Evolution of MDR-TB

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10th National TB Conference
Washington DC, 19th April 2017
### Global TB Estimates (2016)

<table>
<thead>
<tr>
<th>Type of TB</th>
<th>Number of cases</th>
<th>Number of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>All forms of TB</td>
<td>10.4 million</td>
<td>1.8 million</td>
</tr>
<tr>
<td>MDR-TB</td>
<td>480,000</td>
<td>190,000</td>
</tr>
<tr>
<td>XDR-TB</td>
<td>~48,000</td>
<td>~24,000</td>
</tr>
</tbody>
</table>

"Only" ~5% MDR / XDR

Entering Post-Antibiotic Era!
“Drug-Resistant Bacteria Are Less Fit”
Proportion MDR among new TB (2015)
The Relative Fitness of DR *Mtb* is Heterogeneous

Borrell & Gagneux 2009 *IJTLI* 13: 1456-66
Exploring the Role of Epistasis

Drug-resistance mutation(s)

Epistasis

Compensatory mutations

Strain genetic background

“Success” of MDR *Mtb*

Borrell & Gagneux 2011 *Clin Microbiol Infect* 17: 815–820
Evolution of Drug Resistance

- DS
- DR
- Compensation

Borrell & Gagneux 2009 *IJTLD* 13: 1456-66
Compensatory Mutations in *rpoA/C*

Comas et al. 2012 *Nature Genetics* 44: 106–110
**In clinico** Fitness of *rpoA/C* Mutations

- **Global**:
  - 12% with CMs
  - 20% with CMs

- **High-burden**:
  - 21% with CMs
  - 31% with CMs

* P < 0.05

Comas et al. 2012 *Nature Genetics* 44: 106–110
Genetics in *M. smegmatis*

**In vitro growth**

**Transcription efficiency**

Song et al. 2014 *Mol Microbiol* 91: 1106–19
• RIF$^R$ strains: N=1,488
• + RpoA: N=73
• + RpoC: N=729
**Mtb** Lineage Impacts INH Resistance Levels

- 134 clinical INH\(^R\) isolates

### Percentage

<table>
<thead>
<tr>
<th>MIC &lt;3.0 mg/L</th>
<th>MIC &gt;3.0 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.004</td>
<td>0.011</td>
</tr>
</tbody>
</table>

### Lineages

- **Lineage 1**
- **Lineage 2**
- **Lineage 3**
- **Lineage 4**
- **Lineage 5**
- **Lineage 6**
- **Lineage 7**

### Mutations

- **KatG** 315 mutations
- **InhA pro -15** mutations

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Fenner et al. AAC 2012 56: 3047-53
Positive Sign Epistasis in DoubleR

rpoB / gyrA mutations

Borrell et al. 2013 Evol Med Publ Health eot003: 65-74
Conclusion (1): Epistasis matters!

Drug-resistance mutation(s) (e.g. rpoB/gyrA)

Compensatory mutations (e.g. rpoA/C)

Strain genetic background (e.g. L1 vs L2)

Epistasis

- Success of MDR Mtb
- MICs
- Patient outcome (?)
A Web of Epistasis Mediates MDR in Mtb

Trauner et al. 2014 *Drugs* 74: 1063-72
What about **within**-host evolution?
Simple population
Antibiotic treatment
Simple population + 1
Antibiotic treatment
Case Study From Switzerland

- Tibetan refugee (HIV-neg.)
- **Primary** resistance to:
  - isoniazid
  - rifampicin (+ compensatory mutation in *rpoC*)
  - pyrazinamid
  - streptomycin
  - ethionomide
  - fluoroquinolones
  - linezolid (!)
Resistance mutations to 7 drugs
“Clinical Cure”

Relapses

Percentage of sequencing reads with mutant allele


Rv0678  bedaquilineR  clofazimineR

Bloemberg et al. 2015 NEJM 373: 1986-8
“Clinical Cure”

Relaps

Percentage of sequencing reads with mutant allele

0%
20%
40%
60%
80%
100%


Rv0678
capreomycin\textsuperscript{R}

bedaquiline\textsuperscript{R}
tly\textsubscript{A}
clofazimine\textsuperscript{R}

Bloemberg et al. 2015 *NEJM* 373: 1986-8
“Clinical Cure”

Relaps

Percentage of sequencing reads with mutant allele


0% 20% 40% 60% 80% 100%

Rv0678
bedaquiline$^R$
clofazimine$^R$
capreomycin$^R$
tlyA

Bloemberg et al. 2015 *NEJM* 373: 1986-8
“Clinical Cure”

Relaps

Percentage of sequencing reads with mutant allele

0% 20% 40% 60% 80% 100%


Rv0678
clofazimine
bedaquiline
capreomycin

delamanid

tlyA
fbdA
fgd1

Bloemberg et al. 2015 NEJM 373: 1986-8
“Clinical Cure”

Relaps

Percentage of sequencing reads with mutant allele

0%

100%

Jan 2011
Mar 2013
Aug 2013
Dec 2013
Mar 2014
Jun 2014
Jul 2014

Rv0678
bedaquilineR
clofazimineR
capreomycinR
tlyA
fgd1
fbiA
delamanidR

Bloemberg et al. 2015 *NEJM* 373: 1986-8
What about other patients?

