Executive summary:

Tuberculosis (TB) still remains a huge threat to global health, representing a leading cause of morbidity and mortality from a single source of infection. It was thought that the combination of mass BCG vaccination and DOTS (Directly Observed Treatment, Short-Course) chemotherapy will steadily eliminate TB. The international community realized soon after that such a strategy is far from being effective, inasmuch as one third of the actual world's population carries Mycobacterium tuberculosis, the major etiologic agent of human TB. More worrying is the worldwide emergence of the difficult-to-treat multidrug resistant TB (MDR-TB), which tends to evolve to extensively drug resistant TB (XDR-TB) or totally drug resistant TB (TDR-TB), leaving no hopes for curing patients. Hence, it becomes obvious that effective control of TB should address additional challenges. In this regard, investing in the early detection of TB cases and surveillance of TB transmission appears to be of uppermost importance. Indeed, the failure to quickly recognize and treat TB patients invariably results in increased drug resistance and ongoing transmission.

The advent of molecular tools has revolutionized the way we diagnose and survey TB. Recently, a fully automated molecular device to detect M. tuberculosis and rifampicin-resistant strains has been conceived and successfully assessed worldwide. This novel, WHO-endorsed approach, detects TB and its resistant forms within 100 minutes, thus providing the proof of principle for the huge potential of molecular tools in the rapid detection of TB and its drug resistant forms. Furthermore, TB genotyping has proved very effective in tracking outbreaks and rapidly expanding clones. Genotyping could therefore be a valuable complement to guide National TB programs.

Effective implementation in developing countries of TB molecular tools must be accompanied with a reinforcement of their TB research capacity. This implies updating knowledge, enhancement of technical skills, and upgrading of equipment platforms. For this purpose, Mediterranean Partner Countries (MPC) TB research entities from Morocco (Institut National d'Hygiène, INH), Algeria (Institut Pasteur d'Algérie, IPA), and Tunisia (Institut Pasteur de Tunis, IPT), solicited the contribution of European leading TB research centres from France (Institut Pasteur, IP), Guadeloupe (Institut Pasteur de Guadeloupe, IPG), Germany (Research Center Borstel, FZB), Spain (Instituto Aragonés de Ciencias de la Salud, I+CS), and Bulgaria (Stephan Angeloff Institute of Microbiology, SAIM), to establish a Euro Mediterranean network, EUMEDNETVSTB, aimed at strengthening and promoting TB research and surveillance in the Maghreb. Such initiative received funding from the European Commission (EC,) by participating to the FP7-REGPOT-2009-2 call.

The EUMEDNETVSTB main objectives consist in:
(i) establishing the foundation for an active Euro Mediterranean TB network,
(ii) enhancing the knowledge on TB research and surveillance in the Euro Mediterranean region,
(iii) increasing the skills and competitiveness of MPC TB research entities, and (iv) raising awareness of the Euro Mediterranean community on TB issues.

Since its implementation, in March 2010, the EC-funded EUMEDNETVSTB supported the organization of two international symposia, three

workshops, and two practical trainings, thus significantly contributing to knowledge updating, brain gaining, and skills reinforcement. Owing to EUMEDNETVSTB, several trainings of MPC young scientists in European leading TB research centers were organized. EUMEDNETVSTB also provided complement salaries for MPC Post Doctoral training in a European TB laboratory and for hiring young researchers in MPCs. EUMEDNETVSTB funding allowed the three MPC TB research entities to upgrade and reinforce their technical platform by acquiring and implementing new equipments, which are actually being used to advance research twinning activities, established with the European partners. These trainings and twinning actions brought the MPC TB research to the pathogenomic era and endowed them with sufficient skills to conduct future research in relation to host-pathogen interaction.

Overall, EUMEDNETVSTB laid the foundation for a favorable TB research context in the Maghreb, which undoubtedly will benefit to TB diagnosis and surveillance. Through the organized symposia and meetings, and communication means, TB professionals, stakeholders, and the MPC community at large, were sensitized with TB issues and became aware of the new tools that might help to better control TB.

