Educational Workshop

EW01: A new threat from an old foe: XDR tuberculosis
arranged with ASM (American Society for Microbiology)

Convenors: William Bishai, Baltimore, US
Achim Schwenk, London, UK

Faculty: Veronique Vincent, Geneva, CH
Shaheen Mehtar, Tygerberg, ZA
William Bishai, Baltimore, US
Clifton E. Barry III, Rockville, US
Phenotypic tests

- **Solid medium (egg-based, agar-based)**
  - Resistance defined by critical proportion of growth in drug-containing media
    - Proportion method
    - Absolute concentration method
    - Resistance ratio method

- **Liquid medium**
  - Resistance defined by selective growth in the presence of drug
    - BACTEC (Becton Dickinson)
    - MGIT (Becton Dickinson)
    - MBBacT (bioMérieux)
    - Bacti-Alert (Organon Technika)
Solid versus liquid media

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity AFB/ ml</th>
<th>Time per Test</th>
<th>Time DST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>&gt; 5,000</td>
<td>2 h</td>
<td></td>
</tr>
<tr>
<td>Culture solid</td>
<td>~ 100</td>
<td>16 d smear + 29 d smear -</td>
<td>7 weeks</td>
</tr>
<tr>
<td>Culture liquid</td>
<td>~ 10</td>
<td>8 d smear + 16 d smear -</td>
<td>2 weeks smear+ 4 weeks smear-</td>
</tr>
</tbody>
</table>

Liquid culture increases sensitivity (smear-) and reduces delays.

Culture in liquid media

Liquid medium Bactec radiometric

Liquid medium Non radioactive

Automates for liquid culture
First-line DST methods

- Methodology for 1st-line drugs (H, R, S, E) well-established and standardised
- Results very similar using different methodologies, including liquid medium
- Performance characteristics well-described
- WHO recommendations for the use of liquid cultures

DST inaccuracy: Sources

- Drug powder quality, storage & expiry
- Correct drug concentration: Potency!
- Drug dissolution and dilution
- Medium preparation (heating) and storage
- Strain viability
- Inoculum size & dispersion
- Too early reading
- Neglect to use positive and negative controls

QA is essential

Tuberculosis supranational reference laboratory network, 2006
Role of the NRL

- NRL performs DST
- specimens transported to the NRL for culture and DST
- if regional culture labs, cultures transported to the NRL
- NRL ensures the quality of microscopy / culture/ DST within the lab network
- establishes a regular on-site supervision programme
- provides training and QA for lab procedures
- Critical issue: HR, adequate training, adequate staffing
- In countries where culture/DST is decentralized, DST results have to be QA by the NRL

Risk of occupational tuberculosis in National Tuberculosis Programme laboratories in Korea


TB developed among 7/15 DST technicians compared to only 2/59 culture/non-DST technicians. Compared to non-laboratory workers, the relative risk for DST technicians was 21.5 (95%CI 4.5–102.5).

CONCLUSION: DST led to the highest relative risk among all types of mycobacteriology work, while performing smear microscopy alone did not pose an elevated risk compared to clerical work.
Biosafety cabinet

- HEPA filter traps 99.97% of particles
- BSCs should be ducted or vented to the outside
- Whatever the type I or II
- No recirculation allowed into the room

Evaluation of effectiveness

- Operations performed by the manufacturer or qualified professional, with specific equipment well calibrated

BSL 3
The mycobacteriology laboratory
BSL2 or BSL3 facilities?

- The BSL-2 laboratory area: specimen processing for culture in a BSC
- The BSL-3 laboratory area: culture identification, drug susceptibility testing, and other tests that require concentrated cell suspensions.
- Knowledgeable personnel working under close supervision.

Pre-requisites for upgrading BSL-3

For most high burden countries, there are major constraints to the successful establishment, staffing and maintenance of BSL3 labs.

Upgrading of BSL3 should be planned, financed and implemented according to a short-term plan, a responsibility of the country.
Policy Guidance on Drug Susceptibility Testing (DST) of Second-line Anti-Tuberculosis Drugs

DRAFT

The document provides an interim policy framework for the lab component relevant to programmatic implementation of MDR TB strategies.

The document addresses:
- Drug efficacy of SLD
- Reliability and reproducibility of SLD
- Cross resistance
- DST methodology and critical concentrations for SLD

Policy Guidance on Drug Susceptibility Testing (DST) of Second-line Anti-Tuberculosis Drugs

DRAFT

Rational use of DST in programmes for control of drug-resistant TB

As a minimum, lab capacity to reliably detect INH and RIF resistance is a prerequisite.
The use of rapid detection of RIF resistance is recommended in high risk MDR settings.

Routine DST for SLD is not recommended

In order to retain proficiency and expertise, it is recommended that second-line DST only be performed if at least 200 specimens from high-risk patients per year are expected. This implies centralization of laboratory services for SLD or outsourcing such services (e.g. to one of the laboratories in the SRL Network) where programmes involve small numbers of MDR-TB patients.
Rapid tests for DST and new diagnostic tools

- Molecular tests
  - Rapid: few hours
  - Detect resistance on alive and dead bacilli
  - Less sensitive safety issues
  - Limited to detection of RIF INH
  - Require specific training/settings

- Phenotypic tests
  - Slow
  - Detect resistance in viable bacilli
  - Require BSL3
  - Can be used for all drugs

GenoType® MTBDRplus test procedure

1) DNA Extraction
2) Amplification by PCR
3) Hybridization
4) Evaluation

Reaction zones of GenoType® MTBDRplus (examples)
Veronique Vincent
Laboratory diagnosis and drug susceptibility testing in tuberculosis

### Colorimetric tests
(Alamar-blue, MTT & Resazurin)

<table>
<thead>
<tr>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redox-indicators - colour change with oxidation</td>
</tr>
<tr>
<td>MIC - precise for essential drugs</td>
</tr>
<tr>
<td>Inexpensive</td>
</tr>
<tr>
<td>Only from pure culture</td>
</tr>
<tr>
<td>Microtiter plate format, liquid, less safe</td>
</tr>
</tbody>
</table>

- Nitrate reduction detection
  - Precise, inexpensive
  - No special safety problem
- Not properly validated

### Microscopic observation assay
- This assay uses inverted light microscope and Middlebrook 7H9 broth medium to detect mycobacterial growth.
- In a recent large study in Peru it had a median time to culture positivity on average 8 days.
- It may be used for drug susceptibility also.

