

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

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

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 31 March 2009

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, SNPs, deletions). Drug-resistant properties and associated gene mutations of strains (first-line drugs) were tested.
- MTB strains were analysed in macrophage experiments for the pathogenic properties
- The cost-effective combinations of new VNTR markers were suggested for rapid first-line epidemiological screening. Simple PCR method to detect hypervirulent Russian clone B0/Beijing was developed.
- Macroarrays method to detect mutations in *rpoB* gene (marker of MDR TB) was optimized.
- Real-time PCR method to detect FQ resistance is being developed and optimized.
- Six articles were published, two book chapters are in press.
- Patent on method to detect Beijing genotype strains was submitted earlier than planned.
- Virulence related genes were studied; new *M. tuberculosis* family-specific SNPs were identified

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>
- Mapping of IS6110 insertions has been done. This information is being used for development of the fast Real-time PCR method of detection of Beijing genotype and patent preparation.

Payments through NATO Funds: 155,996 EUR

Milestones for the Next Six Months

- To complete development of the method to detect *rpoB* /*gyrA* mutations in MTB strains, as markers of MDR-TB and XDR-TB.
- To complete evaluation of the above genetic method to detect XDR-TB also in clinical samples.
- To complete preparation of Guidelines for use of molecular typing for epidemiological surveillance of *M. tuberculosis* in Bulgaria and Russia

Implementation of Results

- 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 useful discussion with potential end-users representing industry and public health decision makers at the large international Congress on molecular diagnostics in Moscow, 11/2007; these identified an interest of the market in development of the methods proposed in this SFP project.
- Patent application was submitted by Russian team ("Method of detection of Beijing strain")
- Preparation of Guidelines for use of molecular typing for epidemiological surveillance of Beijing genotype in Russia is nearly completed in collaboration with health-authority end-user
- Preparation of Guidelines for use of molecular typing for epidemiological surveillance of *M. tuberculosis* in Bulgaria is nearly completed 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. The IPR Committee has been created.

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 "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; FQ – fluoroquinolones; PCR-polymerase chain reaction.