Andrej Trauner
Mtb in the host

Major (1)
Minor (2)
Rare (10)
Antibiotic treatment

How stable are individual clones?
Context of Treatment Efficacy

Genotypic DST
- DST
- INJ
- FQ
- PZA

Phenotypic DST
- DST
- INH
- RIF
- EMB
- STR

Treatment
- Efficacious treatment
- Non-efficacious treatment

P01
P02
P03
P04
P05
P06
P07
P08
P09
P10
P11
P12

DS

DS

0 2 4 6 8 10 12 14 16 18 20 22 24 time (weeks)

Trauner et al. 2017 Genome Biol, in press.
Emergence of FQ resistance

1 out of 12 patients.

Trauner et al. 2017 *Genome Biol*, in press.
Total variable SNPs = 492
Multiple isolated populations

Diversity driven by drift

Predominant clone

Purifying selection on minor clones

Trauner et al. 2017 *Genome Biol*, in press.
Quantifying the Trajectory of Mutations

**D**: Detected  
**ND**: Not detected

**\( p_D \)**: Stable  
**\( p_{ND\rightarrow D} \)**: Loss

**\( p_{D\rightarrow ND} \)**: Gain  
**\( p_{ND} \)**: Absent

Trauner et al. 2017 *Genome Biol*, in press.
Non-efficacious treatment: ALL SNPs NSY SNPs SYN SNPs

Efficacious treatment: ALL SNPs NSY SNPs SYN SNPs

Trauner et al. 2017 Genome Biol, in press.
Purifying Selection in Efficaciously Treated Patients

Trauner et al. 2017 *Genome Biol*, in press.
Non-effficaciously Treated Patients Accumulate Mutations in DR Genes

<table>
<thead>
<tr>
<th>Gene set</th>
<th>N^a</th>
<th>Excess mutation^b</th>
<th>Excess NSY^c</th>
<th>Excess mutation</th>
<th>Excess NSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Resistance^d</td>
<td>13</td>
<td>0/100 (0.501)</td>
<td>0/0 (1.000)</td>
<td>5/87 (0.001)</td>
<td>5/5 (0.177)</td>
</tr>
<tr>
<td>Drug</td>
<td>166</td>
<td>10/100 (0.545)</td>
<td>4/10 (0.987)</td>
<td>6/87 (0.946)</td>
<td>6/6 (0.121)</td>
</tr>
<tr>
<td>Drug Resistance Associated^e,f</td>
<td>54</td>
<td>3/100 (0.881)</td>
<td>1/3 (0.964)</td>
<td>5/87 (0.229)</td>
<td>3/5 (0.876)</td>
</tr>
<tr>
<td>Mycolate Superpathway^g</td>
<td>54</td>
<td>3/100 (0.881)</td>
<td>1/3 (0.964)</td>
<td>5/87 (0.229)</td>
<td>3/5 (0.876)</td>
</tr>
<tr>
<td>MTBCT-Cell Antigens^h</td>
<td>300</td>
<td>14/100 (0.153)</td>
<td>10/14 (0.426)</td>
<td>6/87 (0.550)</td>
<td>6/6 (0.121)</td>
</tr>
</tbody>
</table>

^a Number of genes in the gene set. ^b Proportion of mutations in gene set, p-value calculated with a one-sided binomial test. ^c Proportion of NSY mutations in geneset, p-value calculated with a one-sided binomial test. ^d Walker et al., ^e Zhang et al., ^f Farhat et al., ^g O'Neill et al., ^h Coscolla et al.
Conclusions (2)

- Most new mutants are unstable.
- Mutations with no (little) functional effect are more stable.
- This is particularly true in efficaciously treated patients.
- Non-efficaciously treated patients accumulate mutations in DR genes.
Thanks to... Collaborators

University of Valencia
  Iñaki Comas
New York University
  Joel Ernst
University of Cape Town
  Rob Wilkinson
  Helen Cox
Stellenbosch University
  Rob Warren
University of Bern
  Matthias Egger
University of Zurich
  Erik Böttger
UCSF
  Midori Kato-Maeda
Sanger Institute
  Simon Harris

University of Ghana
  Dorothy Yeboah-Manu
Institute Trop. Med. Antwerp
  Bouke de Jong
Fudan University
  Qian Gao
ETH Zurich
  Ruedi Aebersold
  Uwe Sauer
  Tanja Stadler
  Jörg Stelling
  Christian Beisel
Forschunszentrum Borstel
  Stefan Niemann
Max-Planck Institute Jena
  Johannes Krause