MOORE DAJ, et al, Microscopic observation drug susceptibility assay, a rapid reliable diagnostic test for MDR suitable for use in resources-poor setting, J Clin Microbiol 2004, 42: 4432 - 4437
New rapid tests

Rapid phenotypic tests:
- Still in development
- Early validation stage or early field demonstration phase
- No rapid identification tests available for SLD

Conclusion

- Molecular tests for the detection of resistance to RIF (+INH) well evaluated on cultures in laboratory based studies
- Current evaluation for feasibility, cost effectiveness and cost-benefit in the field
- Field testing on samples (FIND studies)
- WHO recommendation scheduled in 2008
Shaheen Mehtar
Epidemiology and infection control principles of XDR tuberculosis

Resistant Tuberculosis- a Global and South African review

Prof Shaheen Mehtar
Head Unit of IPC
Tygerberg Hospital & Stellenbosch Uni
Cape Town, S Africa

Nearly 2 million people die from TB every year

Almost 9 million new TB cases occurred in 2004 — 80% of them in 22 countries

TB is curable, but kills 50 000 people every day

Global tuberculosis notification trends
1993 - 2003

Africa Region
South-East-Asia Region
Western Pacific Region
Eastern Mediterranean Region
European Region
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Epidemiology and infection control principles of XDR tuberculosis

**TB in South Africa**

- TB: 930/100,000 pop
- HIV: 27% of total pop

**Tuberculosis Historical Background**

In southern Africa linked to the discovery of diamonds and gold, industrialization and massive migrant labour to the (then) Transvaal republic.

- Western Cape used as a TB sanatorium for European TB patients in 1800’s
- Cape Town always been a “TB hot spot”

**TB 2005**

<table>
<thead>
<tr>
<th>World</th>
<th>South Africa</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.9 m new cases</td>
<td>280,000 new cases</td>
</tr>
<tr>
<td>62 / 100,000 pop</td>
<td>&gt; 900 / 100,000</td>
</tr>
</tbody>
</table>

Current Global Trends
- 2000-2020: Estimated 35 million TB deaths
- Fatality: 7.4% (HIV)

WHO Global Tuberculosis Report 2006

Current SA trends
- 2000-2020: Estimated 5 million TB deaths

Fatality: 7.4% (HIV)
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Western Cape: 2005
- TB burden 47,603 Cases
  - Incidence: > 900/100,000
  - Treatment outcomes NSP
  - Cure 70%, Completion 79%
  - Death 3.2%
  - Failure 1.8%
  - Transfer 3.3%
  - Default 11.8% (19.5% in 1996)
  - High Re-treatment burden: 30%
- MDR Drug Resistance Prevalence
  - MRC Survey 1995 & 2001-2002:
    - Western Cape
      - New: 1%
      - Re-treatment: 4%

The TB pandemic growing and becoming more complicated because of:
- Poverty and overcrowding
- Population dynamics: population growth, migrancy and urbanization
- The spread of HIV/AIDS
- Increase in other risk factors and chronic diseases which lower the body’s defences against TB
- Poorly managed health services

TB statistics - SA, UK & USA 2005

<table>
<thead>
<tr>
<th></th>
<th>S Africa</th>
<th>UK</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notification rate (annual)</td>
<td>570</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>New cases (est)</td>
<td>285000</td>
<td>8494</td>
<td>13500</td>
</tr>
<tr>
<td>Incidence</td>
<td>600</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>HIV prevalence in TB</td>
<td>57</td>
<td>6.7</td>
<td>15</td>
</tr>
</tbody>
</table>

WHO, Global TB Bull, 2005
Shaheen Mehtar
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Incidence of TB + % of adults with TB and AIDS 2004

Adherence to Treatment:
- following treatment at a level above which treatment goals are likely to be met.

Determinants of adherence
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**Factors affecting TB control programme outcomes in South Africa**

### Patient Factors
- Poverty & overcrowding
- Poor access to services
- Traditional beliefs regarding illness and treatment
- Treatment side effects
- Stigmatization and fear
- Direct and indirect costs
- Substance use
- Social mobility
- External locus of control
- HIV / AIDS

### Clinic Factors
- Inadequate teamwork
- Discontinuity of care
- Task orientation
- Little patient education
- Rigid opening hours
- Long waiting times
- Overcrowding

### Health Care Organisation Factors
- Ineffective management
- Interruptions to drug supply
- Access to diagnostic facilities
- Inadequate strategies for case management and patient recall
- Oriented to acute conditions
- Organizational restructuring
Multi-drug-resistant TB (MDR TB) (resistance to at least INH and rifampicin)

- Costly (e.g., a single course of drugs cost > R20,000)
- Therapy takes 18 – 24 months

MRC study (K Waye)
followed up 300 MDR TB patients in 2000 for 2 years:

Outcome
- 50%, overall cure rate
- 30% interrupted treatment
- 12% died

- MDR TB in new sputum positive patients varied
  0.3% in W. Cape & 3.5% in Mpumalanga

Risk Factors
- HIV status = not significant (p=0.57)
- Previous treatment and/or admission to hospital = (p =0.004)

- An MDR TB rate > 1% in new patients indicates an ineffective the TB control programme

M(X)DR TB

- Resistant to rif & INH, PLUS
- Any fluoroquinolone
- and capreomycin, amikacin and kanamycin

Cure rate is low but not zero.

