# Inaugural Lecture:
# Prof Du Toit Loots

**Infectious Disease Metabolomics Laboratory, Centre for Human Metabolomics, Biochemistry, North-West University, Potchefstroom, 2520, South Africa**

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# Special Thanks:

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- Mom and Dad (Winifred and Du Toit Loots)
- Prof Japie Mienie – MSc and PhD Promoter
- Stellenbosch University Medical Faculty – Prof Nulda Beyers
- Academic Medical Centre in Amsterdam – Prof Ries Duran
- CEN - Prof Este Vorster
- CHM - Prof Frikkie van Niekerk / Prof Carools Reinecke
- All Colleagues at Biochem (including all support and administrative staff) and International collaborators
- Marlien and Gemma
- Derelize and Lizelle

# William Shakespeare – Hamlet (1623)

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**"To be, or not to be, that is the question—**

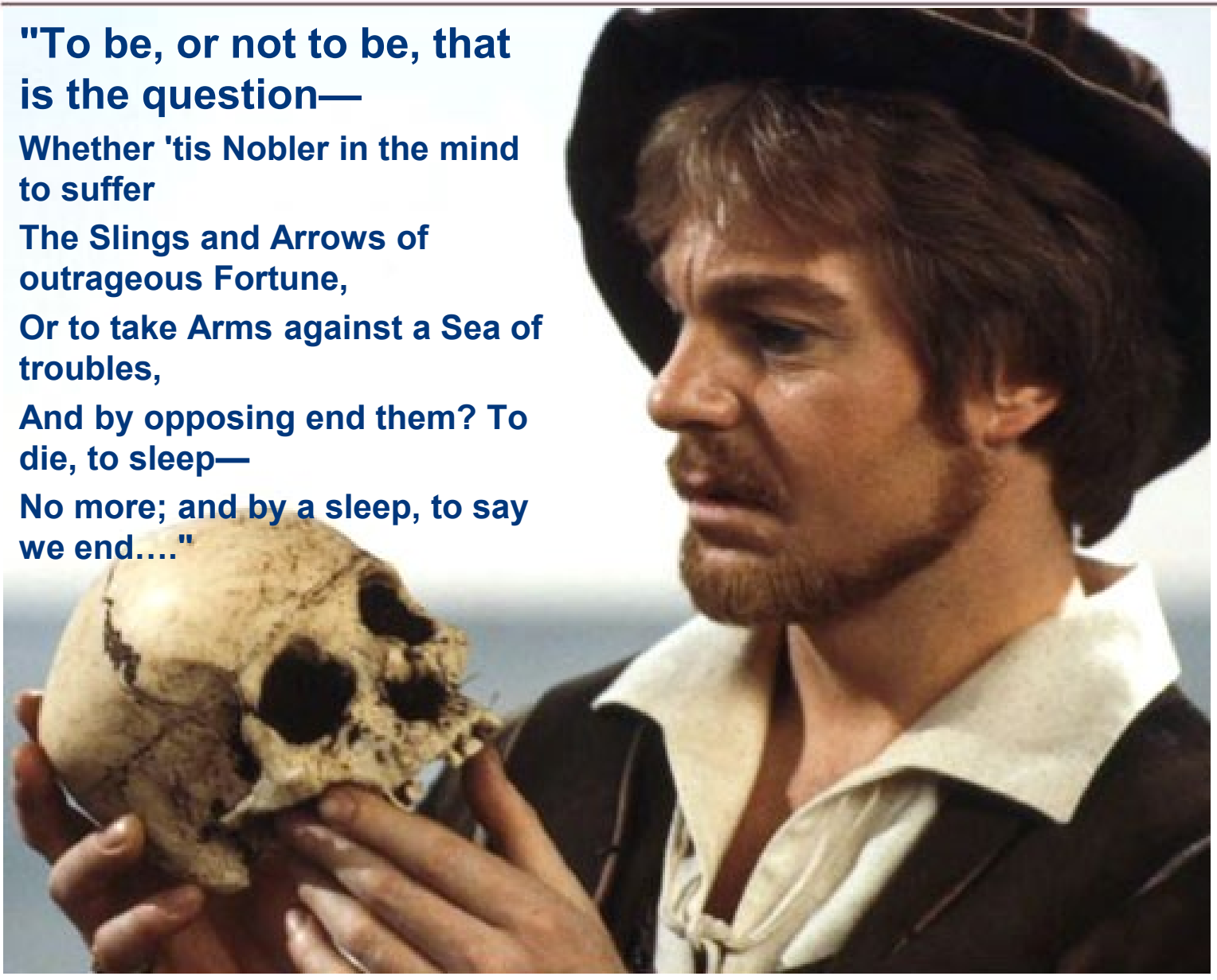
**Whether 'tis Nobler in the mind to suffer**

**The Slings and Arrows of outrageous Fortune,**

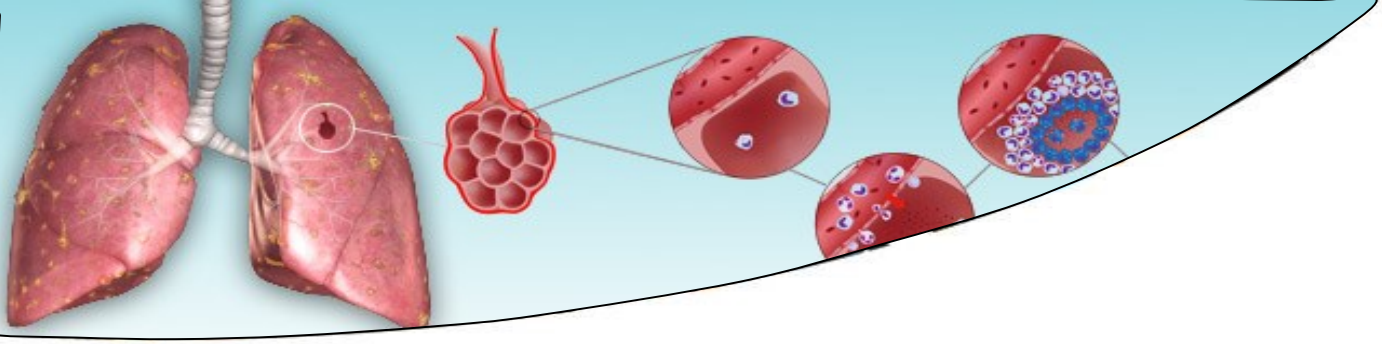
**Or to take Arms against a Sea of troubles,**

**And by opposing end them? To die, to sleep—**

**No more; and by a sleep, to say we end…."**



**In the speech, a despondent or feigning Prince Hamlet contemplates death and suicide. He bemoans the pains and unfairness of life but acknowledges the alternative might be still worse.**



"TB, or not TB?"

