Inaugural Lecture:
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Special Thanks:

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"To be, or not to be, that is the question—
Whether 'tis Nobler in the mind to suffer
The Slings and Arrows of outrageous Fortune,
Or to take Arms against a Sea of troubles,
And by opposing end them? To die, to sleep—
No more; and by a sleep, to say we end...."
"TB or not TB"
That is the question -
Understanding and Diagnosing TB using Metabolomics
History of TB

- Robert Koch - 1882
History of TB

150 million years ago - Jurassic period

- 3 million years ago – infection in early hominids (TB meningitis in Homo erectus)
• 1.7 million years ago - Migration out of East Africa

• 30,000 years ago – migration into America

• Peruvian mummies show TB well before this migration
Evolutionary timeline = TB timeline
TB was first identified in 460BCE by Hippocrates and initially named "phthisis" which means "consumption" in Greek. Throughout history, TB has had many names, including "white plague". However, since Dr. Koch's discovery, "tuberculosis" became the more common medical term.

- 1882: Robert Koch discovered TB using the microscope.
- 1895: Development of chest X-ray diagnostic test.
- 1907: Tuberculosis skin test developed.
- 1921: BCG vaccine introduced.
- 1936: Solid culture first used to identify TB.
- 1943: First anti-TB drug discovered: Streptomycin.
TB Timeline Continued…

1952
First anti-TB regimen used: Streptomycin, PAS, Isoniazid

1963
Rifampicin and Capreomycin discovered

1974
British Medical Research council trials added Rifampicin and Pyrazinamide

1980
Emergence of MDR-TB*
Liquid culture developed

1994
Emergence of TB-HIV co-infection*
Directly Observed Treatment, Short-course (DOTS)

USAID’s Tuberculosis Program Began
TB Timeline Continued…

Emergence of XDR-TB*

1998
- Rifapentine approved

2009
- ILED microscope, line probe assay developed

2010
- GeneXpert MTB/RIF® rapid test for TB receives CE IVD marketing approval

2011
- New drug development approach: CPTR (critical path to TB [drug] regimens)

GeneXpert MTB/RIF® assay is the new molecular test that can detect TB and mutations associated with Rifampicin resistance in fewer than 2 hours with far greater accuracy than smear microscopy.

One Day We Hope to Have….

- A tool that can diagnose TB and MDR-TB within 24 hours for children, adults, and HIV-infected individuals
- A shorter treatment regimen that can cure TB in 10 days or less that will also work with antiretroviral drugs
- A vaccine that can prevent new TB infections or recurrences of the disease

*Notes
- MDR-TB: Multi-drug resistant tuberculosis
- TB: Tuberculosis
- HIV: Human Immunodeficiency Virus
- XDR-TB: Extensively drug-resistant tuberculosis
Tuberculosis: Shocking Statistics

- Infectious disease of primarily lungs – *M. tuberculosis*
- 2nd most deadliest infectious disease (after HIV)
- ⅓ of global population infected
- 9 million new cases per year
- 1.4 million deaths per year of which 25% are HIV co-infected
- MDR-TB, XDR-TB is on the rise globally
- Despite fervent research efforts since it's discovery in 1882 – TB is still considered a global epidemic
- New approaches are needed = Metabolomics?
Metabo-WHAT-ics????
Systems Biology

The “Omics” Cascade

What can happen
- Genome

What appears to be happening
- Transcriptome

What makes it happen
- Proteome

What has happened and is happening
- Metabolome

DNA
RNA
Proteins
Metabolites

Systems / Integrative Biology
Metabolomics workflow

Patient sample collection (e.g., blood, urine)

Metabolite identification and quantification (NMR/GC-MS/LC-MS)

Database Production

Identify and compare treatment classes

Computational Analysis

Quantify putative biomarkers

Map to metabolic pathways

 Identify metabolite interactions

Metabolite Markers
Our Approach?

1) **TB Diagnostics:**

2) **TB Characterization:**
1. Total Lipid Extraction Method

2. "Total Metabolome Extraction Method"


Applications of Methodologies

Cell Cultures

1) Total Lipid Extraction Method

Applications?

