Advances in Tuberculosis

Abraham Aseffa, MD, PhD
Armauer Hansen Research Institute

Sixth EDCTP Forum
9-12 October 2011
Addis Ababa, Ethiopia
Outline

• Global TB burden
• Genomics and Pathogenesis
• Biomarkers and Diagnostics
• Vaccines
• Treatment
• Conclusions
TB is still out of control

- In 2009
  - The largest number of TB cases ever reported in history with 9.4 million incident cases in the year
  - The largest number of death from TB with 1.68 million deaths

Incidence: 137 cases per 100 000 population

WHO report 2010
Global trends in TB rates: 1990-2008

1995-2008:
- 36 million cured,
- 6 million lives saved

Slow decline

Western Europe: incidence was falling at 4-5%/yr before chemotherapy and 7-12%/yr with drugs

At 70% case detection rate, ≥85% cure and no HIV: 5-10%/yr/capita

Borgdorff MW Bull WHO 2002

HIV fueled TB in Africa

500% increase in some countries!

With rise in the proportion of women with TB

Africa: 30% of the prevalent and 80% of the HIV co-infected patients

Incident cases of HIV in newly diagnosed TB:
35-39% in Africa and below 9% in other regions

TB is out of control in Africa

1990-2008 (WHO report, 2010)

1990-2009 (UNAIDS report, 2010)
Multi-drug resistant TB: a global menace

More serious challenge than ever!

In 2008

- Estimated 440,000 MDR cases
- 3.6% of all newly diagnosed cases
- Only 7% of them diagnosed/notified

Proportion of MDR-TB among new TB cases, 1994–2009

WHO report 2010
XDR TB: a frightening crisis of unknown magnitude

XDR so far

772 cases in 2007
963 cases from 33 countries in 2008
Over 40,000 reported from 58 countries in 2009

Countries with at least one reported XDR case as of Jan 2010

<table>
<thead>
<tr>
<th>WHO region</th>
<th>No. of countries reporting second-line anti-TB drug resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>African (46)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Americas (35)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Eastern Mediterranean (21)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>European (53)</td>
<td>31 (58)</td>
</tr>
<tr>
<td>South-East Asia (11)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Western Pacific (27)</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Total (193)</td>
<td>46 (24)</td>
</tr>
</tbody>
</table>

S Africa: the largest number of XDR reported so far, 6% of all MDR

.................a function of testing? Burden “remains unquantified in Africa”
A long and persistent relationship with **M. tuberculosis** complex

*M tuberculosis complex* comprises:

- the classic human pathogen *M. tuberculosis, M. africanum*
- the animal pathogenic (sub-)species: *M. bovis* (cattle),
  - *M. caprae* (goats),
  - *M. microti* (voles), and
  - *M. pinnipedii* (seals, sea lions)
- *M. canetti*

---

*Djelouadji D et al.* the Lancet online June 13, 2011 DOI:10.1016/S1473-3099(11)70093-7
Out of Africa migration

Back to Africa and global spread

Global phylogeny of *M. tuberculosis*

A. Large sequence polymorphism

B. Single nucleotide polymorphism

Global phylogeography of *M. tuberculosis* ...

... adaptation to specific human populations

Relative contribution of social factors?

Lineage 7

Whole genome sequencing

Comas et al. 2010 unpublished
Firdessa et al 2011 manuscript in preparation
Population structure of *M. tuberculosis* in Ethiopia

- Euro-Am (Lineage 4)
- CAS (Lineage 3)
- Ethiopian (Lineage 7)
- Ancient (Lineage 1)
- Beijing (Lineage 2)
- *M. bovis*

ALL SITES  n = 932

Firdessa et al. Unpublished
Modern lineages tend to inhibit innate immunity

“Modern” lineages elicit lower pro-inflammatory cytokines than “ancient” lineages in human monocyte derived macrophages

More virulence with population growth and urbanization?


Ancient lineages are relatively less likely to progress to disease

Lineage matters:

- Gambians infected with Lineage 6 (M. africanum) have lower response to ESAT-6 antigen (Elispot).