Very high mortality in those with HIV infection and those infected with one of the more virulent strains.
Epidemiology and infection control principles of XDR tuberculosis

### MDR & XDR-TB global (% of all reported cases) (MMWR 55/11)

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Industrialised</td>
<td>20</td>
<td>3</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>Central &amp; South America</td>
<td>48</td>
<td>6</td>
<td>55</td>
<td>6</td>
</tr>
<tr>
<td>Europe/ West Asia</td>
<td>55</td>
<td>9</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td>Africa &amp; Middle East</td>
<td>17</td>
<td>0</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Asia excl South Korea</td>
<td>81</td>
<td>0</td>
<td>70</td>
<td>1</td>
</tr>
</tbody>
</table>

### Mean of Resistance to specific Drugs in New Cases by WHO Regions - 1994-'07

![Graph showing mean resistance to specific drugs in new cases by WHO regions]

### Drug resistance in Previously treated cases - '99-'07

![Graph showing drug resistance in previously treated cases]
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![Chart showing MDR-TB among TB cases - '94-'07](image)

### Numbers & Proportions of MDR-TB among all TB cases

<table>
<thead>
<tr>
<th>Region</th>
<th>No of MTR TB cases</th>
<th>No of MDR TB cases</th>
<th>Low 5% CI</th>
<th>High 5% CI</th>
<th>% MDR TB</th>
<th>Low 5% HIV+</th>
<th>High 5% HIV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Established Market Economies</td>
<td>1,235,756</td>
<td>1,311</td>
<td>1.4%</td>
<td>1.6%</td>
<td>1.2%</td>
<td>1.1%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Central Europe</td>
<td>59,052</td>
<td>621</td>
<td>1.0%</td>
<td>1.3%</td>
<td>2.3%</td>
<td>1.3%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>494,531</td>
<td>636</td>
<td>1.3%</td>
<td>1.4%</td>
<td>1.2%</td>
<td>1.3%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Latin America</td>
<td>340,279</td>
<td>397</td>
<td>1.2%</td>
<td>1.2%</td>
<td>2.1%</td>
<td>1.2%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Eastern Mediterranean Region</td>
<td>673,226</td>
<td>740</td>
<td>1.1%</td>
<td>1.2%</td>
<td>1.2%</td>
<td>1.3%</td>
<td>1.1%</td>
</tr>
<tr>
<td>African/low/HIV incidence</td>
<td>375,981</td>
<td>945</td>
<td>1.3%</td>
<td>1.3%</td>
<td>1.2%</td>
<td>1.3%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Africa/high/HIV incidence</td>
<td>1,695,422</td>
<td>467,716</td>
<td>1.2%</td>
<td>1.2%</td>
<td>2.0%</td>
<td>1.2%</td>
<td>1.3%</td>
</tr>
<tr>
<td>South East Asia</td>
<td>1,668,379</td>
<td>194,700</td>
<td>1.2%</td>
<td>1.2%</td>
<td>1.0%</td>
<td>1.2%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Western Pacific Region</td>
<td>2,173,333</td>
<td>190,667</td>
<td>1.2%</td>
<td>1.2%</td>
<td>1.2%</td>
<td>1.3%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Surveied countries</td>
<td>1,903,053</td>
<td>451,320</td>
<td>1.3%</td>
<td>1.3%</td>
<td>1.2%</td>
<td>1.3%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Non-surveyed countries</td>
<td>2,230,383</td>
<td>69,834</td>
<td>1.0%</td>
<td>1.1%</td>
<td>1.7%</td>
<td>1.0%</td>
<td>1.3%</td>
</tr>
<tr>
<td>All countries (n=157)</td>
<td>10,102,089</td>
<td>409,718</td>
<td>1.4%</td>
<td>1.5%</td>
<td>1.3%</td>
<td>1.4%</td>
<td>1.3%</td>
</tr>
</tbody>
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### XDR-TB - S Africa

**XDR-TB Report from KZN (2007)**
- 544 patients with MTB
- 221 were MDR TB
- 53 MDR-TB patients had XDR-TB
- 44/53 were tested and were HIV +
- 15 on ARV
- 52/53 died - median survival = 16d (2-210d)
- Now known to be genetically linked - nosocomial transmission

Gandhi et al. Lancet 2006; 368
Shaheen Mehtar
Epidemiology and infection control principles of XDR tuberculosis

Challenges for IPC

• Understaffing in healthcare- 40% (nurses & doctors)
• Overcrowded healthcare facilities
• Infrastructure for health varied by province
• Little structured IPC training healthcare workers
• Oscillatory migration between poorer provinces and richer provinces in search of a better life
• Population carries its own concepts of health influenced by stigma, prejudice and fear.
• Influenced by entrenched bureaucracy
• Lack of trust between colleagues & staff

IPC in TB- TBH- 6/12

• During study period to TBH = 33263 admissions
• MTB confirmed cases = 394 (1.2%)
  – OPD- 17.3%
  – IP— 83%
• HIV status (199/394) 50.5% pos
• Microbiology on 394 cases
  – Smear pos 110 (28%)
  – Culture pos 306 (77.7%)
  – Sensitivity testing 140 (46%)
    • MDR 13/140 (9.3%)

Potential Exposure & IPC Risk

• TAT for results
  • Smear 9 hr
  • Culture 27d
  • Sensitivity 42d
• LOS
  – MDR – LOS- 36 days
  – Non MDR- LOS- 17 days
• Potential exposure to MTB - 22 days (mean)
• Mortality
  – Overall: 3.1%
  – Mortality associated with TB= 9.4%
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Epidemiology and infection control principles of XDR tuberculosis

PPE reported by Nurses

<table>
<thead>
<tr>
<th>In place</th>
<th>yes</th>
<th>%</th>
<th>no</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gloves</td>
<td>20</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curtains around beds</td>
<td>17</td>
<td>85</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Anti-TB drugs</td>
<td>16</td>
<td>80</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Surgical masks</td>
<td>14</td>
<td>70</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Isolation</td>
<td>11</td>
<td>55</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>N95-masks</td>
<td>8</td>
<td>40</td>
<td>12</td>
<td>60</td>
</tr>
</tbody>
</table>