Project Context and Objectives:

Tuberculosis (TB) is an ancient disease that has affected mankind for more than 4,000 years. It remains a leading cause of morbidity and mortality from a single source of infection among adults. In countries where the disease is relatively brought under control, tight surveillance must be applied to avoid emergence of outbreaks or new TB transmission chains. Discovery of effective anti-tubercular drugs and adoption of the standardized directly observed treatment short course (DOTS) did not significantly reduce the disease burden, especially in poor countries where HIV co-infection is common. The alarming TB statistics prompted the World Health Organization (WHO) to declare TB as a global health emergency. According to the latest WHO global report, there were, in 2011, an estimated 8.7 million new cases of TB (13% co-infected with HIV) and 1.4 million deaths, including almost one million deaths among HIV-negative individuals and 430 000 among people who were HIV-positive. The association between poverty and TB is well-recognized, and the highest rates of TB were found in the poorest section of communities. Poverty is a cause of malnutrition which may be associated with immune dysfunction. Furthermore, poverty is generally associated with overcrowded living conditions, poor ventilation, and poor hygiene-habits. These factors are likely to increase the risk of transmission of TB.

Vaccination with BCG, the oldest means to prevent TB, has been shown to be very effective against TB meningitis in children, and to lesser extent against miliary TB, but the protective effects of the vaccine decrease with time. BCG does not offer lifetime protection against TB infection or TB disease. In countries where mass BCG vaccination, at birth and at entry to school, was practiced, a general sharp decrease in TB prevalence was observed, but this measure alone, could not allow reducing the TB burden to acceptable levels or completely eliminated TB, as for other infections.

DOTS has considerably contributed to declining rates of TB, especially in the developed country, but its misuse has a major negative impact, which consists in the emergence of drug resistance, a huge global threat tackling the achievement of the Millennium Development Goals (MDG). Better understanding of the prevalence of drug resistance against tuberculosis is one of the key elements in the control of TB. Drug resistance, in combination with other factors, especially HIV infection, results in increased morbidity and mortality due to TB. Although the overall rates of TB are declining thanks to DOTS, drug-resistant strains of TB is emerging worldwide, and highly expandable epidemic clones, such as the Beijing genotype, have crossed several continents, emphasizing the need to confer a top priority for combating the drug resistance issue. A global rise in both multidrug-resistant TB (MDR-TB) and extreme drug-resistant TB (XDR-TB) has been reported by the WHO. Unlike drug sensitive TB, both treatment and management of MDR-TB/XDR-TB cases are well beyond the capacity of any developing country. Therefore, it becomes increasingly clear, that effective TB surveillance and prevention of drug resistance emergence requires a concerted action at the global at regional levels. Within countries, tight collaboration among government, non-government and private organizations is highly recommended.

Development of drug resistant TB is mainly due non-adherence to therapy, limited or interrupted drug supplies, poor quality of drugs, widespread availability of anti-TB drugs without prescription, poor medical management, and poorly-managed national TB control programmes (NTP). The

failure to quickly recognize and treat TB patients, particularly those harbouring resistant strains, lead to increased mortality and resistance, as well as ongoing transmission. Basically, detection of drug resistant TB cases relies on drug susceptibility testing (DST) after mycobacterial culture. Using solid culture, this process could take more than two months. Liquid culture DST has reduced the turnaround time to less than one month, but it is financially demanding. The first generation nucleic acid-amplification technologies for the detection of tuberculosis and its multidrug-resistant forms (line probe assays for instance) have significantly reduced the time to diagnosis to 1 or 2 days, however, the need for molecular biology skilled technicians and the associated infrastructure, restricts the use of such tests to highly qualified reference laboratories. Recently, a fully automated molecular device to detect M. tuberculosis and rifampicin-resistant strains (and therefore the majority of MDR-TB cases) has been conceived and successfully assessed in several laboratories throughout the world. This second-generation, WHO-endorsed approach, detects TB and its resistant forms within 100 minutes, thus providing the proof of principle for the huge potential of molecular tools in the rapid detection of TB and its drug resistant forms. In addition, this molecular device not only impressively reduced the turnaround time, but offers the unprecedented advantage of not necessitating skills in molecular biology. Nonetheless, these skills remain strongly required for molecular typing of M. Tuberculosis in detecting and surveying outbreaks, or highly expandable clones. Endemic developing countries are therefore urged to upgrade their skills and knowledge to be able to take advantage of TB molecular tools for effective TB surveillance.

There are several challenges which need to be addressed for effective control of TB and drug resistance in endemic developing countries:

- Reinforcement of the intervention capacities of National TB programs
- Upgrading of the surveillance system
- Accelerating the identification of cases coupled to tight surveillance of contact cases
- Expansion of DOTS to hard-to-reach areas
- Strengthen DOTS in urban settings
- Implementation of treatment facilities for MDR cases
- Reinforcement of the laboratory facilities
- Increasing the skills of laboratory technicians
- Involvement of private practitioners
- Increase the detection rate of TB among children and extra-pulmonary cases
- Ensure effective coordination among health care providers

Strategically, these measures could have a more positive impact if they involve effective actions to strengthen the TB research capacities of developing countries. This has been clearly stated in the WHO report of 2012.