# "TB or not TB"
# That is the question -
## Understanding and Diagnosing TB using Metabolomics

[redacted]

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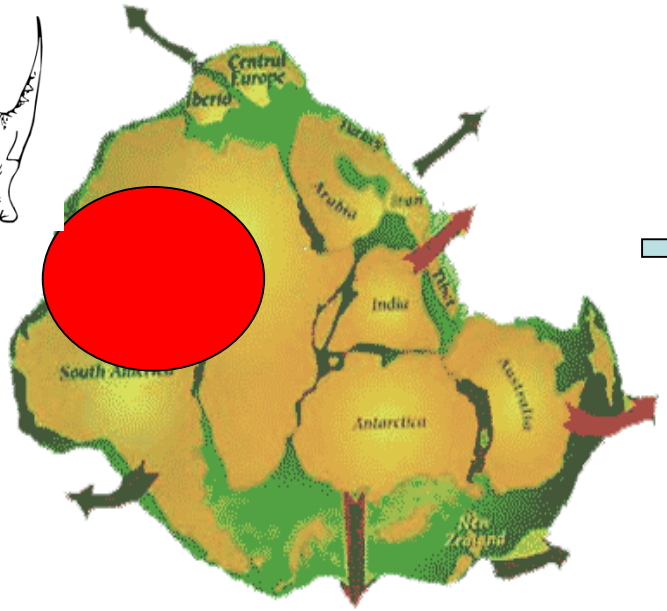
# History of TB