- Capilia TB lateral flow assay: lower sensitivity in W Africa (Lineage 5 mpb64 gene mutations).
  Comas I et al. Trends in Microbiology xx (2011) 1–9

Strains of the ‘ancient’ Lineage 6 (M. africanum), were three times less likely to progress to active TB compared to other MTBC lineages

Comas I, Gagneux S. Trends in Microbiology xx (2011) 1–9
**M. tuberculosis** diversity affects drug and possibly vaccine response

- Beijing genotype is often associated with drug resistance (MDR/XDR)
- *M. africanum* less prone to drug resistance than the Euro-American lineage of *M. tuberculosis* prevalent in Ghana
- BCG efficacy was reduced in mice when challenged with Beijing strain than with the Euro American lineage H37Rv lab strain
  - Geographical variation in BCG efficacy
- Success of TB program in India could partly be because ancestral lineage is less rapidly disseminating at the population level and less prone to acquisition of drug resistance
Global TB burden by strain lineages (2002 estimate)

What lineages do most new candidate vaccine strains belong to?
Overview of the immune response in TB
Pathogenesis

• *M. tuberculosis* conserves its epitopes to be recognized by the T cells of the host
  – Establishes latency
  – Induces host destruction and escape
    • Implications for diagnostics: *limited diversity, possible wide geographic application*
    • Implications for vaccines: *could vaccine induced immunity also lead to inadvertent dissemination*?

Comas I et al. Nature genetics 2010;42(6)498-503
...a major area of current interest is to understand the granuloma

- Mycobacteria exploit the granuloma to their advantage
  - Use host cholesterol
  - Defend against oxidative stress through mycobacterial proteasome

- Mycobacteria inhibit autophagy to enhance intracellular growth
  - IFN γ induces IGRM1 to regulate autophagy and suppress proliferation
  - Polymorphism in IGRM protects against *M. tuberculosis* ss but not against *M. africanum* in West Africa

Inflammation or resolution?

B cells?
Neutrophils?

Huynh K et al. Current Opinion in Immunology 2011, 23:464–472
TB Biomarkers

Multiple types and degrees of pathological processes within the same host: dynamic continuum

Biomarkers would provide prognostic information

Resistance
Latency
Protection
Diagnosis

Walzl G et al. Nature reviews 2011
Biomarkers

• Correlates of risk of TB disease:
  – Limitations of IFN-γ
    • Th1 specific cytokine responses as well as polyfunctional T cells measured at 10 weeks post immunization did not correlate with risk of disease in BCG vaccinated newborns followed for two years

• IGRAs do not have a high predictive value for the development of active tuberculosis
  – Rangaka et al. Lancet Infect Dis 2011;Epub 2011/08/19
Biomarkers…

• Alternatives under investigation:
  – Poly-cytokine bio-signatures
  – mRNA transcripts for
    • IL-8, FOXP3,
    • IL-12 β in ESAT-6 stimulated PBMC?
  – T cells secreting only TNF α as indicators for active TB? Or only IFN γ?

• Harari A et al. Nature Medicine 2011;17(3)372-6
• Casey R et al. PloS One 2010;5(12)e15619
Biomarkers:

- Sputum culture conversion at two months after treatment
  - Predictor of non-relapse, used in drug trials
    - Not so predictive at the individual level?
    - Need to wait two months to distinguish
    - Relies on sputum, difficult in children
  - Alternatives under investigation:
    - Changes in cytokine ratios (IFN $\gamma$/IL-4; IL-4/IL-4$\delta$2) or proportion of T cells secreting different cytokines
    - Bactericidal activity in whole blood culture
- Serological tests disappointing so far; quest goes on
  - Steingart et al Plos Medicine 2011;8(8):e1001062 Epub 2011/08/23
Biomarkers...

- An “-omics” or systems approach to biomarker discovery in the next years:
  - High throughput screening for antibodies against the entire proteome of *M. tuberculosis* has recently identified a small pool of antigens recognized in sera of patients with active TB.
  - Proteome fingerprinting of serum (- serum amyloid A and transthyretin -) to differentiate TB from other differential diagnoses
  - Transcriptional profiles that could distinguish between active and cured or active and latent TB

Agranoff D et al. Lancet. 2006;368(9540):1012-21
Diagnostics

• Rapid detection of MDR critical!
• Important advances recently - targeted focus!
  – Tests endorsed by WHO for use in disease endemic countries for rapid detection of MDR (interim):
    • Microscopically observed drug susceptibility test (MODS), nitrate reductase assay, indirect colorimetric redox indicator methods
  – Commercial liquid culture systems
  – Nucleic acid based tests, line probe assay
• **Xpert MTB/RIF**
  