In fact, the Global Fund, Stop TB partnership, and their leading partner, the WHO, devoted a considerable amount of funding and provided logistic means in more than 100 countries to help increasing their national TB control capacities. Countries of the Maghreb (Morocco, Algeria, and Tunisia), through their respective NTP programs, have benefited of this international funding, and undertook several measures to tighten TB control at different levels (provide second-line drugs, increase peripheral diagnostic capacities, building treatment facilities for MDR

cases, implementing liquid culture, organizing workshops for laboratory staff...).

However, such funding was not meant, at least at this step, to support research activities and to foster application of new molecular-based tools for the rapid identification of TB cases, particularly its drug resistant forms. Likewise, population-based molecular studies aiming at characterizing TB transmission dynamics, which could help guiding NTP programs, could not be supported by such international funding. Furthermore, since TB re-emergence, funding of research activities was mainly restricted to developed countries with little collaboration with researchers from endemic developing countries. Consequently, there is now an important gap in TB research capacities between northern and southern endemic countries. Such a divide has recently increased with the advent of new financially demanding technologies, which require skilled personnel with high level, and updated knowledge in fundamental science.

The challenges facing the TB community of the Maghreb is as follows:

- Increased North-South gap in TB research capacity
- Risk for loss of expertise in TB research in Mediterranean Partner Countries (MPC)
- Flat funding and low participation of MPC to international funding opportunities
- Unconsciousness of decision makers and the community about the real and new TB challenges

The FP7-REGPOT2 call launched by the European Commission, under the seventh framework program (FP7), addressed the aforementioned funding gaps. Indeed REGPOT2 supports capacity building projects with the ultimate aim to increase the ability of endemic developing countries to reinforce their research capacity. The project EUMEDNETVSTB "Building a cooperative strategy between Europe and Mediterranean Countries for upgrading tuberculosis research and control" was thus proposed in response to the REGPOT2 call. Its overall objective consists in upgrading TB research in the South Mediterranean region by creating a favourable context connecting TB research laboratories from this region with European TB reference and centres of excellence. EUMEDNETVSTB involves research institutions from three MPCs, (National Institute of Hygiene from Morocco, Institut Pasteur d'Alger form Algeria and Institut Pasteur de Tunis from Tunisia), three European centers (Institut Pasteur of Paris, Instituto Aragonés de Ciencias de la Salud from Spain, Research Centre Borstel from Germany), one institution from a convergence region (The Stefan Angeloff Institute of Microbiology from Bulgaria), as well as a research center from an outermost region (Institut Pasteur of Guadeloupe).

EUMEDNETVSTB intends to set up and realize a number of strategic measures with the ultimate goal to strengthen and upgrade the overall TB research potential of MPCs. Specific actions aimed at reinforcing the technical and human resources, as well as the competitiveness and visibility of the participating MPCs' entities, are planned. The overall strategy consists in building on and accelerating the ongoing efforts relating to TB research in MPC research entities through the development of a durable focused network involving European reference research laboratories, for the purpose of sharing information and know how, coordinating activities and working towards a high quality research agenda, as well as mutual research funding activities.

EUMEDNETVSTB participants will join their efforts to meet the following specific objectives:

- Update and increase the MPC scientific knowledge on emerging issues in TB
- Upgrade the MPC equipment platform and to ensure their optimal use
- Reinforce the MPC research potential and researches skills by performing training in leading European TB research entities and two way visits.
- Raise awareness of MPC health professionals, stakeholders, and the general community on TB issues (TB global trends, expansion of drug resistance, and emergence of outbreaks...), and the new perspectives and opportunities for better control and surveillance (new rapid diagnostic tests, genotyping-based control...).

The objectives of EUMEDNETVSTB will be achieved through the following 4 workpackages (WP):

- WP1. Project Coordination and Management
- WP2. Updating the scientific knowledge and skills on basic and newly emerging technologies relating to TB
- WP3. Adaptation and upgrading of the equipment platform
- WP4. Scientists' exchange program and skills/know how reinforcement
- WP5. Dissemination of the project outcomes

EUMEDNETVSTB targets three major TB research themes:
(i) M. tuberculosis genotypic diversity,
(ii) drug resistance, and
(iii) M. tuberculosis interaction with the host.

