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

Background

Tuberculosis (TB):

- Important cause of death in Sub-Saharan Africa
- In best-case of TB treatment scenario, **≈ 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**

MRC SA (Durban,, **study site)**

Cape Town University

(PK/ PD)

Study design and population

Rafagene study participants from 2 clinical trials:

- *OFLOTUB trial (completed)*
- *RAFA trial (almost completed)*

Oflotub project

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

A Four-Month Gatifloxacin-Containing Regimen for Treating Tuberculosis

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

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

N Engl J Med 2014; 371:1588-1598

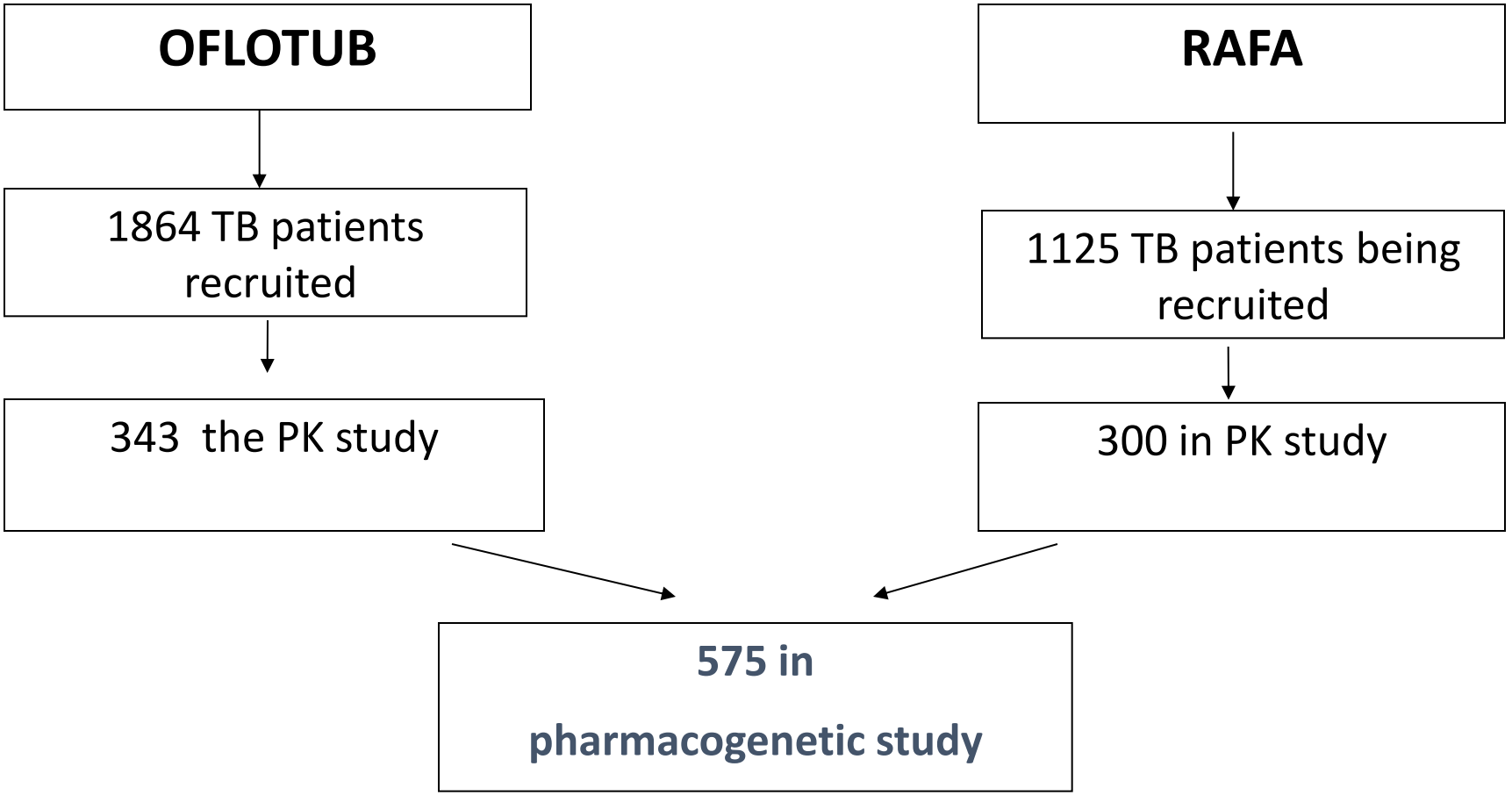
RAFA

To improve TB/ HIV co-infected treatment

Arm	Intensive phase	Continuation phase	Particularity
1	2ERHZ	4RH	ART at 15 days
2	2ERHZ	4RH	ART at 2 months
3	2ERHZ	4RH	ART at 2 months + High R

Study completed, abtsract submitted to International AIDS Society Conference

RAFAgene nested in OFLOTUB and RAFA



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 litterature).

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

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

Mangement 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