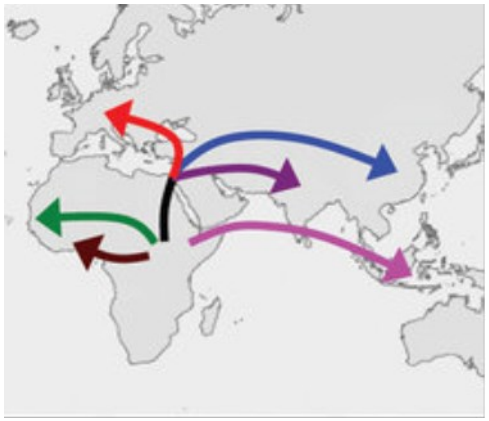
- **Robert Koch - 1882**

# History of TB

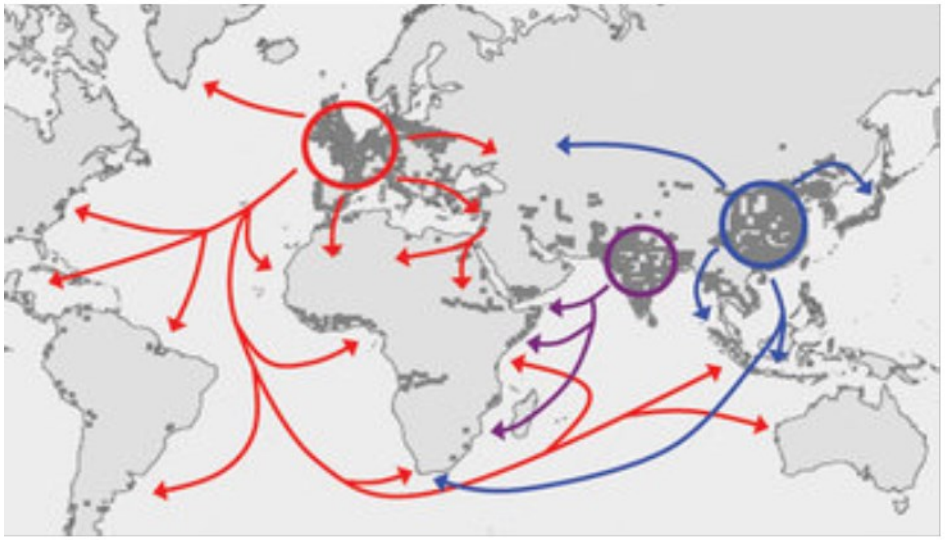



150 million years ago - Jurassic period

- 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

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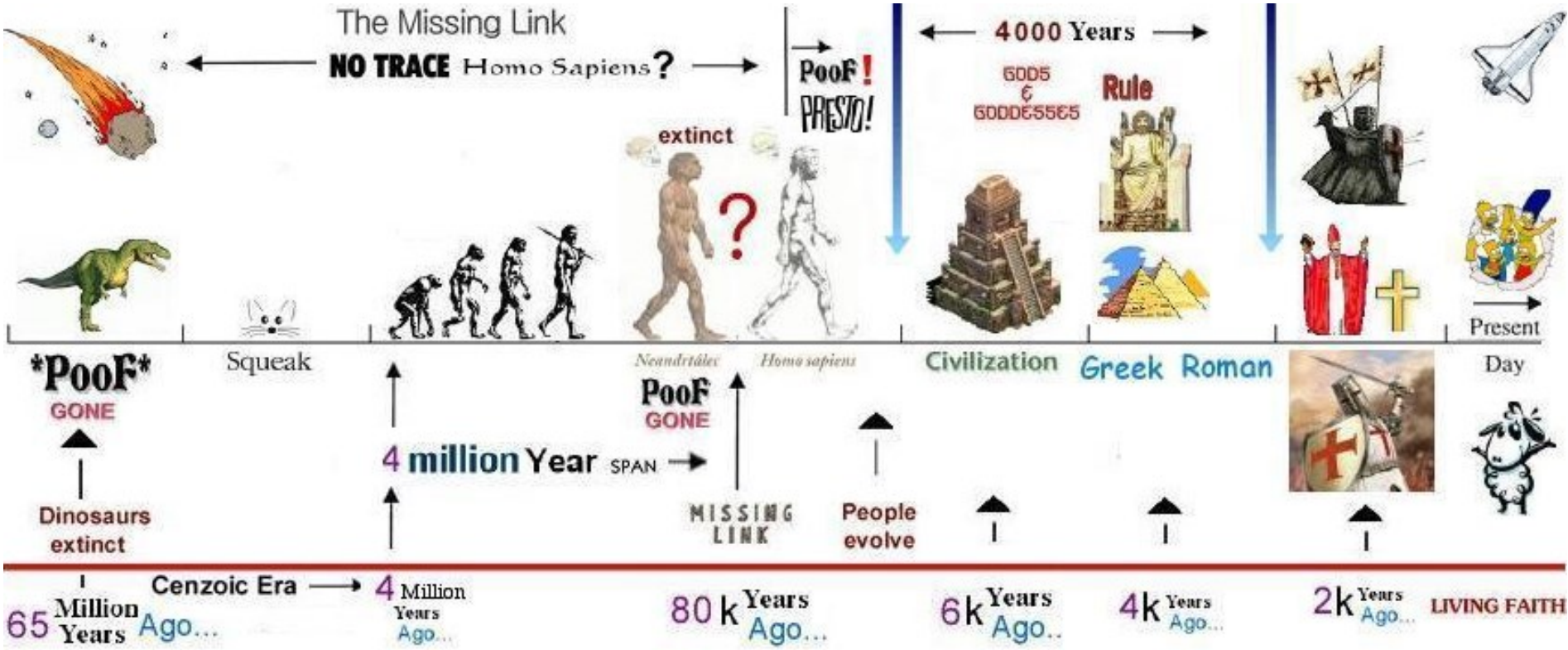
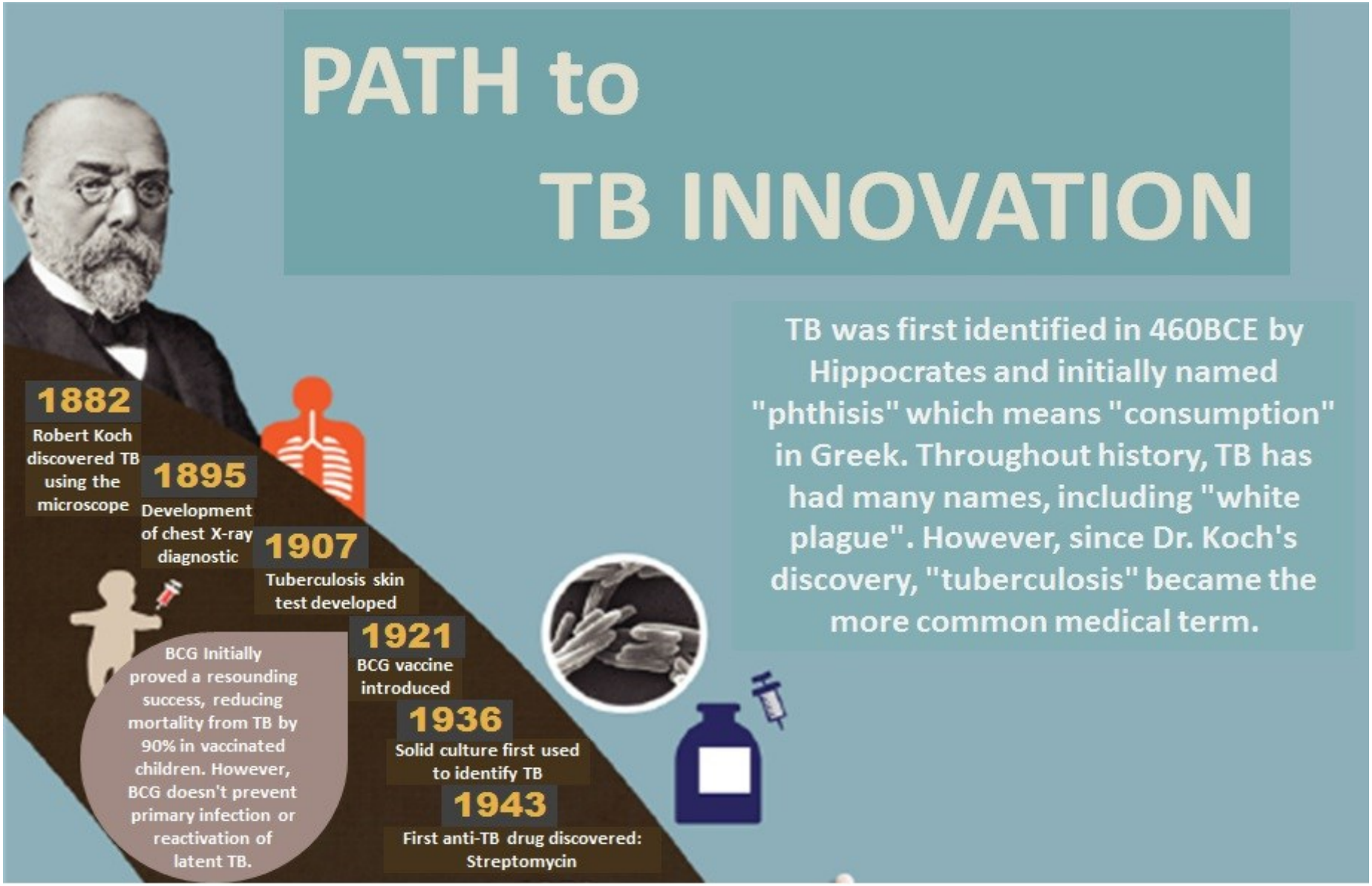