These themes are critical for the implementation of effective control measures against TB, and are of common interest to the participants.

Over the past ten years, the MPC research entities that participated to EUMEDNETVSTB have developed and carried out research activities pertaining to these themes and the data indicate that in the three countries, M. tuberculosis is subject to similar evolutionary dynamics. This conclusion might be linked to the fact that the host populations of the three countries are genetically closely related, have been vaccinated with similar BCG vaccine strains, and evolve in a very low-incidence HIV context. The rationale behind EUMEDNETVSTB is to provide MPC TB researchers with the required means to expand upon these findings. Based on the existing MPC research potential, EUMEDNETVSTB will support the development of human resources and build capacities around the following specific research topics:

- Establishing the genetic structure of the M. tuberculosis population circulating in the three MPCs
- Identifying genetic and genomic markers of the most successful clones and multidrug-resistant strains circulating in MPCs
- Evaluating the extent of homologous recombination-mediated deletions in PE/PPE genes and their impact in the natural success and adaptation of M. tuberculosis
- Delineation of host immunological and genetic determinants underlying the epidemiological success of strains found to be stably adapted to the North African population

Overall, and in accordance with the terms of the FP7-REGPOT2 call, EUMEDNETVSTB aims to create a dynamic and interactive Euro Mediterranean TB research space that will benefit from advanced science approaches and cutting-edge technologies in order to address the above research themes. Interaction with the European TB research leader centers will not only be critical to promote excellence in science of MPC institutions, but in return, will benefit from the regional specificities of MPCs and institutions from convergence and outermost regions. Bridging the two regions will ultimately enable MPC TB research institutions to be more scientifically competitive which ensures their participation to future international funding. The TB research entities from both convergence and outermost regions have been chosen based on their prior involvement in the targeted TB research topics. With no doubt, inclusion in the network of TB research entities from outermost and convergence regions will significantly enrich the consortium by bringing additional experiences and new regional specificities. Successful achievement of EUMEDNETVSTB' aims is expected to promote scientific excellence and enhanced visibility of MPCs in the aforementioned prioritized TB research topics, namely molecular epidemiology of M. tuberculosis,drug resistant TB, and TB host-pathogen interaction.

Project Results:

EUMEDNETVSTB is primarily a capacity building project, whose general goal consists is establishing a Euro Mediterranean network aimed at strengthening and promoting TB research and surveillance in the Maghreb. Towards this end, MPC research entities have worked during the last three years in concert with European TB leading research centers to upgrade their scientific and technical skills. For a more comprehensive description of the main EUMEDNETVSTB S&T results, this section will be dealt with according to the following project's outputs rather than a workpackage-by-workpackage description:

- Creation of a Euro Mediterranean TB network
- Knowledge updating and brain gaining in current TB research themes
- Skills development and reinforcements in TB diagnosis and research
- Trainings/Twinning actions
- MPCs technical platform upgrading
- Development of human resources

1. Creation of a Euro Mediterranean TB network

EUMEDNETVSTB, has been created to build a cooperative strategy between Europe and Mediterranean Countries for upgrading TB research and control in the Maghreb. It consists of a network made of three MPC research entities (IPT, IPA and INH), three European TB leading research centres (IP, FZB, I+CS) one research laboratory from a convergent region (SAIM) and one TB reference laboratory from an outermost region (IPG). This network was made effective through coordination meetings, participation to international events, organization of trainings, and establishment of twinning actions. A web site (see http://www.eumednet-tb.org online) was dedicated to the network.

During this 3-year project, 4 coordination meetings were organized in accordance with the project's agenda:

-The kick-off meeting took place at the Institut Pasteur (Paris), 27th-28th April, 2010, two months after the official beginning of the project, and involved all the participants. They introduced their work and expressed their respective needs in terms of training, technical and scientific support. During this meeting, the EUMEDNETVSTB website was presented and commented. The way to interact and to establish twinning actions was also discussed.

-The first coordination meeting was organized in Tunis, September 19th 2010, one day before the international symposium. We took advantage of this international meeting to spare travel and accommodation funds. It provided the opportunity to discuss the budget and to establish a preliminary agenda of the scientific exchange program. Most of EUMEDNETVSTB participants attended this meeting and presented in details their key field expertise in order to establish a wise agenda for trainings and twinning actions.