Usage of PPE by nursing staff = 98%

Audit of TB facilities TBH- '07

<table>
<thead>
<tr>
<th>Spec</th>
<th>wds</th>
<th>Masks</th>
<th>glove</th>
<th>apron</th>
<th>N95</th>
<th>SR door</th>
<th>SR Curtains</th>
<th>SR NPV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&amp;E</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>ObGy</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td></td>
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<tr>
<td>I Med</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>7</td>
<td>0</td>
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<tr>
<td>ICUs</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>0</td>
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<tr>
<td>Paed</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Surg</td>
<td>9</td>
<td>5</td>
<td>9</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Negative Pressure Ventilation

Total number of beds= 1269; single rooms (SR)= 292 (23%)

CDC- Guidelines for MTB- can SA implement these?

- **Level I.- CDC**
  - Written plan for rapid identification, isolation and effective treatment
  - Training and counselling of HCW dealing with TB
  - Supervision by well trained staff

- **In South Africa**
  - TB load high
  - Training inadequate: being extended
  - Implementing effective work practice
  - Screening of workers for TB infection & disease
  - Protection of HIV positive workers
  - Need to include the Community

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CDC guidelines (Level II)

Adequate ventilation in all high risk areas
- Local area exhaust ventilation- in all patient areas
- Directional airflow from clean to less clean
- Dilution and removal of contained areas- exhaust ventilation- 220CFM/person through HEPA filter
- Disinfection of air by UV light

- South Africa
- For Western Cape this would mean ALL Healthcare areas where patients are seen
- Exhaust ventilation cannot be maintained
- Too expensive
- UV light not proven valuable in uncontrolled environment

Natural ventilation effect

- Used CO₂ clearance from
  - Mechanically ventilated rooms
  - Natural ventilation
- 368 experiments carried out
- Natural ventilation clearance = 28 ACH
- Mechanical negative- pressure rooms = 12 ACH
- Wells-Riley airborne infection model prediction
  - 33% in negative pressure rooms
  - 11% in natural ventilated rooms

MDR ward- alternative

Open windows & door for ventilation
Bed curtains around patients' beds

Care givers: instructed in IPC
Windows open, sunlight
Same precautions as HCW if tending patient
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CDC guidelines (level III)

- Personal Protective clothing
  - Respiratory masks
  - Surgical masks inadequate for MTB protection
  - N95 or equivalent
  - 95% to 99.97% filtration instead of HEPA
    - Fit well - face seal fitting test
    - Respiratory inspection and checking

- In South Africa
  - Surgical masks recommended
  - Bed curtains for in patients
  - N95 masks for MDR and XDR TB
  - Cough rooms for sputum sampling - exhaust ventilation not common
  - Engineering Maintenance difficult

Cough (droplet) demonstration

- Coughing - 3m
- Hanky - 0.5m
- Surgical mask - 0.5m

Aerosol demonstration

- Coughing - 2m
- Hanky - 1m
- Surgical mask - 0.25m
Cough etiquette for OPD

Cough Rooms

- Small confined space where patient goes in to produce sputum
- Usually it is a toilet or sluice which doubles up as a cough room
- Patient may or may not be supervised while producing sample
- Exhaust ventilation is usually not present
- Sometimes patients nebulised to produce a good sample.
- STAFF AT RISK!

Isolation facilities

- For confirmed cases of MDR/XDR TB
- Single room with negative pressure vent (6-12 ac/hour)
- Keep trolley with all equipment outside the room
- Garb before entering
- Discard contaminated gloves, aprons & masks inside the room at end of procedure
- Wash and dry hands
- Discard waste as infectious waste
Shaheen Mehtar
Epidemiology and infection control principles of XDR tuberculosis

Additionally in South Africa

• Inadequate decontamination of mechanical ventilation and bronchoscopic equipment
  – Inspection
  – Maintenance
  – Decontamination
  – Written Standard Operating Procedures (SOP)
• Development of decontamination & sterilization facilities and practices needed.

Summary

TB continues to be a growing problem- 1% each year

In Africa the TB epidemic is fuelled by HIV.

Drug resistance in MTB is emerging associated with poor adherence to treatment

Nosocomial transmission of MDR TB can be reduced with good IPC management of respiratory secretions
Preclinical Development of Combination Regimens for Resistant TB Infections

William Bishai, MD, PhD
Johns Hopkins Univ.
April 19, 2008

Why so much drug? Why so long?

<table>
<thead>
<tr>
<th>Tuberculosis*</th>
<th>Other bacterial pneumonias</th>
</tr>
</thead>
<tbody>
<tr>
<td>• INH 47 g</td>
<td>• Azithromycin 1.5 g</td>
</tr>
<tr>
<td>• RIF 37 g</td>
<td>• Self-supervised</td>
</tr>
<tr>
<td>• PZA 76 g</td>
<td>• Cost $35</td>
</tr>
<tr>
<td>• ETB 65 g</td>
<td></td>
</tr>
<tr>
<td>• ~60 DOT visits*</td>
<td></td>
</tr>
<tr>
<td>• Cost &gt; $15,000</td>
<td></td>
</tr>
</tbody>
</table>

*Denver regimen for susceptible M. tb: daily therapy for 2 wk, then 2x/wk for 24 wk for a 70 kg adult

Can modern technology lead to: more potent and short-course anti-TB agents?
William Bishai
Pre-clinical testing of combination regimens for resistant TB infections

Why does it take so long?

