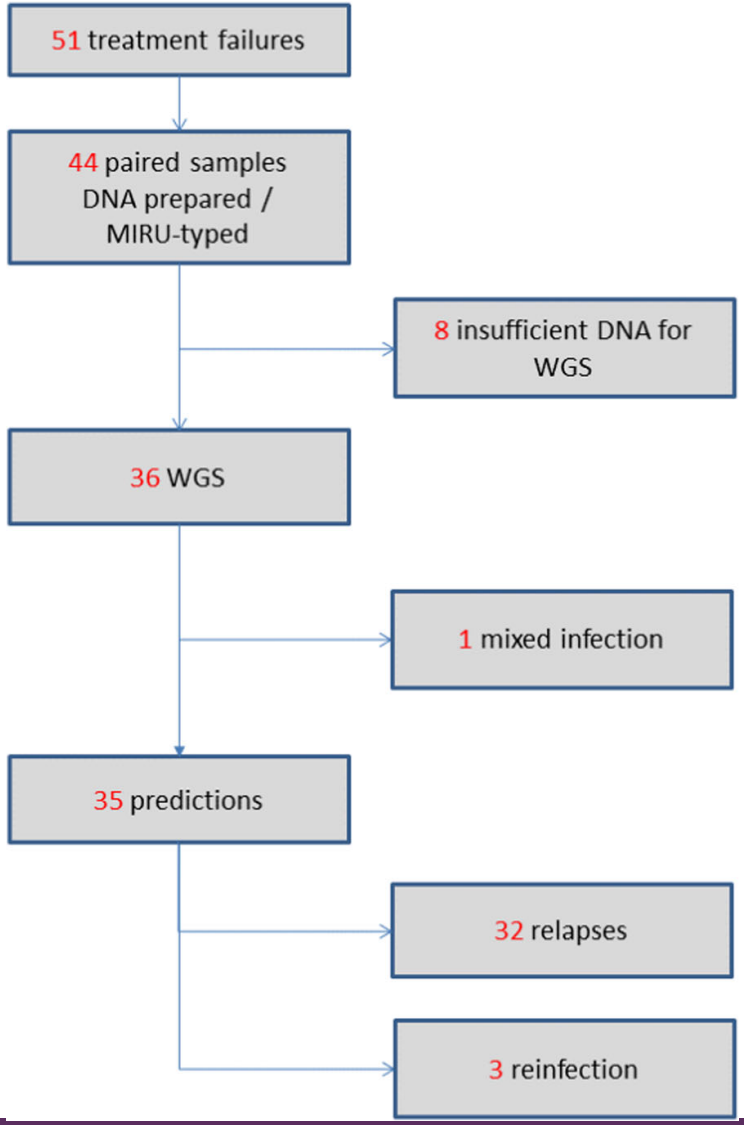
- 33 Relapses
  - Pairs differing by mean of 0.47 SNPs (all with $\leq$ 6 SNPs)
- 3 Re-infections
  - All had more than 1306 differences (mean SNP distance 1355 between isolates)

WGS in RIFAQUIN Trial to Distinguish Relapse from Reinfection

- Patients with DS TB
- Standard 6 month HRZE regimen
  - vs 4 mo regimen with moxifloxacin replacing INH and rifapentine
  - vs 6 mo regimen with moxifloxacin replacing INH and high dose rifapentine

Witney AA et al. BMC Medicine 2017. 15:71

WGS in RIFAQUIN Trial to Distinguish Relapse from Reinfection



N=32 with ≤ 5 SNP differences
N=3 with high # SNP differences
(737 & >1000)

Categorization of Relapse vs Re-infection in a Trial

Based on Currently Available Data

- Thresholds used in TB Alliance trials to categorize:
  - ≤ 12 SNPs different = Relapse
  - > 12 and < 100 SNPs = Indeterminant, to be evaluated case by case
    - Note that in our trials we have not yet seen an “indeterminant” value
  - ≥ 100 SNPs = Reinfection

Case Study from the Nix-TB Trial

- **Protocol Title: A Phase 3 open-label trial assessing the safety and efficacy of bedaquiline plus pretomanid plus linezolid in Subjects with pulmonary infection of either extensively drug-resistant tuberculosis (XDR-TB) or treatment intolerant / non-responsive multi-drug resistant tuberculosis (MDR-TB)**

Nix-TB – Microbiological Characterization

Trial Inclusion Criteria of Note

- For Participants with XDR-TB
  - documented culture positive (for *M.tb.*) results within 3 months prior to screening or
  - *M.tb.* confirmed in sputum based on molecular test within 3 months prior to or at screening
  - Historical documented resistance to isoniazid, rifamycins, a fluoroquinolone and an injectable at any time;

Mycobacteriology Characterization:

- *M.tb*. isolates at baseline and initial relapse (first positive at end of treatment or during follow-up) will be processed at the central lab (UCL in London) for:
  - MIC of bedaquiline, pretomanid and linezolid
  - Drug Susceptibility Testing in liquid culture for rifampicin, isoniazid, streptomycin, ethambutol, kanamycin and moxifloxacin
  - Extraction of bacterial (*M.tb*) DNA for whole genome sequencing

Nix-TB Trial Participant 01-9026-018 with Relapse

- 55 year old male with HIV co-infection (CD4 = 67, viral load = 87 on screening)
- DS TB since 2009
- XDR TB since 15 Oct 2015
  - Prior failed treatment with:
    - Levofloxacin, terizidone, pyrazinamide, amox/clav, PAS and clofazimine
- Screening smear positive
  - Hain LPA confirming MTB resistant to INH and Rif
  - Baseline Culture positive for *M.tb.*
- Enrolled with first dose of trial regimen 29 Jan 2016
  - Last dose of trial regimen 29 July 2016 (183 days – 26 weeks)
    - No interruptions of B-Pa
    - Linezolid decreased from 600 mg bid to 600 mg qd May 10, 2016 (at about month 3.5) for anemia