-The EUMEDNETVSTB network was tightened after the second coordination meeting. During this meeting, the mid-term report was presented and commented (achievements and difficulties) and the program for the second period was discussed. More focus was put on the scientific exchange program (training and visits) as well as the work that has to be finalized in the context of specific twinning actions.

-The overall achievements and outputs of EUMEDNETVSTB were discussed in the final coordination meeting that took place at IP. All participants, but INH, presented their contribution to EUMEDNETVSTB and discussed future collaboration, benefiting of the existing network and twinning activities. Opportunities for future funding were also raised.

Also to ensure the progress of the network's planned activities and to assist host participants in the organization of symposia and/or workshops, 5 coordination visits were performed. The scientific coordinator performed two visits to IP to meet with the project coordinator and the project manager, and two follow-up visits to INH and to IPG. The coordinator also visited IPT to establish a twinning action and to prepare with the scientific coordinator the second EUMEDNETVSTB international symposium.

2. Knowledge updating and brain gaining in current TB research themes

As mentioned in section 2, the advent of new technologies, mainly based on the manipulation of nucleic acids and whole genomes, has urged for reinforcement and/or an update in basic knowledge relating to molecular biology. Enhancing and updating the scientific knowledge of MPC participants is a prerequisite for a better interaction with their colleagues from European leading TB centres. Understanding the molecular basis of the emerging molecular approaches used to dissect the biology of the pathogen and its host is of critical for MPC research entities inasmuch as they have no access to high throughput technologies, such as genome sequencing, whole genome expression profiling, proteomics...

Towards this end, EUMEDNETVSTB participants benefited of two international symposia and three workshops, thus fully achieving what has been planned for.

- International Symposium 1: "Tuberculosis: application of genetics-and genomics-based tools for diagnosis, drug susceptibility testing and epidemiology" was organized by IPT and IP, and took place in Tunis, September 20th-22nd, 2010

- International Symposium 2: "Host-pathogen interaction in M. tuberculosis" organized by IP in parallel with the TBVI (TB Vaccine Initiative) summit meeting "New TB vaccines for the future", Madrid, October 17th-18th 2011

- Workshop 1: "Global phylogeny and host-pathogen compatibility of M. tuberculosis" organized by SAIM Sofia, 16-20 September, 2011

- Workshop 2: "Diagnostic de la Résistance de la Tuberculose aux antibiotiques: Apport des outils moléculaires" organized by IPA, Algiers, 24-24 June, 2012

- Workshop 3: "Tuberculosis Biomarkers: from research to action" organized by INH, Marrakech, October, 4-5, 2012

In accordance with the themes targeted by EUMEDNETVSTB, several aspects of the current and most important issues in TB were covered:

- TB global epidemiology

- Emergence and expansion of multi- and extensively-drug resistant TB

- Molecular basis of drug resistance in M. tuberculosis

- Evolution of M. tuberculosis complex strains as revealed by the new genomics-based markers

- Global, genome-wide-based, phylogeography and adaptability of M. tuberculosis

- Comparative genomics and microgenomics of M. tuberculosis

- New approaches for typing and detection of drug resistance

- Innate and adaptive immune response to TB

- Molecular basis of host-pathogen interaction and susceptibility to TB

- The current and future of TB vaccine development

- New candidate TB vaccines

- Animal models for TB vaccine evaluation

Conferences on these topics were delivered by eminent leader scientists in the field. All EUMEDNETVSTB participants (senior and young researchers as well as PhD students) attended and contributed to these scientific events.

The international symposium held in Tunis attracted 20 participants from 7 MPC countries (Morocco, Algeria, Tunisia, Egypt, Lebanon, Palestine, and Syria), and extended to 9 participants from TB endemic African countries (Sudan, Nigeria, South Africa, Republic of Central Africa, Cameroon, Ghana, Zambia, Burkina Faso, Madagascar), as well as to 3 participants from Cambodia, Guadeloupe and Bulgaria. Ten distinguished mycobacteriologists delivered outstanding talks on their latest findings relating to the three following major:

- Global epidemiology, Molecular typing and host-pathogen interaction

- Drug resistance in Mycobacterium tuberculosis

- Molecular evolution of the Mycobacterium tuberculosis complex

Approximately 100 persons (including the aforementioned participants) attended the conferences and benefited of the exciting and excellent keynote lectures.