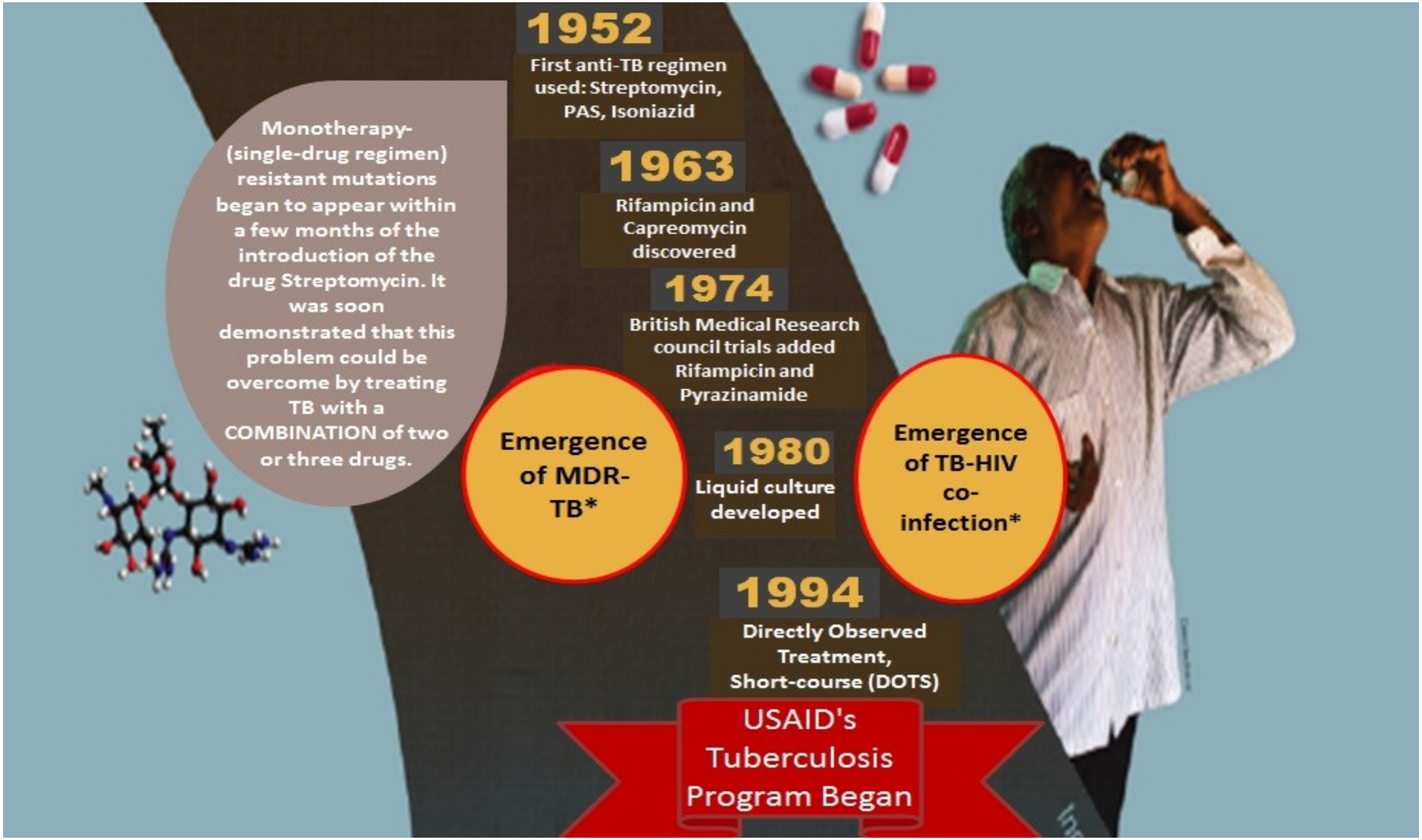
# TB Timeline




PATH to
TB INNOVATION
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
1907
Tuberculosis skin test developed
1921
BCG vaccine introduced
BCG Initially proved a resounding success, reducing mortality from TB by 90% in vaccinated children. However, BCG doesn't prevent primary infection or reactivation of latent TB.
1936
Solid culture first used to identify TB
1943
First anti-TB drug discovered: Streptomycin