  – Automated system
  
  – Integrated sample processing and nucleic acid amplification
  
  – Detects *M. tuberculosis* within 2 hours with high sensitivity and specificity (moderate sensitivity for smear negative)
  
  – Less contamination or infection risk
  
  – Easy to use but still requires adequate lab infrastructure and training
  
  – Costly, does not detect INH resistance
  
  – Data on performance and impact accumulating

---


**Theron G et al.** Lancet 2011;378(9790):481

**Miller MB et al.** J Clin Microbiol 2011. Epub 2011/08/19
Diagnostics... gaps

• Point of care diagnostics needed!!!
• Diagnostics challenges remain:
  – TB in children
  – HIV co-infection
  – Smear negative tuberculosis
  – Extra-pulmonary tuberculosis
Making the best with what exists...

- WHO recommends same day diagnosis and start of treatment with 2 consecutive spot-spot sputum smears
  - Spot-spot is 2.8% less sensitive than spot-morning; with same specificity of 98%; benefit in reduced default and early treatment

### Same-day diagnosis of tuberculosis by microscopy. WHO policy statement 2010

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Smear Result</th>
<th>Culture</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>Positive</td>
<td>453 (63.6)</td>
<td>63.6% (59.7%–67.5%)</td>
<td>97.4% (93.5%–99.9%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>259 (36.4)</td>
<td>2,160 (97.4)</td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>Positive</td>
<td>550 (64.8)</td>
<td>64.8% (61.3%–68.3%)</td>
<td>97.8% (94.3%–99.9%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>299 (35.2)</td>
<td>2,524 (97.8)</td>
<td></td>
</tr>
<tr>
<td>SSM</td>
<td>Positive</td>
<td>500 (70.2)</td>
<td>70.2% (66.5%–73.9%)</td>
<td>96.9% (93.2%–99.9%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>212 (29.8)</td>
<td>2,149 (96.9)</td>
<td></td>
</tr>
<tr>
<td>SMS</td>
<td>Positive</td>
<td>559 (65.8)</td>
<td>65.9% (62.3%–69.5%)</td>
<td>97.6% (94.0%–99.9%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>290 (34.1)</td>
<td>2,518 (97.6)</td>
<td></td>
</tr>
</tbody>
</table>

Making the best with microscopy

- Fluorescent light emitting diode (LED) microscopy with auramine to replace conventional
  - 84% sensitivity, 98% specificity against culture
  - 6% more sensitivity than conventional ZN microscopy
  - Half the time for reading
  - Scalable platform: malaria dx

Albert H et al. PLoS ONE 2010; 5(12): e15206

FIND-negotiated price for the Primostar iLED microscope is Euro1250 for low and middle income countries

WHO policy statement on LED microscopy. 2010
Vaccines

• Most efficient way to control TB
  – BCG ineffective to stop transmission
  – Two or three different new TB vaccines needed

• Two strategies to develop new TB vaccines:
  – Replace BCG with a whole organism (recombinant BCG or an attenuated *M. tuberculosis*)
  – Enhance protective effect of BCG with booster vaccines (protein-adjuvant or recombinant viral vectored)
### Twelve vaccines under clinical trial in 2010