The second EUMEDNETVSTB international symposium "Host-pathogen interaction in M. tuberculosis" was combined to the TBVI (TB Vaccine Initiative) meeting organized in Madrid, October 17th-18th 2011, by the Ramon Areces Foundation.The idea to jointly organize the two events was the fact that both themes were interrelated since the development of new effective TB vaccine is contingent of a deep understanding of the host immune response to TB infection. Therefore, aside from the vaccine development aspects, several issues in TB immune response were dealt with, thus providing a valuable complement to the EUMEDNETVSTB symposium. Furthermore, several of the distinguished speakers that could have

contributed to the EUMEDNETVSTB symposium, has already been invited to the TBVI event, hence combining the two symposia was beneficial to both.

The program included key note state of the art lecturers delivered by the most distinguished experts in the field. The TBVI/EUMEDNETVSTB event comprised the following five sessions:

- Session I: Development of new TB vaccines (TBVI)

- Session II: New TB vaccine in clinical trials (TBVI)

- Session III: Development of new TB vaccines (TBVI)

- Session IV: Preclinical, models, production, safety, and regulatory issues in TB

- Session V: TB host-pathogen interactions: the new paradigms

A total of 21 talks delivered by eminent TB vaccine developers and immunologists was followed by at least 150-200 attendees. All EUMEDNETVSTB participants took part to this event and have benefited of the latest findings in the field.

To promote tight scientific exchanges and establishment of twinning actions between EUMEDNETVSTB participants, three workshops have been organized.

The first workshop which took place in Sofia (16-20 September 2011) expanded upon the themes covered in Tunis by focusing on the global phylogeny and host-pathogen compatibility of M. tuberculosis. The intended purpose for this workshop was also to gather EUMEDNETVSTB project participants for exchange of scientific information, knowledge and ideas regarding the diversity and evolution of TB strains in different partner countries, as well as to help solidify scientific contacts between researchers. The participants to the workshop have been given the opportunity to describe and discuss their ongoing research and future interests. Four distinguished experts delivered conferences on related themes, allowing all EUMEDNETVSTB participants to enhance and update their knowledge. In total, there were 1 overview lecture (40 minutes), 12 oral presentations (20 minutes each) and 4 key note lectures (45 minutes each), spread over 3 days. As an output of this workshop several twinning actions were initiated.

The second workshop was entirely devoted to the drug resistance issue. It was organized by IPA, at Algiers. Since the fundamental aspects of drug resistance were already covered in the international symposium of Tunis, more practical issues relating to drug resistant TB were dealt with in Algiers. The venue of the Institut Pasteur d'Algérie, as the host for this workshop, was not a mere choice, but was legitimate given the long standing effort of IPA in drug resistance surveillance, training and awareness raising. In fact drug resistant TB does not represent a big issue in the three MPCs compared to other regions in Africa or throughout the world. However, it is of primary importance to maintain a tight control, and work to reduce the existing low rates of drug resistance in these countries. This is why a workshop focused on this aspect was programmed as one of the objectives of EUMEDNETVSTB. The two days-workshop covered the epidemiology and molecular basis of drug resistance, through the delivery of 5 keynote lectures, followed by talks on specific

applications, relevance and possible implementation in MPCs of molecular techniques for the rapid and efficient detection of drug resistance in TB. Given the importance of the WHO-endorsed automated device, GeneXpert, a talk was specifically dedicated to this second generation molecular-based detection of drug resistant TB. A total of 52 participants attended the 2-days conference and contributed to discussions on the feasibility and difficulties when using molecular tools in the real practice and in MPC field conditions. The majority of attendees were high-level laboratory technicians, nurses, public health physicians (practitioners and administrators), decision makers, and NGOs... The event was largely announced and actors at all levels of the TB program in Algeria were invited.

The third EUMEDNETVSTB event was focused on TB immunology and the search for new TB biomarkers with a predictive diagnostic value. The workshop titled "Tuberculosis Biomarkers: from research to action" was organized by INH in Marrakech, October 4-5, 2012. The workshop expanded upon the second international meeting of Madrid (please see above section) by focusing on the determinants of the host immune response and the extent of its variability from one population to another. Issues in relation to the development and validation of new TB markers have been presented. There was also a session dedicated to projects writing. At least 50 participants attended the 2-day workshop which included a dozen of talks. MPC presented their ongoing work on the validation of some latency-associated recombinant antigens for the development of potent serodiagnostic tools, while Morocco presented their three-year project consisting of the establishment of a TB cohort and showed their preliminary data on the immune response to TB of Moroccan individuals.

