

**SfP – Detect Drug-Resistant TB**

SfP – 982319

Multidrug-Resistant and Hypervirulent Tuberculosis Strains: Integral Approach to Rapid Detection

Project Co-Directors:

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Approval Date: 24 October 2006

Effective Date: 1 November 2006

Duration:

3 years; planned to be completed by November 2009

NATO Budget:

188,000 EUR

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Information about the SfP Project through Internet: <http://www.pasteur-guadeloupe.fr/tb/projects/NATO/>

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Abstract of Research

We propose to recognize an increasing worldwide circulation of multidrug-resistant and hypervirulent tuberculosis strains as a specific critical health security problem. Two Partner Countries, Bulgaria and Russia, represent a world region with most alarming situation with Multidrug-resistant tuberculosis (MDR-TB). Rapid detection of tubercle bacillus and its hypervirulent and drug-resistant hazardous variants presents the first step in fighting TB and is vital for both patients and community health.

Conceptually, the proposed project merges problems of an already recognized emergence of Multidrug-resistant TB with not yet appreciated circulation of the Hypervirulent TB strains, and proposes to consider them as a combined problem of increasing propagation of MDR-HV TB strains. Methodologically, this project aims to develop, evaluate and implement a fast, high-throughput and inexpensive Macroarrays and PCR method for detection of MDR-HV TB strains.

DNA Macroarray approach will be used in this project as a technical backbone for the above proposed strategy to detect TB strains resistant to the main drugs. In this method, specific oligonucleotides corresponding to wild-type ("drug susceptible") and mutant ("drug resistant") sequences of a gene are immobilized onto a membrane and subsequently hybridized with biotin-labeled PCR-amplified gene fragment; the hybrids are revealed by luminescence. This method will be used in combination with simple PCR method to detect specific IS6110 insertions characteristic for MDR-HV genotypes thus permitting to identify such dangerous genotypes directly in clinical samples.

*M. tuberculosis* is an intracellular pathogen infecting primarily macrophages which are the key cells of the innate immune response to mycobacteria. The macrophage cultures infected with pathogenic mycobacteria will be used as a suitable model for the screening of new drugs and vaccines designed to kill intracellular mycobacteria either directly or through macrophage activation. The peculiarities of pathogenesis and immune response to different *M. tuberculosis* genotypes mentioned above suggest that the therapeutic effect of new anti-mycobacterial drugs should be verified not only against laboratory strains of *M. tuberculosis*, but against clinical isolates of the predominant *M. tuberculosis* genotypes as well. Consequently, to evaluate pathogenic properties, we will screen selected and representative clinical strains in macrophage cell culture.

The knowledge about potential hypervirulent properties of MTB strain is no less critical for correct choice of patient's treatment than diagnosis of drug resistance only. Therefore early identification of the hazardous hypervirulent genotypes/variants is critical for adequate treatment, along with early TB diagnostics and detection of drug resistance.

Objectives

- To develop sensitive and specific method for early and rapid detection of multidrug-resistant and hypervirulent *Mycobacterium tuberculosis* strains directly in clinical samples from TB patients.
- To submit patents (involving "commercial end-user") before end of project.
- To transfer methods and know-how, to implement the developed methodology into TB Control Programs in the involved Partner countries, by collaborating with health authority "end-users" and participants.
- To establish a panel of "human-successful" MTB strains, as optimal candidates for studies on vaccine development and for assessing therapeutic efficacy of new drugs.
- To evaluate new markers for molecular epidemiology of TB. To determine genomic variations among MTB strains linked to different epidemiological and clinical characteristics of patients.
- To assess the role of the identified genotypes/variants in TB transmission in the target areas and neighboring regions by comparison with international databases.

## Overview of Achievements since the Start of the Project until 30 September 2008

### PPD laboratories in Russia and Bulgaria:

- The *M. tuberculosis* strains were characterized using traditional and more recent markers for molecular epidemiology (IS6110-RFLP, VNTR, spoligotyping). Drug-resistant properties and associated gene mutations of strains (first-line drugs) were tested.
- Candidate MTB strains were selected for macrophage experiments of the pathogenic properties
- New 27 VNTR markers and their different combinations were tested. The cost-effective combinations of the reduced VNTR sets of the five VNTR markers were suggested to be used in Russia and Bulgaria for rapid first-line epidemiological typing. Simple PCR method to detect Beijing B0 cluster was proposed.
- Microarrays method was optimized and evaluated in three laboratories to detect mutations in *rpoB* gene (marker of MDR TB)
- Six articles were published, one was accepted, one more was submitted to international journals
- Patent on method to detect Beijing genotype strains was submitted earlier than planned

### NPD laboratory in Institut Pasteur de Guadeloupe, France:

- The results obtained in PPD laboratories were compared with international database of *M. tuberculosis* SITVIT2 created at the NPD laboratory.
- Mutations in *gyrA* gene and mutations in new gene targets *gyrB* were identified and will serve to upgrade the method to detect FQ-resistant and XDR TB
- website of the project has been updated: <http://www.pasteur-guadeloupe.fr/tb/projects/NATO/index>

Payments through NATO Funds: 120,184 EUR

### Milestones for the Next Six Months

- To continue development of the method to detect *gyrA* mutations in MTB strains, as markers of XDR-TB.
- To evaluate the genetic method to detect XDR-TB in clinical samples.
- Mapping of IS6110 insertions in the major strains circulating in Russia and Bulgaria
- To obtain information on ESAT-6 gene region related to different virulence properties in strains
- To start preparation of Guidelines for use of molecular typing for epidemiological surveillance of *M. tuberculosis* in Bulgaria and Russia

### Implementation of Results

- first results were reported to the commercial and health-service end-users. New schemas were proposed for use for corrected treatment of TB patients and for epidemiological interventions.
- Two scientists from Russian team had oral presentations at the large international Congress on molecular diagnostics in Moscow, 28-30.11.2007. Useful discussion with potential end-users representing industry and public health decision makers identified an interest of the market in development of the methods proposed in this SFP project.
- Patent application submitted by Russian team, on method of detection of Beijing genotype strain
- Preparation of Guidelines for use of molecular typing for epidemiological surveillance of Beijing genotype in Russia started by Russian team in collaboration with health-authority end-user
- Preparation of Guidelines for use of molecular typing for epidemiological surveillance of *M. tuberculosis* in Bulgaria started by Bulgarian team in collaboration with health-authority end-user.

### Other Collaborating Institutions

#### Commercial end-user:

- The Department of New Technologies / St. Petersburg Pasteur Institute

#### Health-authority end-users:

- Professor Yuri N. Levashov, Chief Phthisiatrician of St. Petersburg/ Russian Ministry of Health;
- Research Institute of Phthisiopulmonology (Haskovo), Ministry of Health, Bulgaria

#### Key participant:

- Laboratory of Microbiology of Tuberculosis St. Petersburg Research Institute of Phthisiopulmonology/Ministry of Health, Russia

### Intellectual Property (IP) Rights

Companies and other organisations interested in providing funds for commercialisation of project results can request further information from the Project Co-Directors or from the SFP Programme Director ([www.nato.int/science](http://www.nato.int/science) "How to contact us"). Release of information requires Co-Directors' authorisation.

Abbreviations: MTB – Mycobacterium tuberculosis; MDR-HV TB – multidrug-resistant and hypervirulent tuberculosis; RFLP – restriction fragment length polymorphism; Spoligotyping – spacer oligonucleotide typing; VNTR – variable number of tandem repeats; XDR TB – extensively drug resistant tuberculosis