# TB Timeline Continued…





# TB Timeline Continued…

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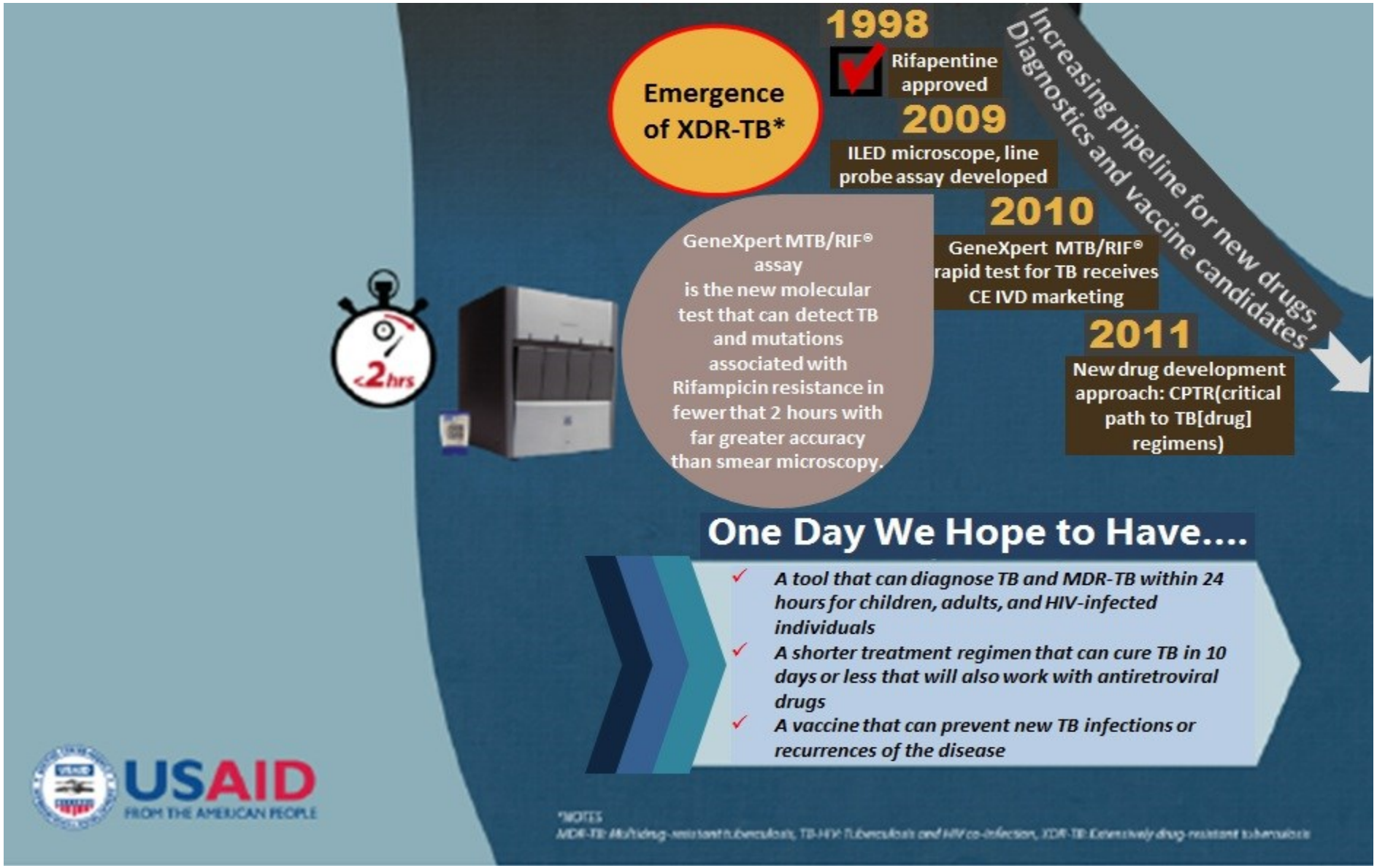
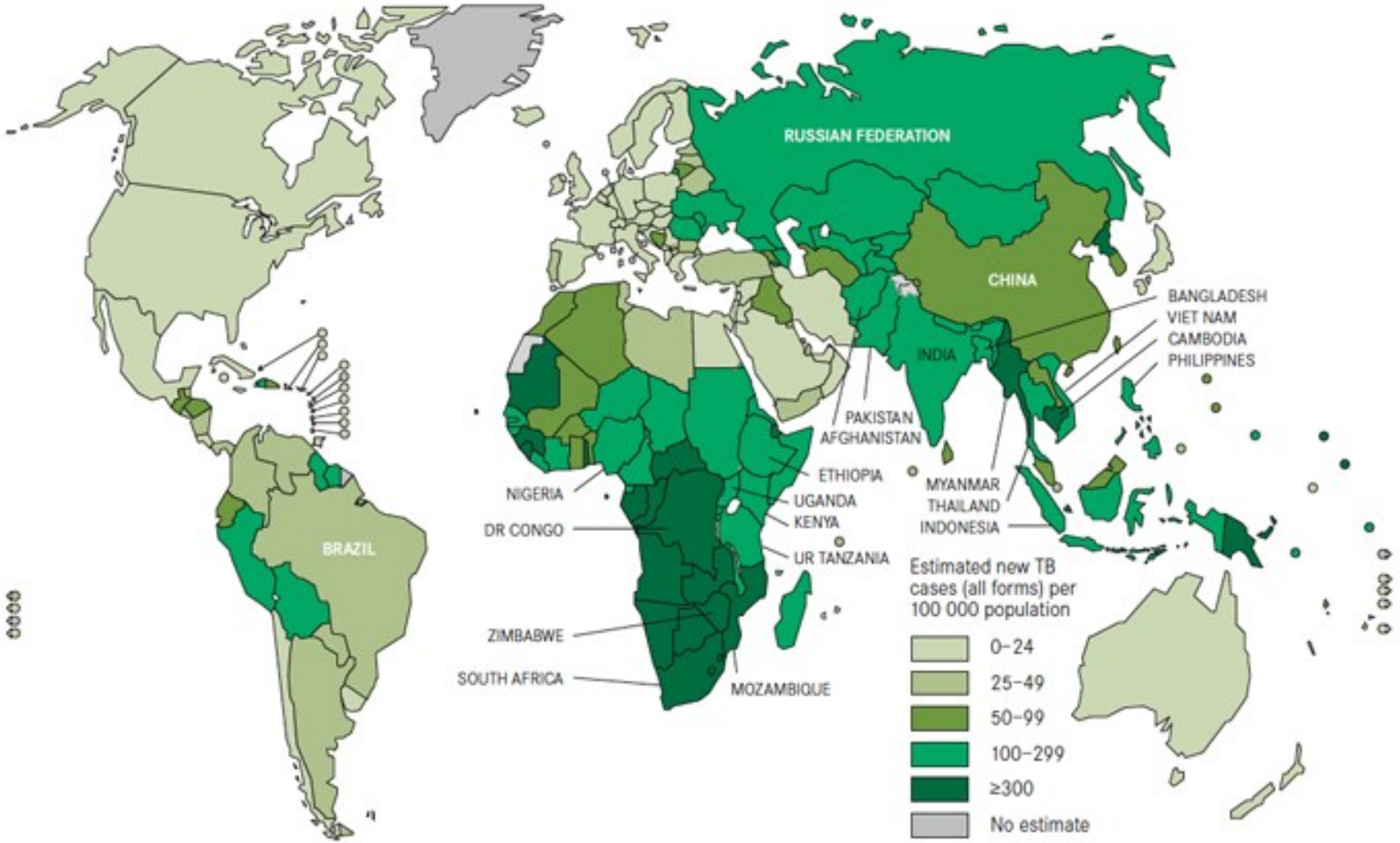


# Global TB prevalence





# Tuberculosis: Shocking Statistics

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- 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????

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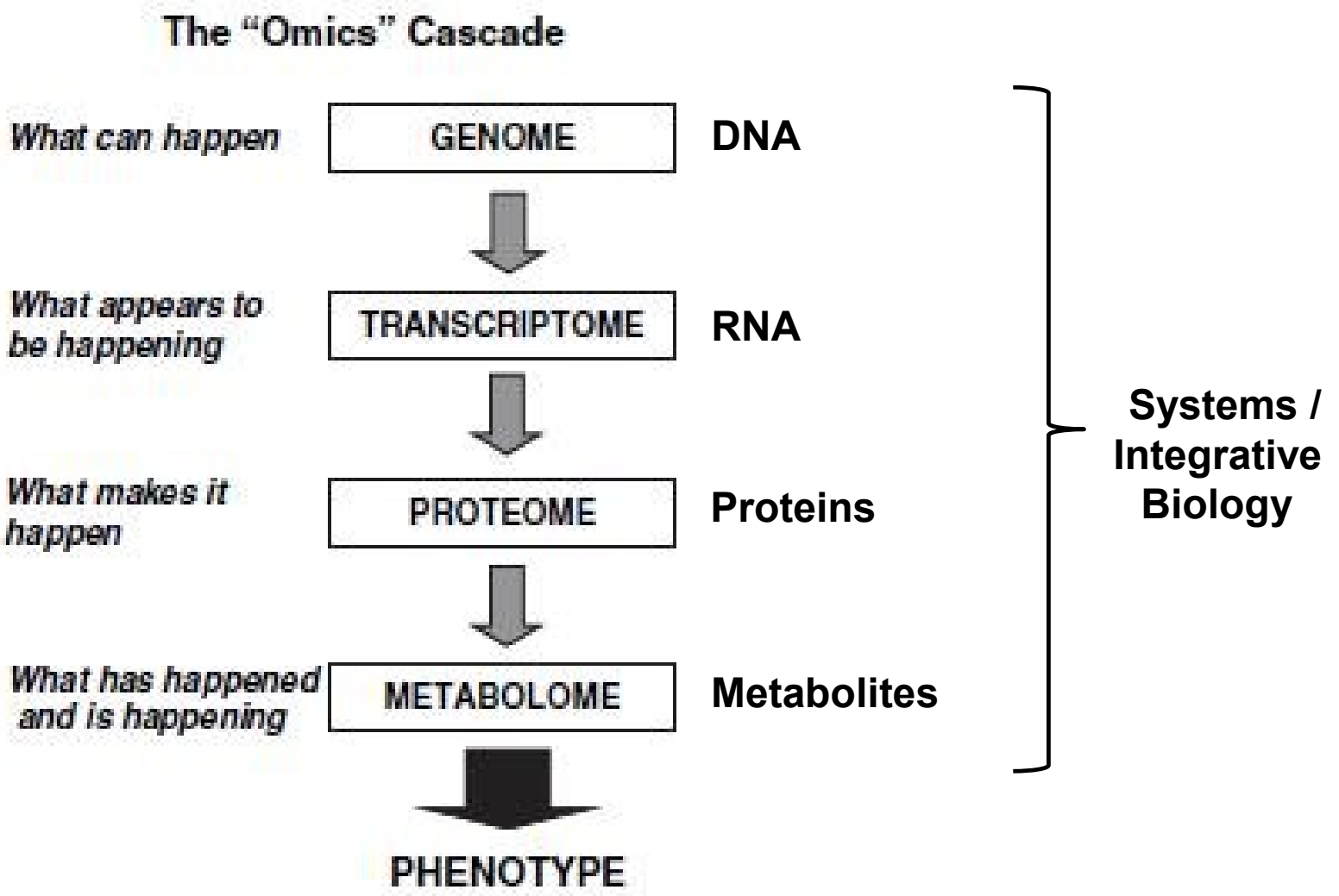
# Systems Biology