2) Total Metabolome Extraction Method

a) Virulence
   Meissner-Roloff et al. (2012). *Metabolomics* (hyper vs hypo)
   Swanepoel et al. (2013). *Metabolomics* (ESX-1)

b) Growth
   Loots et al. (2013). *Metabolomics* (ESX-3)
   Loots et al., submitted, *Metabolomics* (Iron)

c) Drug Resistance
   INH: Loots 2014. *Antimicrobial Agents and Chemotherapy*

b) Diagnostics
   Patient sputum samples

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a) Diagnostics
   Olivier & Loots. (2012). *Journal of Microbiological Methods*
   Patent: PCT/IB2012051995

b) Drug Resistance
1. Sputum:
   • **Homogenization:** Schoeman et al. 2012. *Journal of Microbiological Methods*
   • **Applied to 95 Patient sputum samples:** du Preez & Loots. (2013). *Tuberculosis*

2. Urine:
   • Early Prediction of Treatment Outcome: *De Villiers et al.*

3. Blood
New Metabolic Pathways - Drug Resistance / Virulence

Cell wall synthesis

↓ Methyl branched fatty acids

SAM

Methionine

MUFA

dehydrogenase

FADH₂

O₂

FAD

2H⁺ + 2cyt b₅ Fe²⁺

NADP⁺

NADPH + H⁺

Riboflavin metabolism

GTP → RNA

Fatty acid

Acetyl CoA

β-oxidation

Lipid peroxides

↑ Alkanes

↑ Alcohols

Alkanes

Fatty Acids

↑ Glycolytic surfactants
(D-glycerol-L-mannoheptanoic acid)

ENERGY

ATP

H₂O + O₂

↑ H₂O₂ → Free Radicals

Inhibition

Urease

Nε-Nε

Reperoxide

Scavenger

↑ Cadaverine

↑ p-OH-benzoic acid

↑ Ascorbic acid

INH Resistance

Rif Resistance
Adaptations of Mycobacteria to Host in Competition to Survive

The diagram illustrates various metabolic pathways such as the Glyoxylate cycle, Citramalate cycle, and Krebs cycle. These pathways are interconnected, showing how mycobacteria adapt to host conditions. Key metabolites include acetyl-CoA, CoA, oxaloacetic acid, citric acid, pyruvic acid, isocitric acid, citramalic acid, fumaric acid, glycine, glutamic acid, succinic acid, and GABA (gammabarbituric acid).
Adaptations of Host to Mycobacteria in Competition to Survive

Macrophage containing $M. \text{ tuberculosis}$

$\text{glucose + O}_2 \xrightarrow{\text{glucose oxidase}} \text{H}_2\text{O}_2$

$\text{d-gluconic acid d-lactone}$

$\text{lipid peroxidation}$

$\text{butanal ethane}$

$\text{ETC deficiency}$

$\text{Neurotransmitters}$

$\text{GABA, norepinephrine}$

$\uparrow \text{amino acid degradation}$

$\uparrow \text{fatty acid } \beta\text{-oxidation}$

$\text{3,4-dihydroxybutanoic acid}$

$\text{normetanephrine}$

$\text{sebacic acid}$

$\text{glutaric acid}$
**TB Diagnostics**

**Current**

- **TB or not TB?**
  - Culturing (2-8 weeks)
  - Smear microscopy
    - Poor detection limit -10,000 cells/ml
  - Gene amplification
    - Poor sensitivity & false positives

- **Drug Resistance**
  - Culturing (8-16 weeks)
  - Gene amplification
    - Poor sensitivity & false positives

- **Speciation**
  - Gene amplification

- **Treatment and monitoring**
  - Predicting treatment outcome

**Future with metabolomics**

- **Symptomatic Patient**
  - 3 Markers sputum
    - 98% sensitivity
    - 100% specificity
    - 1 Hour turnaround time
    - Inexpensive after infrastructure purchased

- **Point of care?**

- **Metabolomics Drug Resistance Markers?**

- **Patent: PCI/IB 2012051995**

  PCA plot: Treatment response-Cured vs Failed
Simple, fast, inexpensive, sensitive and specific, point of care TB diagnostics
In Closing: The Key to Success