6 candidates in preclinical studies and GMP; next generation in the pipeline: 33

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Products</th>
<th>Description</th>
<th>Sponsor</th>
<th>Indication</th>
<th>Status as of 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recombinant Live</strong></td>
<td>VPM 1002</td>
<td><em>rBCG</em> Prague strain expressing listeriolysin and carries a urease deletion mutation</td>
<td>Max Planck, Vakzine Projekt Management GmbH, TBVI</td>
<td><img src="image" alt="Phase Ib" /></td>
<td>Phase Ib</td>
</tr>
<tr>
<td></td>
<td>rBCG30</td>
<td><em>rBCG</em> Tice strain expressing 30 kDa Mtb antigen 85B; phase I completed in U.S.</td>
<td>UCLA, NIH, NIAID, Aeras</td>
<td><img src="image" alt="Phase I" /></td>
<td>Phase I [completed]</td>
</tr>
<tr>
<td></td>
<td>AERAS-422</td>
<td>Recombinant BCG expressing mutated PfoA and overexpressing antigens 85A, 85B, and Rv3407</td>
<td>Aeras</td>
<td><img src="image" alt="Phase I" /></td>
<td>Phase I</td>
</tr>
<tr>
<td><strong>Viral Vectored</strong></td>
<td>Oxford MVA85A / AERAS-485</td>
<td>Modified vaccinia Ankara vector expressing Mtb antigen 85A</td>
<td>Oxford Emergent Tuberculosis Consortium (OETC), Aeras</td>
<td><img src="image" alt="Phase IIb" /></td>
<td>Phase IIb</td>
</tr>
<tr>
<td></td>
<td>AERAS-402/ Crucell Ad35</td>
<td>Replication-deficient adenovirus 35 vector expressing Mtb antigens 85A, 85B, TB10.4</td>
<td>Crucell, Aeras</td>
<td><img src="image" alt="Phase IIb" /></td>
<td>Phase IIb</td>
</tr>
<tr>
<td></td>
<td>AdAg85A</td>
<td>Replication-deficient adenovirus 5 vector expressing Mtb antigen 85A</td>
<td>McMaster University</td>
<td><img src="image" alt="Phase I" /></td>
<td>Phase I</td>
</tr>
<tr>
<td><strong>Recombinant Protein</strong></td>
<td>M72 + AS01</td>
<td>Recombinant protein composed of a fusion of Mtb antigens Rv1196 and Rv0125 &amp; adjuvant AS01</td>
<td>GSK, Aeras</td>
<td><img src="image" alt="Phase II" /></td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>Hybrid-I+IC31</td>
<td>Adjuvanted recombinant protein composed of Mtb antigens 85B and ESAT-6</td>
<td>Statens Serum Institute (SSI), TBVI, EDCTP, Intercell</td>
<td><img src="image" alt="Phase I" /></td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>Hybrid-I+CAF01</td>
<td>Adjuvanted recombinant protein composed of Mtb antigens 85B and ESAT-6</td>
<td>SSI</td>
<td><img src="image" alt="Phase I" /></td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>HyVac 4/AERAS-404, +IC31</td>
<td>Adjuvanted recombinant protein composed of a fusion of Mtb antigens 85B and TB10.4</td>
<td>SSI, Sanofi-Pasteur, Aeras, Intercell</td>
<td><img src="image" alt="Phase I" /></td>
<td>Phase I</td>
</tr>
<tr>
<td><strong>Whole Cell, Inactivated or Disrupted</strong></td>
<td><em>M. vaccae</em></td>
<td>Inactivated whole cell non-TB mycobacterium; phase III in BCG-primed HIV+ population completed; reformulation pending</td>
<td>NIH, Immodulon</td>
<td><img src="image" alt="Phase III" /></td>
<td>Phase III [completed]</td>
</tr>
<tr>
<td></td>
<td>Mw [<em>M. indicus pranii</em> (MIP)]</td>
<td>Whole cell saprophytic non-TB mycobacterium</td>
<td>Department of Biotechnology (Ministry of Science &amp; Technology, Government of India), M/s. Cadila Pharmaceuticals ltd</td>
<td><img src="image" alt="Phase III" /></td>
<td>Phase III</td>
</tr>
<tr>
<td></td>
<td>RUTI</td>
<td>Fragmented Mtb cells</td>
<td>Archivell Farma, S.I.</td>
<td><img src="image" alt="Phase II" /></td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td><em>M. smegmatis</em></td>
<td>Whole cell extract; phase I completed in China</td>
<td>–</td>
<td><img src="image" alt="Phase I" /></td>
<td>Phase I [completed]</td>
</tr>
</tbody>
</table>

Stop TB Partnership Working Group on New TB Vaccines: Tuberculosis Vaccine Pipeline - 2010
### Global Clinical TB Vaccine Pipeline