# Metabolomics workflow

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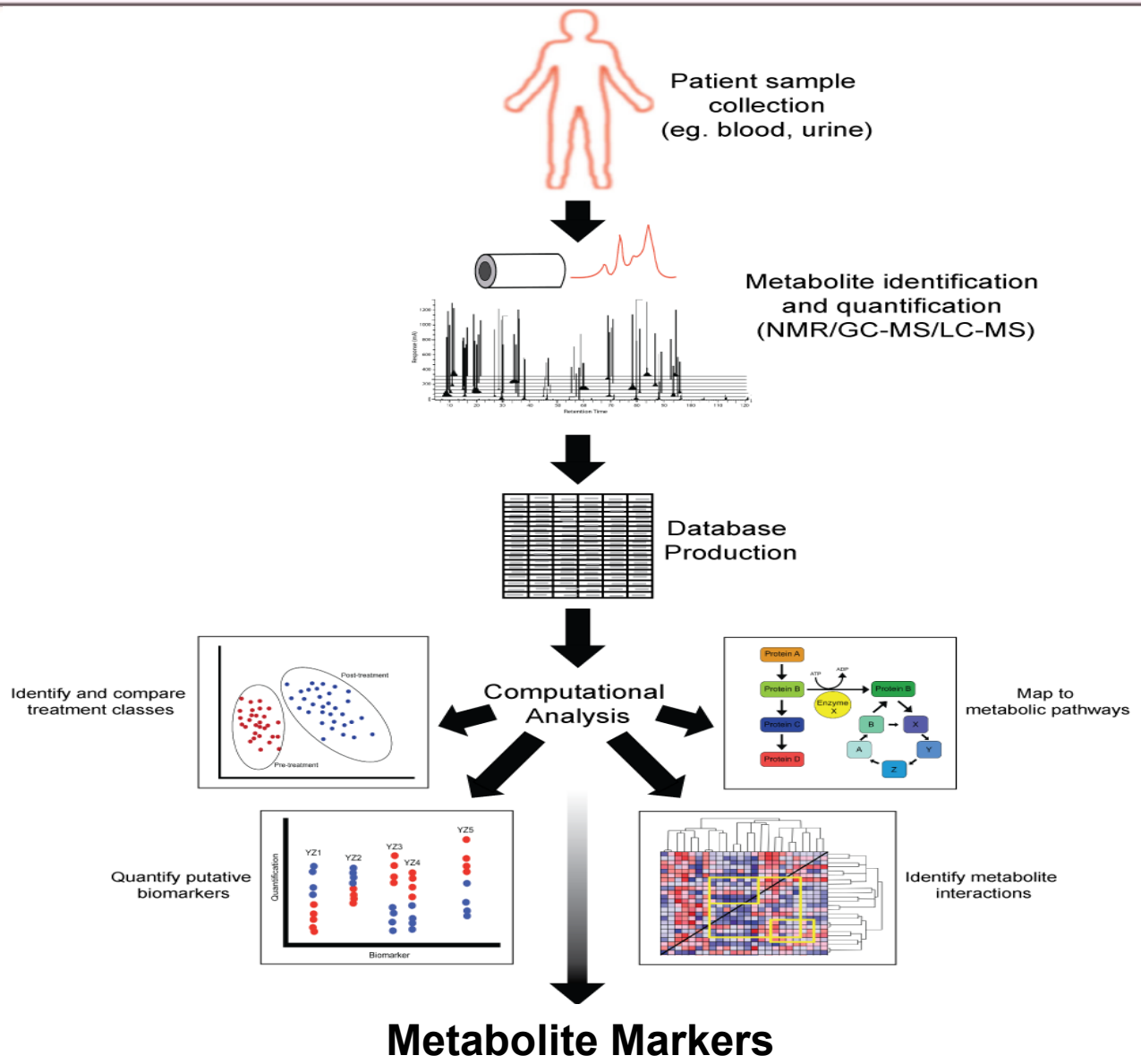

# Our Journey

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## Our Approach?

1) **TB Diagnostics:**
*I. Olivier & Du T. Loots. (2011). An overview of tuberculosis treatments and diagnostics. What role could metabolomics play? J Cell Tissue Research, 11(1): 2655-2671.*

2) **TB Characterization:**
*J.C. Schoeman & Du T. Loots. (2011). Improved disease characterization and diagnostics using metabolomics: A review. J Cell Tissue Research, 11(1): 2673-2683.*

# Metabolomics Methodology:

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1. **Total Lipid Extraction Method**

2. **"Total Metabolome Extraction Method"**

Olivier & Loots. (2012). *African Journal of Microbiology Research*

Du Preez & Loots. (2013). *African Journal of Microbiology Research*

# Applications of Methodologies

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## Cell Cultures

### 1) Total Lipid Extraction Method

### 2) Total Metabolome Extraction Method

**Applications?**

#### a) Diagnostics

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

#### b) Drug Resistance

Rif: Olivier & Loots. (2012). Omics.