Bactericidal activity: INH (H), Streptomycin (S)
Sterilizing activity: Rif (R), Pyrazinamide (Z)

Our goal
Activity of INH+SM, INH+RIF, or INH+RIF+PZA in mice (and in humans) against “persistors”
RIF and PZA are more active vs. persistors than INH and SM

The drug development process

Likelihood of a successful anti-TB drug by 2010 is <5%; for 2 successful anti-TB drugs, the likelihood is <1%

New drugs: Despite the invigorated TB drug pipeline, it will be challenging to attain a truly novel regimen in the near future

Existing drugs: need to be exploited to their greatest potential

Glickman et al., Science, 2006;311:1246

The Drug Discovery Pipeline for Anti-TB Drugs:
William Bishai
Pre-clinical testing of combination regimens for resistant TB infections

Chronology of TB Drugs

• 1945 Streptomycin
• 1946 PAS
• 1946 Thiacetazone
• 1952 Isoniazid
• 1952 Pyrazinamide
• 1955 Cycloserine
• 1958 Ethionamide
• 1960 Capreomycin
• 1963 Ethambutol
• 1967 Rifampin
• 1998 Rifapentine
• 1999 Moxifloxacin (not FDA-approved for TB)

Antibiotics (new and old) under study for improving TB therapy

1. Rifampin (R)
2. Isoniazid (H)
3. Pyrazinamide (Z)
4. Ethambutol (E)
5. Rifapentine (P)
6. Moxifloxacin (M)
7. Linezolid (L)
8. PA-824 (PA)
9. OPC-67683 (O)
10. TMC-207 (J)
11. SQ-109 (S)
12. LL-3858 (U)

Traditional “first line” agents

FDA approved for TB

FDA approved, possible “off-label” role

Diarylquinoline ATP synthase inhibitor, Tibotec

Nitroimidazole, TB Alliance, Chiron

Nitroimidazole, Otsuka

Ethambutol derivative, Sequella

Pyrrole, Lupin

Outline

Preclinical
Nitroimidazoles: PA-824 & OPC67683
Diarylquinolines: TMC-203

Clinically available drugs
Moxifloxacin
Rifapentine
Combinations

Does the mouse predict human?
Innovations
Pre-clinical testing of combination regimens for resistant TB infections

The mouse model of TB chemotherapy

Recapitulation of the short-course regimen in the mouse...as in humans

Outline

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Does the mouse predict human?

Innovations
William Bishai
Pre-clinical testing of combination regimens for resistant TB infections

The nitroimidazo-oxazine PA-824
- MIC: 0.12-0.25 µg/ml
- Cmax of 20µg/ml and T1/2 of 12-14 hr in mice after 100mg/kg dose
- Concentration-dependent activity
- Might inhibit protein and cell wall mycolate synthesis

Early work
- PA824 alone as bactericidal as INH in 0-8 wk
- PA824 alone nearly as sterilizing as INH-RIF in 8-24 wk

Tyagi et al., AAC 2005; 49: 2289

Treatment shortening potential of PA-824 in mice. Lung CFU counts over 6 months

Conclusion 1: PA-824 adds activity to RZ, (but not RHZ) probably via bactericidal & sterilizing activity

Conclusion 2: Replacement of H associated with treatment shortening

The nitro-imidazo-oxazole OPC-67683
- MIC: 0.012 µg/ml
- Cmax of 0.3 µg/ml and t1/2 of 5.9h in mice treated with 3 mg/kg
- Concentration-dependent activity
- Might inhibit protein and cell wall mycolate synthesis
- ~20-fold more potent than PA-824
William Bishai
Pre-clinical testing of combination regimens for resistant TB infections

Comparative activity of OPC-67683 with other TB drugs in the mouse model

Comparative activity of 2O\(R_5Z_{100}/2OR\) and 2R\(H_{10}E_{100}Z_{100}/2RH\) in the mouse model of TB

Outline
Preclinical
- Nitroimidazoles: PA-824 & OPC67683
- Diaryquinolines: TMC-207
Clinically available drugs
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Does the mouse predict human?
Innovations
The diarylquinoline TMC-207

- Inhibits ATP synthase
- No cross-resistance with known drugs
- MIC of 0.06 µg/ml
- C\text{max} of 0.5µg/ml and t\text{1/2} of 24h
- Time-dependent activity: after single dose, serum concentrations exceed MIC for 3d!

Comparative bactericidal activity of TMC-207 (J), rifampin (R), isoniazid (H), moxifloxacin (M), and pyrazinamide (Z) in the mouse model of TB

Comparative bactericidal activity of daily RHZ, RMZ, and JMZ in the mouse model of TB
William Bishai
Pre-clinical testing of combination regimens for resistant TB infections

Outline
Preclinical
Nitroimidazoles: PA-824 & OPC67683
Diarylquinolines: TMC-203
Clinically available drugs
Moxifloxacin
Rifapentine
Combinations
Does the mouse predict human?
Innovations

MICs (μg/ml) of main quinolones against M. tuberculosis

Moxi in combinations in mouse TB

Conclusion 1:
M adds activity to RZ, (but not RHZ) probably via increasing bactericidal & sterilizing activity

Conclusion 2:
Replacement of H associated with treatment shortening

Nuernberger et al. AJRCCM 2004
William Bishai  
Pre-clinical testing of combination regimens for resistant TB infections

Outline

Preclinical
- Nitroimidazoles: PA-824 & OPC67683
- Diarylquinolines: TMC-203

Clinically available drugs
- Moxifloxacin
- Rifapentine
- Combinations

Does the mouse predict human?