In conclusion, all EUMEDNETVSTB planned meetings aimed at upgrading knowledge on current TB research themes were organized. These scientific events endowed the participants with the most advanced and state of the art approaches used in TB research, thus fully achieving one of the main objectives of EUMEDNETVSTB. The MPC partners have particularly benefited of these meetings as they had the opportunity to be introduced to the international TB research community, and to interact with their cognates from European TB research laboratories, as well as from other parts of the world. The EUMEDNETVSTB-supported events represented an unprecedented Euro Mediterranean forum for discussing TB issues, and for bringing in close contact European and North African TB actors. Furthermore, these events significantly contributed to raise awareness in the three countries about the epidemiological trends of TB and the huge issue relating to the emergence of drug resistance.

3. Skills development and reinforcements

As a complement to conferences, EUMEDNETVSTB supported the organization of two practical workshops:

- International practical workshop 1: "TUBERCULOSIS: Molecular tools for diagnosis, drug susceptibility testing, and typing" organized by IPT/IP, Tunis, 24 September-01 October, 2010

- Practical workshop 2: "Détermination des marqueurs génétiques de la résistance de M. tuberculosis aux antibiotiques", Laboratory of TB and mycobacteria, Institut Pasteur d'Algérie, Algiers, Annex Hamma, 26-28 June, 2012

EUMEDNETVSTB not only funded logistic and organizational aspects but also financially supported selected MPC participants in terms of travel and accommodation.

Both workshops focused on drug resistant TB, particularly the new molecular tools for the rapid detection of TB resistant cases. The workshop of Tunis, aside from dealing with drug resistance, covered also the two gold standard typing methods (Spoligotyping and MIRU-VNTR), currently used to survey TB outbreaks and expansion. The overall aim of these training workshops consists in promoting the rational and clever use in developing countries of such molecular techniques, as a complement to the classically used approaches.

The training course of Tunis took place immediately after the international symposium, and therefore, the participants had already been infused with basics in genetics and molecular biology of the tubercle bacillus. The participants were introduced to the various state of the art molecular techniques in M. tuberculosis typing (spoligotyping, manual and automated MIRU-VNTR typing) and rapid detection of drug resistance (mutational analyses, HAIN test, and GeneXpert), by including a number of conferences on the utility, advantages/disadvantages and applicability of the available techniques. In parallel, the participants got a hands-on practice on such techniques, with the continuous assistance of eight facilitators.

Initially programmed to involve EUMEDNETVSTB participants only, this international training course was extended to several TB endemic African countries, owing to the financial contribution of the IAEA (International Atomic Energy Agency), and therefore MPC participants were sensitized to specificities of the TB issues in central and southern Africa, notably the association with HIV infection.

The practical workshop that was held at the Institut Pasteur d'Algérie, involved 14 participants that have also attended the two-day conferences on drug resistance. The candidates were selected based on their profile, affectation, motivation, and commitment in the fight against TB. As a complement to the conferences, they benefited of a three-day hands on practice on the most advanced techniques for the detection of drug resistant TB, namely HAIN and GeneXpert. They were supervised by the technicians of the laboratory of TB and mycobacteria of the Institut Pasteur d'Algérie, with the very kind contribution of European highly experienced and skilled facilitators. The program of the practical workshop consisted mainly of the following steps: sample preparation, PCR amplifications, processing by HAIN and/or GeneXpert, data acquisition and results interpretation. The practical workshop was ended with a general discussion on the utility, versatility, and constraints posed by these new molecular approaches.

Overall, the two practical courses organized in the context of EUMEDNETVSTB were of particular impact as they have been voluntarily organized immediately after a symposium or a workshop, to maximize the benefit of the participants. With no doubt, these courses will greatly facilitate the implementation in MPCs of the new rapid molecular tools for TB diagnosis and surveillance.

4. Trainings/Twinning actions

For an effective EUMEDNETVSTB network, it is critical to reinforce and tighten direct interactions between MPCs and their European partners. One of the best ways to achieve this goal consists in the establishment a scientific exchange program. The latter initiative is expected to (i) enhance the skills of MPC young scientists, (ii) promote twinning actions, (iii) foster technology transfer to MPC research entities, and (iv) to prompt joint participation to future funding opportunities. As stressed out in the proposal, the three MPC TB research entities have already developed TB research activities, and therefore should rely upon their existing research potential to benefit of such scientific exchange program with the European leading TB research centres.

To achieve these goals EUMEDNETVSTB supported research trainings of promising MPC doctoral students in the European TB reference centers. In this context seven short-term (one month or less) and one 9-month long training have been organized. These trainings have been arranged earlier during the scientific and/or coordination meetings, and it was agreed that these training would preferably be conducted in the context of twining actions.