DST History and Trial Culture Results

Historical DST – from Provincial Lab

Drug	Method	Date	Result
Isoniazid	Molecular	15 OCT 2015	Resistant
Kanamycin	Molecular	15 OCT 2015	Resistant
Ofloxacin	Molecular	15 OCT 2015	Resistant
Rifampicin	Molecular	15 OCT 2015	Resistant

Sputum Culture History – in MGIT

- Baseline Positive
  - Positive 2 samples at Weeks 1, 2, 4, 6 and 8
  - Negative 2 samples at Weeks 12, 16, 26, and Follow up off therapy at Month 1 and 2
  - New positive, confirmed MTB at follow up Month 3 with multiple positive confirmations

Phenotypic Profile from Central Lab at UCL

Baseline DST - MGIT Liquid Culture

Drug	Date	Test date	DST MGIT Result
Ethambutol	28JAN2016	16FEB2017	Resistant
Isoniazid	28JAN2016	16FEB2017	Resistant
Kanamycin	28JAN2016	16FEB2017	Resistant
Moxifloxacin	28JAN2016	13OCT2017	Resistant
Pyrazinamide	28JAN2016	16FEB2017	Resistant
Rifampicin	28JAN2016	16FEB2017	Resistant
Streptomycin	28JAN2016	16FEB2017	Resistant

Post Week 16 DST - MGIT Liquid Culture

Drug	Visit	Date	Result
Ethambutol	Fup M 3	20OCT2016	Resistant
Isoniazid	Fup M 3	20OCT2016	Resistant
Kanamycin	Fup M 3	20OCT2016	Resistant
Moxifloxacin	Fup M 3	20OCT2016	Resistant
Pyrazinamide	Fup M 3	20OCT2016	Resistant
Rifampicin	Fup M 3	20OCT2016	Resistant
Streptomycin	Fup M 3	20OCT2016	Resistant

MIC of Trial Drugs at Baseline and F/U Month 3

Method is MGIT Liquid Culture
At University College London

Isolate	Bedaquiline (µg/ml)	Linezolid (µg/ml)	Pretomanid (µg/ml)
Baseline (Day 1)	0.5	0.5	0.12
F/U Month 3	4*	0.5	0.12

Phenotypic DST from NICD National Lab, South Africa

Same Results on Baseline and Follow up Month 3 Isolates:

- Extended testing on panel of 17 drugs
- Resistant to all except:
  - Linezolid (susceptible below 1.0 ug/uL)
  - p-aminosalicylic acid (susceptible below 4.0 ug/uL)

WGS at NICD to Predict Resistance – Baseline Isolate

Gene	Mutation	Genotypic prediction (high confidence)
Rifampicin/Rifabutin		
*rpo*B	Ser450Leu	Rifampicin/Rifabutin resistant
Isoniazid		
*inh*A promoter	wt	Isoniazid resistant
*kat*G	Ser315Thr	
Ethambutol		
*emb*B	Met306Val	Ethambutol resistant
Pyrazinamide		
*pnc*A	Met175Val	Pyrazinamide resistant
Fluoroquinolones		
*gyr*A	Asp94Gly	Fluoroquinolone resistant
*gyr*B	wt	
Aminoglycosides		
rrs	G1401A	Aminoglycoside resistant
*rps*L	Lys43Arg	
Linezolid		
rrl	wt	*
*rpl*C	Thr41Ala	
Bedaquiline		
*atp*E	wt	Refer to phenotypic testing
*pep*Q	wt	
Rv1979c	wt	
Rv0678	wt	

*Insufficient evidence that this mutation is a resistance determinant

WGS at NICD to Predict Resistance – Month 3 f/u Isolate

The only difference from baseline: mutation in the Rv0678 gene

- Correlates with an increase the MICs for both bedaquiline and clofazimine

Bedaquiline		
atpE	wt	Refer to phenotypic testing
pepQ	wt	
Rv1979c	wt	
Rv0678	138_139insG	*

*Insufficient evidence that this mutation is a resistance determinant

Bedaquiline MIC and the *Rv0678* Mutation

- Study of 387 BDQ naïve patients in South Africa
  - MGIT Epidemiologic cutoff values determined
    - ≤ 1 ug/mL susceptible; ≤ 2 ug/mL intermediate
  - Note, no clinical breakpoint has been determined
  - 3 with the *Rv0678* mutation
    - Codes a drug efflux pump regulator
    - Correlates with increased MIC to bedaquiline and clofazimine
    - 2 had MIC ≤ 1 and 1 had MIC ≤ 2

Ismail, N.A., et al., Defining Bedaquiline Susceptibility, Resistance, Cross-Resistance and Associated Genetic Determinants: A Retrospective Cohort Study, EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.01.005

WGS to Determine Relapse vs Re-infection

- Thresholds specified in SAP to categorize:
  - ≤ 12 SNPs different = Relapse
  - > 12 and < 100 SNPs = Indeterminant, to be evaluated case by case
  - ≥ 100 SNPs = Reinfection
- Nix WGS conducted through University College London
  - Methods as described in: *Whitney A et al. BMC Medicine 2017*
- WGS of Baseline and Month 3 followup Isolate on Patient 01-9026-018:
  - 5 SNPs different; thus a Relapse

Note that no Regulatory Authority to date has agreed that a "re-infection" based on a difference in WGS from baseline may be considered not to be a failure (relapse) in the review of an investigational drug for market approval

Thank You!

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