LABORATORY BASED DST FOR BEDAQUILINE AND INTRODUCTION IN COUNTRIES

NDWG Annual meeting 2015, Cape Town, South Africa

Rigouts Leen, Institute of Tropical Medicine, Antwerp, Belgium
Bedaquiline (BDQ) – Sirturo®

Andries et al., Science 2005

- Diarylquinololone
- Inhibits ATP synthase enzyme
- Molecular target = *atpE* gene

2012 – Food and Drug Administration (FDA)
- approved for adult MDR-TB
- after Phase II clinical trial (C209)
  *under provisions of accelerated approval regulations for "serious or life-threatening illnesses" (21CFR314.500)*

Cross resistance BDQ - clofazimine (CLF)
(Hartkoorn RC et al., AAC 2014; Andries et al., PloseOne 2014)
High level resistance
Mutations in *atpE* gene
8- to 133-fold increase in MIC
MICs 0.25 to 4.0 µg/mL

Low level resistance
Mutations in *Rv0678* gene
2- to 8-fold increase in MIC
MICs 0.25 to 0.50 µg/mL
Cross-resistance BDQ-CFZ due to Rv0678 mutations

Slide from Koen Andries
Phenotypic drug-susceptibility testing (DST)

- MIC ranges *M. tuberculosis* : 0.03 – 0.12 µg/ml
  - Reference strains on agar
  - Clinical strains in BACTEC - radiometric
Phenotypic drug-susceptibility testing (DST)

- Data from Phase II clinical trial
  - Minimal inhibitory concentration (MIC)
  - BDQ: Stock in DMSO (10,000 µg/ml stock)
    Working solutions in sterile distilled water
  - QC by H37Rv and BK12 (Andries et al., aptE mutant)

- Middlebrook 7H11 agar medium (Diacon et al., 2009 – 2012 – 2014)
  - 21 days incubation at 37°C + 5% CO2
  - Polystyrene tubes

- REMA (Palomino et al., 2012)
**Mycobacterium tuberculosis**

Listed breakpoints have been set in parallel with marketing authorisation by EMA. Breakpoints for other agents have not yet been established.

<table>
<thead>
<tr>
<th>MIC breakpoint (mg/L)</th>
<th>S ≤</th>
<th>R &gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delamanid</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Bedaquiline</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Objective: To evaluate performance of the MGIT960 for BDQ susceptibility in Mtb
QC range and ECOFF (Torrea et al., 2015)

**QC range H37Rv in MGIT**
- 10 different days
- 3 different batches MGIT

H37Rv MICs and ECOFF in MGIT ~ 4-fold 7H11
Accuracy / trueness (Torrea et al., 2015)

- 74 Probable-susceptible (PS) strains
- 18 Probable resistant (PR) strains (AtpE or Rv0678 mutants)
- Parallel testing

Bimodal curves
Breakpoints close to ECOFF
### Reproducibility – Repeatability (Torrea et al., 2015)

<table>
<thead>
<tr>
<th>Time points</th>
<th>MIC values (mg/L)</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>H37Rv</td>
<td>BK12</td>
<td>Medium</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Week1-op1</td>
<td>0.5</td>
<td>≥4</td>
<td>1</td>
<td>0.5</td>
<td>≥4</td>
</tr>
<tr>
<td>Week1-op2</td>
<td>0.5</td>
<td>≥4</td>
<td>1</td>
<td>0.25</td>
<td>≥4</td>
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<tr>
<td>Week2-op1</td>
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<td>≥4</td>
<td>1</td>
<td>0.5</td>
<td>≥4</td>
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<tr>
<td>Week2-op2</td>
<td>0.5</td>
<td>≥4</td>
<td>1</td>
<td>0.25</td>
<td>≥4</td>
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<tr>
<td>Week3-op1</td>
<td>0.5</td>
<td>≥4</td>
<td>1</td>
<td>0.25</td>
<td>≥4</td>
</tr>
<tr>
<td>Week3-op1</td>
<td>0.5</td>
<td>≥4</td>
<td>1</td>
<td>0.25</td>
<td>≥4</td>
</tr>
<tr>
<td>Week3-op1</td>
<td>0.5</td>
<td>≥4</td>
<td>1</td>
<td>0.25</td>
<td>≥4</td>
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<tr>
<td>Week3-op2</td>
<td>0.5</td>
<td>≥4</td>
<td>1</td>
<td>Invalid</td>
<td>≥4</td>
</tr>
</tbody>
</table>

2 operators
Different weeks

100% (39/39) replicates differed by ≤ 1 dilution

5 strains

Same day testing
MGIT960 is reproducible and accurate vs. M7H11 agar
- ECOFF ~ Breakpoint
  - 1,00 µg/ml for MGIT960 and 0,25 µg/ml for M7H11
MGIT960 and M7H11 ECOFFs being only a 2-fold concentration lower than the lowest MICs for mutant strains ~ risk to misclassify mutant strains as susceptible, if not performed meticulously.
Further PK/PD studies and clinical data from a larger number of susceptible and MDR strains will be needed to finally set the clinical breakpoint.
• MIC distribution and ECOFF
• 10 Pan-susceptible strains, BDQ-treatment naive
• 12 MDR and pre-XDR isolates
• BDQ tablets

Non-significant difference between medians pan-susceptible and MDR/XDR

ECOFF > Torrea: tablets?

• Drug stability test in MGIT (0-3 weeks pre-incubation)
Multicenter evaluations

- Two studies performed
- 8 different laboratories and locations
- 7H10 and 7H11 agar medium versus 7H9 broth medium
- QC data H37Rv (personal communication D. Cirillo)
  - 0.015 to 0.12 μg/mL on 7H10 et 7H11 agar
  - 0.015 to 0.06 μg/mL in 7H9 broth
• Löwenstein-Jensen: not suitable ~ protein binding

• Stability stock solution in DMSO
  – Minimum 3 months at <-18°C

• Stability diluted solutions in broth (REMA plates)
  – Maximum 2 weeks at 2-8°C
## Summary phenotypic DST

<table>
<thead>
<tr>
<th>Medium</th>
<th>ECOFF (µg/ml)</th>
<th>Breakpoint (µg/ml)</th>
<th>QC range H37Rv (µg/ml)</th>
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</thead>
<tbody>
<tr>
<td>7H10 agar</td>
<td>0.015 - 0.12</td>
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<tr>
<td>7H11 agar</td>
<td>0.25</td>
<td>0.25</td>
<td>0.015 - 0.12</td>
</tr>
<tr>
<td>7H9 broth (REMA)</td>
<td>0.06 – 0.015</td>
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<td></td>
</tr>
<tr>
<td>MGIT960</td>
<td>0.12 – 0.5</td>
<td></td>
<td></td>
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<tr>
<td>LJ</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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How to proceed if you want to test in your lab?
Order BDQ-resistant reference strains:
http://bccm.belspo.be/ OR BCCM.ITM@itg.be
Remaining challenges

- Not yet included in EQA proficiency panels
- Molecular testing
  - No *atpE* mutants in clinical isolates so far
  - Broad variety in Rv0678 mutations
  - Rv0678 mutations ~ MIC levels: to be determined
Any Questions?