Outline

1. Overview of the Project

2. Progress to date

3. Challenges

4. Next steps
Overview of the project
RAFAgene project

Contribution of Genetic Variation to Pharmacokinetic Variability and Toxicity in Patients Undergoing Multi-drug Tuberculosis Treatment in Sub-Saharan Africa
Tuberculosis (TB):

- Important cause of death in Sub-Saharan Africa
- In best-case of TB treatment scenario, $\approx 10\%$ of patients cannot be cured
- Complex relationship between TB pathogen, drugs and host
- Genetic variability of the host might play an important role for treatment response
General Aim

- To conduct:
  - a pharmacogenetic study
  - of TB drugs (Rifampicin, Isoniazid, Ethambutol, Pyrazinamide and Gatifloxacin)
  - in TB patients in Sub-Saharan Africa
Specific aims

1. To assess the **role of host genetic variation on the pharmacokinetics (PK)** of TB drugs

2. To assess the role of **genetic variation in host** genes governing PK on the **efficacy and safety** of TB treatment

3. To validate **functional mechanisms** for putative associations.

4. To strengthen pharmaco(genetic) **research capacities** in Africa
RAFAgene partners

University of Liverpool (UK) (Genetic analysis)

London School of Hygiene & Tropical Medicine (UK) (Genetic epidemiology)

National TB program (Dakar), study site

National TB program (Conakry) study site

National Hospital for TB and Pulmonary Diseases (Cotonou, Benin) study site

Cape Town University (PK/ PD)

MRC SA (Durban, study site)
Rafagene study participants from 2 clinical trials:

- **OFLOTUB trial (completed)**
- **RAFA trial (almost completed)**
### Original Article

**A Four-Month Gatifloxacin-Containing Regimen for Treating Tuberculosis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental Group</th>
<th>Control Group</th>
<th>Adjusted Difference, Experimental Group–Control Group†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified intention-to-treat analysis</td>
<td></td>
<td></td>
<td>percentage points (95% CI)</td>
</tr>
<tr>
<td>Primary outcome: unfavorable outcome 24 mo after the end of treatment — no./total no. (%)</td>
<td>146/694 (21.0)</td>
<td>114/662 (17.2)</td>
<td>3.5 (−0.7 to 7.7)</td>
</tr>
</tbody>
</table>

Corinne S. Merle et al. *A Four-Month Gatifloxacin-Containing Regimen for Treating Tuberculosis.*

*N Engl J Med* 2014; 371:1588-1598
To improve TB/ HIV co-infected treatment

<table>
<thead>
<tr>
<th>Arm</th>
<th>Intensive phase</th>
<th>Continuation phase</th>
<th>Particularity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2ERHZ</td>
<td>4RH</td>
<td>ART at 15 days</td>
</tr>
<tr>
<td>2</td>
<td>2ERHZ</td>
<td>4RH</td>
<td>ART at 2 months</td>
</tr>
<tr>
<td>3</td>
<td>2ERHZ</td>
<td>4RH</td>
<td>ART at 2 months + High R</td>
</tr>
</tbody>
</table>

Study completed, abstract submitted to International AIDS Society Conference
RAFAgene nested in OFLOTUB and RAFA

**OFLOTUB**
- 1864 TB patients recruited
- 343 in the PK study

**RAFA**
- 1125 TB patients being recruited
- 300 in PK study

575 in pharmacogenetic study
Pharmacokinetic (PK) analysis

- **Serial blood samples** (pre-dose and at various hours after TB treatment dosing)
- Samples processed and analyzed by liquid chromatograph mass-spectrometry (LC-MS)
- **Area under the curve (AUC) measured**
- Already performed in the parents study (Oflotub and RAFA)
Genetic analysis

Comprehensive approach to identify relevant genetic markers relevant to drug absorption, distribution, metabolism, and elimination (ADME):

- **Affymetrix DMET Plus Premier Pack** comprising 1936 **polymorphisms** in ~230 genes relevant to ADME

- **Sequenom iPlex platform or real-time PCR** for a number of hypothesis-driven targeted variants in genes not covered in Affimetrix (based on literature).
Functional validation

If association PK/PD and genetic analysis, **mechanistic relevance of single nucleotide polymorphisms (SNPs)** will be evaluated:

- To confirm the biological plausibility
- To help understand underlying mechanisms of identified SNP associations

A range of in vitro techniques will be employed
### Capacity building

#### Capacity strengthening

Building of a large team with:

- Expertise in (pharmaco) genetics, epidemiology, statistical genetics and bioinformatics

- A hub in Benin through training of younger African scientists: 1 PhD, 3 MSc and various courses (PK, TESA courses, genetic epidemiology)
Progress to date
Progress to date

- **Parents studies completed**: PK and PK/PD results available for all patients

- Progress +++ in sampling for genetic studies
### Participants’ recruitment

<table>
<thead>
<tr>
<th></th>
<th>Benin</th>
<th>Guinea</th>
<th>Senegal</th>
<th>South Africa</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number to recruit</td>
<td>186</td>
<td>148</td>
<td>34</td>
<td>200</td>
<td>568</td>
</tr>
<tr>
<td>Number enrolled (whole blood collected)</td>
<td>140 (75.3%)</td>
<td>85 (57.4%)</td>
<td>26 (76.5%)</td>
<td>160 (80.0%)</td>
<td>411 (72.4%)</td>
</tr>
<tr>
<td>Number of refusal</td>
<td>01</td>
<td>00</td>
<td>02</td>
<td>02</td>
<td>05</td>
</tr>
<tr>
<td>Number of deceased</td>
<td>09 (4.8%)</td>
<td>21 (14.2%)</td>
<td>00</td>
<td>20 (10.0%)</td>
<td>50 (8.8%)</td>
</tr>
<tr>
<td>Loss to follow-up</td>
<td>36 (19.4%)</td>
<td>42 (28.4%)</td>
<td>06 (17.6%)</td>
<td>18 (9.0%)</td>
<td>102 (17.9%)</td>
</tr>
</tbody>
</table>
Management of deceased patients

- Use of plasma samples stored from PK studies in genetic studies if whole blood samples are not available
- Ethical approval already obtained
Mangement of loss to follow-up

- Amendments to protocol already submitted in all sites
- Ethical approval obtained in Benin and South Africa, pending in Senegal and Guinea
Genetic studies

DNA extraction started on samples in Cotonou
Challenges
Challenges

- **Difficulties for shipment** of blood samples from Dakar and Conakry to Benin

- **Some administrative/financial issues**
Next steps
Next steps

• DNA extraction of all samples

• **Genetic tests in** Liverpool (by the PhD student based in Cotonou)

• **Data analysis combining already available PK/PD data to genetic tests**
Conclusion

• RAFAgene:
  • A pharmacogenetic study on TB
  • PK/ PD data already available
  • Samples collection for genetic studies almost completed
  • Some issues on samples shipment
Acknowledgements

• RAFA/ OFLOTUB consortium

• NIH
Merci