#### 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*

# Patient samples

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**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

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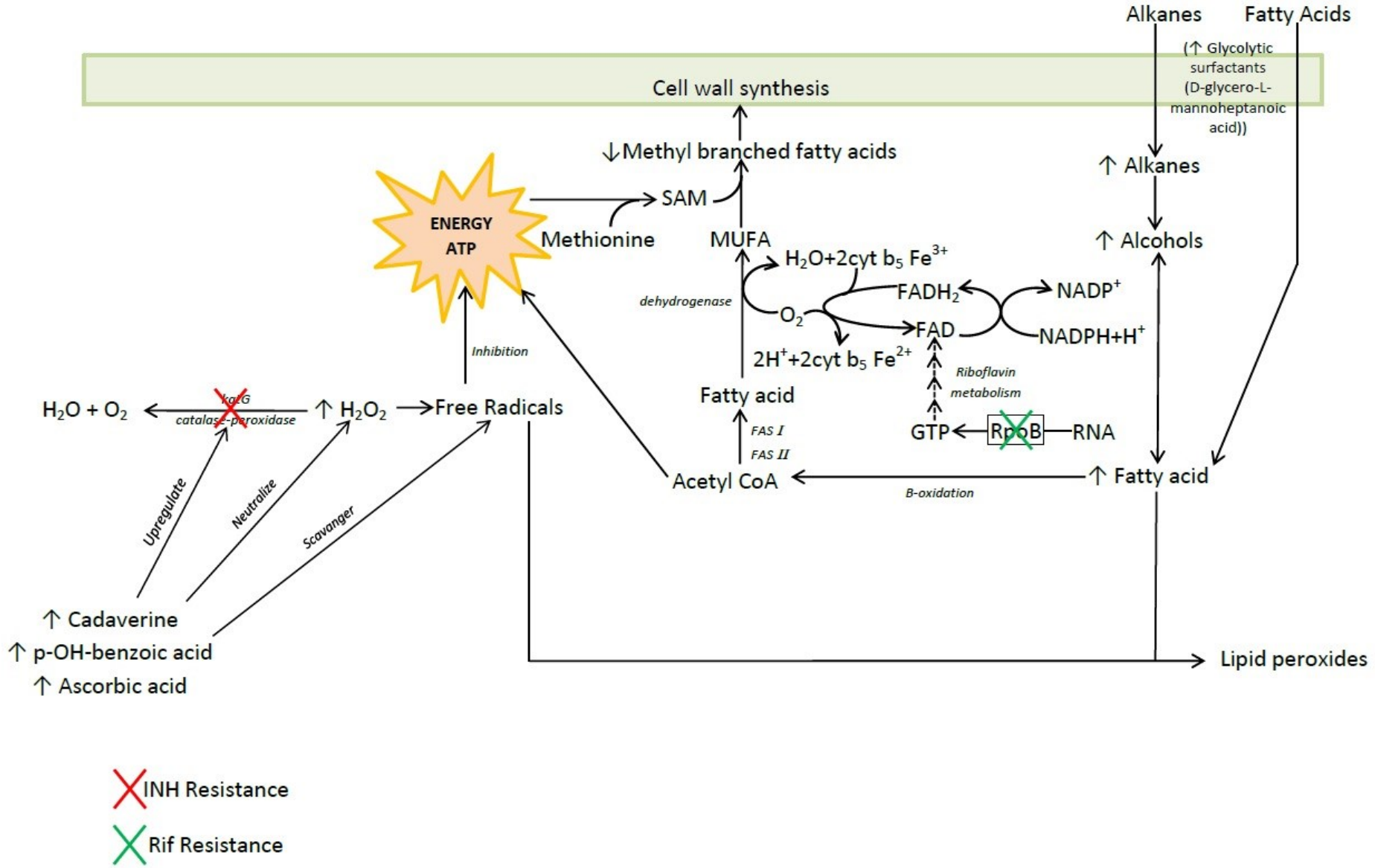

# Adaptions of Mycobacteria to Host in Competition to Survive

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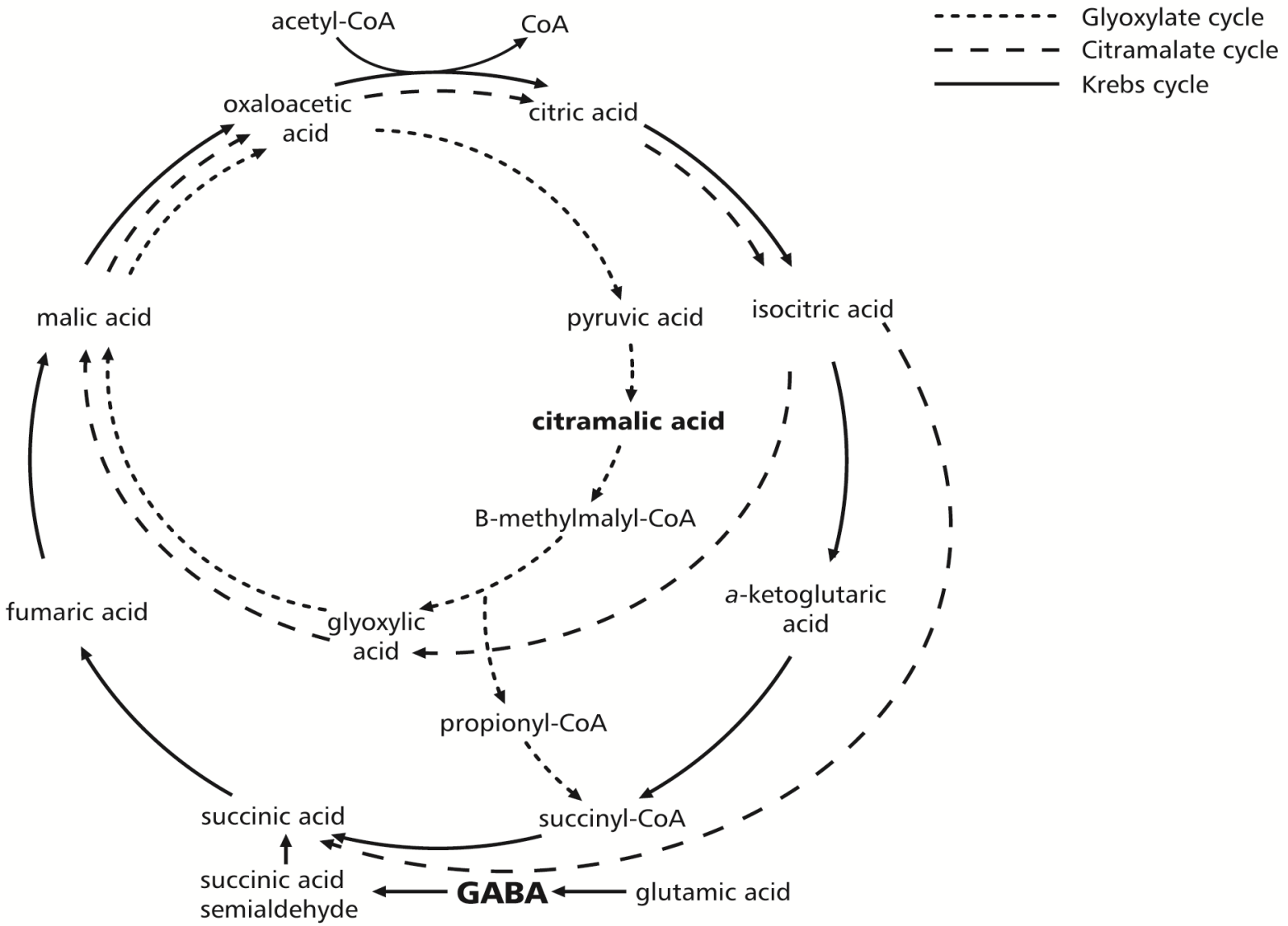