Innovations

Rifapentine (RPT, P)
- \( \text{MIC}_{90} = 0.06-0.125 \, \mu g/ml \)
- Half-life = 10-15 h (vs. 2-3 h for RIF)
- Dose-dependent activity in mouse, EBA studies over a 600-1200 mg range that seems to be well tolerated in humans\(^1\)\(^3\)
- FDA approved dose\(^4\):
  - 600 mg twice weekly (initial phase)
    (~20 mg/kg per week; ~3 mg/kg per day)
  - 600 mg once weekly (continuation phase)

\(^1\)Daniel et al. AJRCCM 2000; 161:1572
\(^2\)Bryal et al. AJRCCM 2005;172:128
\(^3\)Buch et al. AJRCCM 2002; 165:1526
\(^4\)Priftin package insert

Unlocking the potential of the rifamycins

Rifamycins may be underdosed at 10 mg/kg

Jayaram et al. AAC (2003); 47:2118
William Bishai  
Pre-clinical testing of combination regimens for resistant TB infections

**Advantage of RPT in intermittent regimens**

Lung CFU counts after 6 wks treatment with the indicated rifamycin at 10 mg/kg

Rifamycins benefit may improve with increased frequency AND with increased half-life. 

Ji et al, ARD 1993; 148:1541

---

**Effect of dose and frequency on RIF activity in chronic mouse infection**

Lung CFU counts after 6 wks treatment with rifampin 10 mg/kg at the indicated frequency

Ji et al, ARD 1993; 148:1541

---

**Inferiority of intermittent regimens**

Systematic review of published clinical trials with short-course regimens

<table>
<thead>
<tr>
<th>Initial phase</th>
<th>Continuation phase</th>
<th>Odds of relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>Daily</td>
<td>1.0</td>
</tr>
<tr>
<td>Daily</td>
<td>3x/wk</td>
<td>1.6 (0.6 - 4.1)</td>
</tr>
<tr>
<td>Daily</td>
<td>2x/wk</td>
<td>2.8 (1.3 - 6.1)</td>
</tr>
<tr>
<td>3x/wk</td>
<td>3x/wk</td>
<td>2.8 (1.4 - 5.7)</td>
</tr>
</tbody>
</table>

Chang et al, AJRCCM (2006); e-published Aug. 14
**William Bishai**

**Pre-clinical testing of combination regimens for resistant TB infections**

---

**Pharmacodynamics of daily rifapentine and rifampin in mice**

Rifapentine (10mg/kg)  
Rifampin (10mg/kg)

Rifapentine provides greater rifamycin exposure than does rifampin at the same given dose.

---

**Free Drug PD Parameters and Sterilizing Activity**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>$C_{\text{max}} / \text{MIC}$</th>
<th>Weekly (\text{AUC} / \text{MIC})</th>
<th>Time (h) above MIC/week$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Daily Therapy</strong></td>
<td>8.47</td>
<td>405</td>
<td>80/168</td>
</tr>
<tr>
<td>With $R_{\text{FA}}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Twice-weekly Therapy</strong></td>
<td>8.03</td>
<td>531</td>
<td>168/168</td>
</tr>
<tr>
<td>With $P_{\text{FA}}$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^*$ One week = 168h

---

**Figure**: Comparison of rifamycin exposure over time for different combination regimens. The graphs illustrate the concentration of rifamycin over time for various regimens, with a focus on how rifapentine and rifampin compare in terms of exposure and sterilizing activity in a mouse model.
Pre-clinical testing of combination regimens for resistant TB infections

**Outline**

Preclinical
- Nitroimidazoles: PA-824 & OPC67683
- Diary/quinolines: TMC-203

Clinically available drugs
- Moxifloxacin
- Rifapentine
- Combinations

Does the mouse predict human?

Innovations

**Active TB**

Standard Therapy: Denver Regimen

Old Drugs
- H = isoniazid
- R = rifampin
- Z = pyrazinamide
- E = ethambutol

New Drugs (FDA approved)
- P = rifapentine
- M = moxifloxacin

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>HRZE</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Daily doses
- 8 doses

Twice weekly
- 48 doses
Replacing M for H:
Lung CFU counts after 2 Months Treatment

R: Rifampin, I: Isoniazid, M: Moxifloxacin, Z: Pyrazinamide

Varying the “P” in PMZ:
1 month

Varying the “P” in PMZ: 2 mo.
Relapse rate: (2 mo. Tx 3 mo. Follow-up)
William Bishai
Pre-clinical testing of combination regimens for resistant TB infections

Varying the “P” in PMZ: 3 mo

Relapse rate: (3 mo. Tx 3 mo. Follow-up)

![Graph showing varying the “P” in PMZ: 3 mo](image)

Individual Contributions of Rifapentine and Moxifloxacin

Bactericidal Activity

Relapse rates after 10 wks Tx

![Graph showing individual contributions of Rifapentine and Moxifloxacin](image)

Relapse Rates after Treatment

<table>
<thead>
<tr>
<th>Regimen</th>
<th>After 2-mo.</th>
<th>After 3-mo.</th>
<th>After 4-mo.</th>
<th>After 6-mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHZ (5/7)</td>
<td>Cx+</td>
<td>Cx+</td>
<td>90%</td>
<td>0%</td>
</tr>
<tr>
<td>P15MZ (2/7)</td>
<td>_</td>
<td>10%</td>
<td>0%</td>
<td>_</td>
</tr>
<tr>
<td>P20MZ (2/7)</td>
<td>95%</td>
<td>20%</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>P15MZ (3/7)</td>
<td>95%</td>
<td>0%</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>P5MZ (5/7)</td>
<td>60%</td>
<td>5%</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>P10MZ (5/7)</td>
<td>35%</td>
<td>0%</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

(Cx+) All mice tested at treatment completion were culture-positive

Rosenthal et al., PLoS Medicine, 2007

Rosenthal et al., AJRCCM 2007: 175; A509
William Bishai
Pre-clinical testing of combination regimens for resistant TB infections

Bactericidal Activity of daily R\textsubscript{10} HZ, R\textsubscript{10} MZ, and P\textsubscript{10} MZ against \textit{M. tuberculosis} in mice

Recapitulation of the short-course regimen in the mouse... as in humans?

Conclusions
Tubercle bacilli infecting mice treated with rifapentine (2/7) were exposed to more free drug for longer periods of time than those in mice treated with rifampin (5/7)

Enhanced killing of persisters
William Bishai
Pre-clinical testing of combination regimens for resistant TB infections

Outline
Preclinical
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Does the mouse predict human?
Innovations

FQs to Treat Tuberculosis:

![Graph showing mouse model lung CFU counts](image)

Log CFU in entire lung

Duration of treatment (mos.)