**Source:** Tuberculosis Vaccine Candidates – 2010; Stop TB Partnership Working Group on New TB Vaccines

#### September 2011 modified from 2010

<table>
<thead>
<tr>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase IIb</th>
<th>Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AERAS-422</strong>&lt;br&gt;Aeras</td>
<td><strong>M72+AS01</strong>&lt;br&gt;GSK, Aeras</td>
<td><strong>MVA85A/AERAS-485</strong>&lt;br&gt;Oxford-Emergent Tuberculosis Consortium (OETC), Aeras</td>
<td><strong>Mw [M. indicus pranii (MIP)]</strong>&lt;br&gt;Dept of Biotechnology (India), M/s. Cadila</td>
</tr>
<tr>
<td><strong>AdAg85A</strong>&lt;br&gt;McMaster University</td>
<td><strong>RUTI</strong>&lt;br&gt;Archivel Farma</td>
<td><strong>Hyvac 4/AERAS-404</strong>&lt;br&gt;SSI, TBVI, EdCTP, Intercell</td>
<td><strong>M. vaccae</strong>&lt;br&gt;Immudulon, NIH</td>
</tr>
<tr>
<td><strong>Hybrid 56+IC31</strong>&lt;br&gt;Statens Serum Institute (SSI), Aeras, Intercell, TBVI</td>
<td><strong>Hybrid-I+IC31</strong>&lt;br&gt;SSI, TBVI</td>
<td><strong>AERAS-402/Crucell</strong>&lt;br&gt;Ad35&lt;br&gt;Crucell, Aeras</td>
<td></td>
</tr>
<tr>
<td><strong>Hyvac 4/AERAS-404</strong>&lt;br&gt;SSI, Sanofi-Pasteur, Aeras, Intercell</td>
<td><strong>VPM 1002</strong>&lt;br&gt;Max Planck, Vakzine Projekt Mgmt, TBVI</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>M. smegmatis</strong>&lt;br&gt;UCLA, NIH, NIAID, Aeras</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**
- **P** Prime
- **B** Boost
- **PI** Post-infection
- **IT** Immunotherapy

**Preclinical** vaccine candidates are not yet in clinical trials, but have been manufactured under Good Manufacturing Practice (GMP) for clinical use and have undergone some preclinical testing that meets regulatory standards.

* Indicates candidates that have been in clinical trials in the past, but are not currently being tested in clinical trials.

Courtesy of Jelle Thole, TBVI
Vaccines

• Efficacy studies remain a huge challenge!
  – Cost high:
    • The cost of a TB vaccine to be licensed by 2015 would be around 3.5 billion USD!
  – Time too slow
  – Clinical trial sites inadequate
• Back to the basics and forward
  – systems approach to understand TB: processes that take place in pulmonary tissue, B cell compartment, models for studies in TB
    • design better vaccines
    • Identify correlates of protection
Treatment

• TB treatment
  – Too long, too many drugs, with side effects, increasing drug resistance, interaction with ART, no drug for latent TB

• Effective MDR TB treatment is a challenge:
  – Inadequate evidence:
    • Too few centres doing drug sensitivity testing (DST)
    • Existing drugs not effective enough: <69% cured after 18 m
    • Too expensive
  – Recommended treatment duration: > 8 months intensive phase with at least 20 months of therapy
    • Falzon D et al. Eur Resp J 2011;38(3)516-28
New drugs against TB

Lengthy, expensive risky nature of drug development

Financial shortage of over 75%

Inadequate capacity for clinical trials

Clinical trial design challenges

Regulatory requirements

Ma Z et al. Lancet 2010
New drugs in the pipeline


TB/HIV

• High risk of mortality and relapse
• Paucity of data on treatment of TB/HIV co-infection!
  – Duration of treatment with rifamycin should extend over 6 months (recent meta analysis) and is better given daily rather than intermittent
  – Current treatment inadequate
• TB/HIV integration still a challenge
  – Only 7.3% of TB/HIV co-infected patients received ART in 2008; 3Is (intensified case finding, isoniazid preventive therapy, infection control) not being implemented effectively
Conclusions

The advance in TB is for now an advance of the pathogen

Projected tuberculosis incidence: to 2050
Conclusions...

• More understanding of the basics of TB needed: systems approach?

Conclusions....

• Key downstream research needs:
  – **Operational** on how to maximize the yield from existing tools
    • Best ways to tap the local resources - Empowerment of local institutions (financial, policy...)
  – Facilitating conduct of good clinical trials
  – Building genuine lasting capacity to carry out collaborative research
Conclusions...

• New tools ineffective unless **systems** in place to use them
  – Measures to strengthen health systems: systems in governance, financing, health workforce, information management, procurement/supplies, service delivery
  – “Own, sustain and scale up”! (ICASA 2011 Addis
    [www.icasa2011addis.org](http://www.icasa2011addis.org) )
Acknowledgement

• The Armauer Hansen Research Institute is supported through core grants from NORAD and Sida

• We acknowledge grants from Wellcome Trust, EDCTP, WHO, BMGF, DFG, UBS, WHO/TDR and others

• References available on request