# Adaptions of Host to Mycobacteria in Competition to Survive

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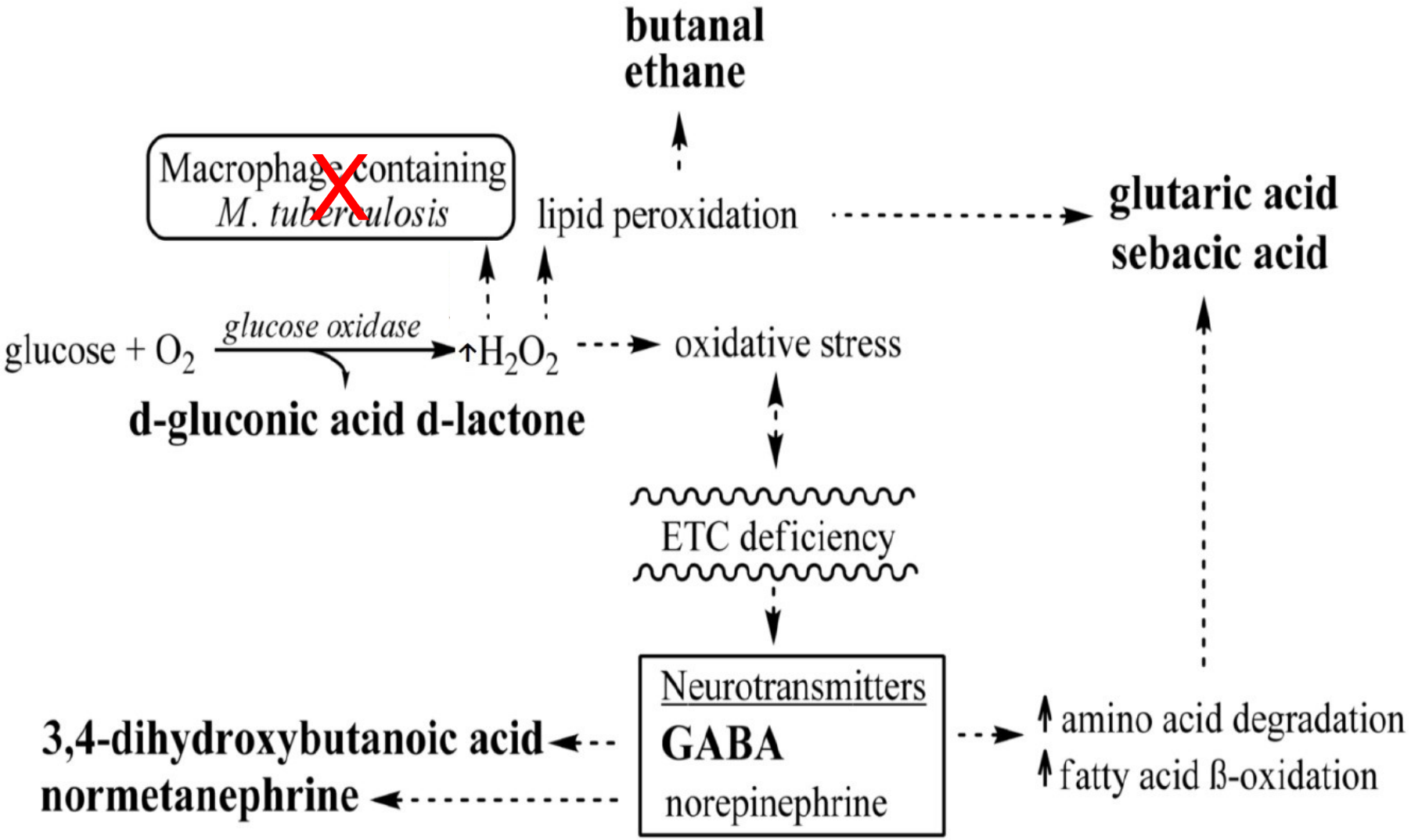

# TB Diagnostics

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## Future with metabolomics

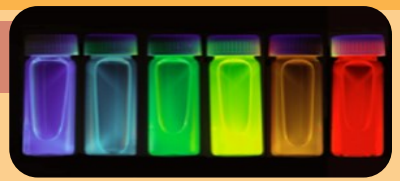
## Symptomatic Patient

## Current

### 3 Markers sputum

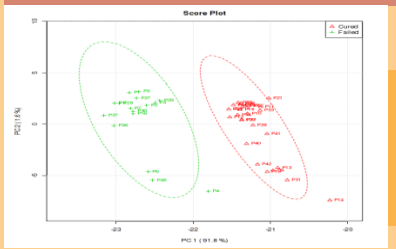
-98% sensitivity
-100% specificity
-1 Hour turnaround time
-Inexpensive after infrastructure purchased

### Point of care?



### Metabolomics Drug Resistance Markers?

### Patent: PCI/IB 2012051995




PCA plot: Treatment response-Cured vs Failed

### 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

# Nobel Prize Winning Research Question

**Simple, fast, inexpensive, sensitive and specific, point of care TB diagnostics**

# In Closing: The Key to Success