CDC TBTC Study 27
(M for E)

FQs to Treat Tuberculosis:

Nuermberger et al. AJRCCM 2004

CDC TBTC Study 27:
placebo-controlled, factorial study – randomization to study drug and treatment frequency

<table>
<thead>
<tr>
<th>Study Drug</th>
<th>Treatment Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moxifloxacin</td>
<td>HRZ Daily (n = 61)</td>
</tr>
<tr>
<td></td>
<td>HRZM Daily (n = 68)</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>HRZE Daily (n = 61)</td>
</tr>
<tr>
<td></td>
<td>HRZE 3x/week (n = 60)</td>
</tr>
</tbody>
</table>
TBTC Study 27 Treatment Arms
Sputum smear+ PTB suspect

randomization

HRZE
For 8 weeks
N = 138

HRZM
For 8 weeks
N = 139

assess for primary endpoints, including sputum culture conversion

ATS/CDC/IDSA-recommended continuation phase regimen

TBTC Study 27
Time to culture conversion – moxifloxacin vs. ethambutol

Median time to culture conversion:
- Moxi: 43 days (28-56) p = 0.01
- Ethambutol: 56 days (41-59)


Mouse Model: Lung CFU counts

Log CFU in entire lung

CDC TBTC Study 28
M for H

Nuermberger et al. AJRCCM 2004
TBTC Study 28: M for H

HRZE vs. MRZE during the initial phase

N: 344 patients, 26 sites

Endpoints: Primary: Sputum culture conversion at 8 wk (liquid & solid)
Secondary: First negative culture at 2, 4, 6 wk

Sites:
- N. America: 40%
- Kampala: 43%
- Barcelona, Rio de Janeiro, Durban: 17%

<table>
<thead>
<tr>
<th></th>
<th>INH</th>
<th>MXF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Median Weight (kg)</td>
<td>56</td>
<td>55</td>
</tr>
<tr>
<td>Male</td>
<td>72</td>
<td>73</td>
</tr>
<tr>
<td>Cavitary TB</td>
<td>76</td>
<td>71</td>
</tr>
</tbody>
</table>

Dorman et al. TBTC Study 28: MXF vs. INH in the 1st 2 mo of treatment for TB. ICAAC 2007 Abstract L736b

Time to culture positivity in liquid statistically faster than solid cultures for African sites

<table>
<thead>
<tr>
<th></th>
<th>INH arm</th>
<th>MXF arm</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC*</td>
<td>344</td>
<td>55%</td>
<td>60%</td>
</tr>
<tr>
<td>MITT</td>
<td>381</td>
<td>52%</td>
<td>57%</td>
</tr>
</tbody>
</table>

* Time to culture positivity in liquid statistically faster than solid cultures for African sites

Dorman et al. TBTC Study 28: MXF vs. INH in the 1st 2 mo of treatment for TB. ICAAC 2007 Abstract L736b
William Bishai
Pre-clinical testing of combination regimens for resistant TB infections

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Innovations

5. Surrogate Markers for TB
the (antiquated) CFU count . . .

Small Animal Imaging Systems

Photo of the Tri-Modality Small Animal Imaging Systems
CT, PET, and SPECT imaging
Computed tomography – anatomic imaging

• CT scans performed for uninfected, TB infected and treated BALB/c mice

Guinea pig imaging

CFU 4.60 ± 0.33

CFU none detected (LLD 2.70)

Rabbit imaging

TRI-MODALITY IMAGING SYSTEM

Gamma camera with SPECT

Optical camera (fluorescence/bioluminescence imaging)

Rabbit containment vessel and gantry insert for X-ray CT mode

Jain, Pomper, & Bishai, in press

Jain, Pomper, Karakousis & Bishai, unpublished
William Bishai
Pre-clinical testing of combination regimens for resistant TB infections

**Fig. 1. In vivo and in vitro experiments with WT and TK- E. coli**

- $^{125}$I-FIAU is a TK substrate that is concentrated in TK+ bacteria.
- Permits visualization of bacterial infection using SPECT.

Bettegowda et al. PNAS 2005; 102: 1145

**SPECT and new TK clones**

- Bettegowda et al. have shown previously that bacteria can be selectively imaged in experimentally infected animals using $^{[125]}$-FIAU, a nucleoside analog substrate for bacterial thymidine kinase (TK).
- TK is absent in mycobacteria. We had previously engineered a *M. smegmatis* strain transformed with TK from *E. coli* UTI89 under the control of hsp60.
- This strain concentrated $^{[125]}$-FIAU and could be imaged using SPECT.
- However, this strain was made using the non-integrating pMV261 plasmid.

Jain, Pomper, & Bishai, in press

**The Drug Discovery Pipeline for Anti-TB Drugs:**
Overall Conclusions

1. Regimens based on the rifapentine, moxifloxacin, and pyrazinamide (PMZ) combination are dramatically more potent than the standard 6-month RHZ regimen.

2. Activity correlates with the better activity of moxifloxacin and the extent of rifamycin exposure.

3. Removal of antagonism (H ↔ R) & use of high exposure to sterilizing drugs (P > M,Z) reduces the persistence phenomenon.

Support and Acknowledgments

NIH-NIAID (N01-AI-40017 & R01 43846)
Global Alliance for TB Drug Development

Center for TB Research (JHU)
Jacques Groset
Ian Rosenthal
Kathy Williams
Mel Spigelman
Maria Freire

CDC-TBTC
Andrew Vernon

National Jewish Medical Research Center
Charles Peloquin

NIH-NIAID

Global Alliance for TB Drug Development
Mel Spigelman
Ann Girardis
Sara Frank

Thank you
Clifton E. Barry III
Drug development and trials of new drugs for TB

- NMTH: National Masan Tuberculosis Hospital
  largest TB referral hospital in Korea

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual newly admitted</td>
<td>798</td>
</tr>
<tr>
<td>patients</td>
<td></td>
</tr>
<tr>
<td>Annual outpatient visits</td>
<td>5,595</td>
</tr>
<tr>
<td>Average no. of inpatients/ day</td>
<td>350</td>
</tr>
<tr>
<td>Mean duration of admission</td>
<td>130</td>
</tr>
</tbody>
</table>

Goal: Understand the basic factors underlying the slow response of TB to chemotherapy and couple this directly to early pre-clinical drug discovery

- The why and how of MDR-TB in the patients at NMTH
- The existence and extent of hypoxia in tuberculous lesions in patients undergoing lung resection surgery
- The utility of a hypoxia-specific drug in TB (metronidazole)
- Employ functional imaging techniques (PET, HRCT, MRI) to learn the detailed kinetics of subclasses of lesion response to Rx
- Evaluation of drug penetration PLC/D in various subclasses of lesion and model penetration rates
Clifton E. Barry III
Drug development and trials of new drugs for TB

Demographic Characteristics of Study Subjects, NMTH, May 2005-Feb. 2006

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>157</td>
<td>54</td>
</tr>
<tr>
<td>Age Median (Range)</td>
<td>46 (21-80)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal education</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Elementary / Middle / High School</td>
<td>88</td>
<td>48</td>
</tr>
<tr>
<td>High School and Above</td>
<td>88</td>
<td>48</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Construction or Factory Worker</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>Unemployed</td>
<td>42</td>
<td>23</td>
</tr>
<tr>
<td>Service Sector</td>
<td>40</td>
<td>22</td>
</tr>
<tr>
<td>Professional / Office Work</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Agriculture / Aquaculture</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Previously Treated</td>
<td>129</td>
<td>69</td>
</tr>
<tr>
<td>Among previously treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix of Poor Compliance</td>
<td>117</td>
<td>83</td>
</tr>
<tr>
<td>Prior treatments Median (Range)</td>
<td>3 (1-15)</td>
<td></td>
</tr>
</tbody>
</table>

CID 2008 (in press)

Prevalence of Drug Resistance among Isolates from Newly Diagnosed and Previously Treated Subjects, NMTH, May 2005-Feb 2005

- MDR
- XDR
- OFX
- KAN
- SM
- PAS
- ETH
- CS

Newly Diagnosed vs Previously Treated
Clifton E. Barry III
Drug development and trials of new drugs for TB

NIAID 07-I-N041 Metronidazole
A Phase II Clinical Trial of Metronidazole
Combined with Standard Second line TB Chemotherapy in Refractory Pulmonary Tuberculosis Patients

Tuberculosis is an extremely complex chronic disease
1956 Mac Vandiviere and colleagues UNC, Chapel Hill
Analysis of surgically removed lesions from TB patients
3 types of lesions:
1) Open Cavities
- thick walled, bronchus
- liquefied, caseous material
- AFB+
- Colonies on plates in 8 weeks
2) Closed Lesions
- caseous, no bronchial connection
- AFB+
- Colonies on plates in 8 months
3) End Stage
- Clean, thin, hard
- AFB-

BASIC HYPOTHESIS
environmental signal hypoxia

M. tuberculosis

Trachea
Bronchi
Non-replicating
persistent phenotype

bacteria in this form persist for months in the face of drugs active against
replicating bacteria and for years in the face of a primed immune response

drug-tolerant phenotype

adaptive response hypoxia regulon

non-replicating persistence
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Nitroimidazoles: History

Metronidazole
- Anaerobic bacteria
- Antiamoebic activity
- Marked cell lysis and toxicity

CGI-17341
- Anaerobic and microaerophilic bacteria
- Potent vs. 890
- Not mutagenic
- Inhibits mtDNA

PA-824
- Anaerobic and microaerophilic bacteria
- Potent vs. 890
- Not mutagenic
- Inhibits mtDNA

OPC-67683
- Anaerobic and microaerophilic bacteria
- More potent vs. 890
- Not mutagenic
- Inhibits mtDNA

In vitro model for Non-Replicating Persistence-Wayne model

Metronidazole in Rabbit Models

- Rabbit: More realistic model for human disease (both aerobic and anaerobic lesions)

- Four weeks therapy
  - M: Metronidazole (20mg/kg bid)
  - R: Rifampicin (10mg/kg qid)
  - C: Controls

Figure: Treatment response of M.bovis-infected rabbits
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Study Design

A randomized, double blind, placebo controlled phase II trial with 60 subjects (30 per arm).

Strata:
1) Fluoroquinolone resistance

Study Drug: Oral metronidazole
500 mg TID PO for eight (8) weeks beginning at time of initiation of second-line antituberculous therapy

Study Objectives

Primary Objective

- To evaluate the impact of adding Metronidazole to standard treatment regimen on the resolution of tuberculosis lesions within multi-drug resistant tuberculosis (MDR-TB) patients using HRCT.

Study Objectives (cont.’)

Secondary Objectives (1)

- To evaluate the safety and tolerability of Metronidazole in patients receiving second-line antituberculosis chemotherapy

- To evaluate the utility of [18F]-fluoro-2-deoxy-D-glucose – positron emission tomography (FDG-PET) as a potential surrogate marker for response to tuberculosis chemotherapy
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Patient Schema

Initial Assessment of Study Patients

E-101: Enrolled 17 December 2006
HR – resistant, Rifabutin sensitive
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Entry 2 Mo 4 Mo
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AFB smear 1+ 2+ 2+ - - -
AFB culture 3+ 1+ 1+ 2+ - -

Monitoring Bacterial Load in Sputum

DTP in liquid culture & CFU counting

After decontamination, add 1 ml of media

DTP: days to positivity

Incubation for DTP

CFU count in 3 weeks
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