4.1. Research Training of MPC young scientists in European TB leading laboratories

- Trainings at the Institut Pasteur (IP)

Meriem Ben Ali, a young scientist initially recruited in IPT, thanks to EUMEDNETVSTB, performed a research training at the Institut Pasteur in the laboratory of Human Evolutionary Genetics headed by Pr. Lluis Quintana-Murci, (June 20-July 1, 2011). Dr Ben Ali is member of Ridha Barbouche's laboratory, renowned for its work on susceptibility to mycobacteria. During her thesis in Barbouche's lab, Dr Ben Ali contributed to studies pertaining to the molecular basis of susceptibility to Bacille Calmette-Guérin (BCG) vaccines and environmental non tuberculous mycobacteria (NTM). After her PhD, Dr Ben Ali carried out a postdoctoral training in L. Quintana's lab, which assesses the impact of natural selection, human demography and lifestyle in the patterns of diversity of the human genome. In particular, the laboratory explores the way infectious diseases exert selective pressures on human genes involved in immunity and host defence in order to unmask immunological mechanisms critical to past and present survival. During her PostDoctoral training, M. Ben Ali particularly focused on selective pressure acting on TLR genes.

EUMEDNETVSTB offered the opportunity to M. Ben Ali to get back to L. Quintana's laboratory to explore more deeply her PhD results on the interleukin-12p40 gene mutation (297del8). During her training in L. Quintana's lab, she performed homozygosity mapping of consanguineous families by using of a set of relevant microsatellite markers overlapping the candidate gene (IL12B). Polymorphic markers encompassing the IL12B gene on 5q31.1-5q33, including flanking dinucleotide repeats (D5S410, D5S5662, D5S412, D5S2038, D5S403, D5S529, D5S422 and D5S621) were genotyped in all the patients.

- Trainings at the Institut Pasteur de Guadeloupe

Since 1993, the TB & Mycobacteria Research Unit led by Nalin RASTOGI, is actively involved in mycobacterial diagnostics, drug-susceptibility testing, and WHO-PAHO referral activity for the Americas. In March 2009,

N. Rastogi's TB lab officially joined the WHO-"TB Supranational Reference Laboratory Network (SRLN). This laboratory has a renowned expertise in the use of modern molecular tools for TB epidemiology, taxonomy and phylogeny. It has also developed one of the biggest TB genotype database worldwide (the latest version SITVIT2 contains data on 71,000 isolates from 160 countries of origin), endowing N. Rastogi's research group with a leadership in deciphering the global transmission of TB due to predominant and emerging clones. Aside from a This Unit multidisciplinary team, this TB lab has all the facilities and equipment needed for the manipulation of infectious material, a P2+ restricted access laboratory facility, as well as a well-developed molecular biology section.

N. Rastogi's TB Lab hosted two research trainings that benefited to a young researcher from IPA and a PhD student from IPT.

Nadir Mezidi from IPA performed a 4-week training (May 21- June 15, 2012) at the Institut Pasteur de Guadeloupe. During this period, he received training on the following subjects:

- Classical membrane-based line-blot hybridization assay for spoligotyping as well as the manual MIRU-VNTRs typing.

- Liquid culture and drug-susceptibility testing of M. tuberculosis complex (MTBC) using the Bactec MGIT960 methodology.

- Molecular identification and determination of drug-resistance mutations using the GenoType Mycobacterium CM/AS and MTBDRplus systems (Hain Lifescience GmbH, Nehren, Germany).

N. Mezidi typed his own M. tuberculosis DNAs that he brought from Algeria, and learned how to use the online international SITVITWEB database. He was trained on the use the BioNumerics (Applied Maths NV, Sint-Martens-Latem, Belgium) software for the management, storage and analysis of genotyping data.

Basically, this training would have endowed, Mr Nadir Mezidi with sufficient skills to carry out studies on the molecular epidemiology of tuberculosis in Algeria.

Likewise, Neira Dekhil, a PhD student from IPT carried out a 1-month training in N. Rastogi's TB Lab (Junuary 15-February 15, 2013). The subject of N. Dekhil's thesis focuses on a severe MDR-TB outbreak identified since 10 years ago, and which involved 51 immuno-competent and nonhospitalized patients residing in northern Tunisia. This outbreak was due to a Haarlem3 ST-50 genotype strain. To better characterize the origin of the outbreak, we performed MIRU-VNTR typing of these strains using the 24 loci format, and extended our typing to all drug-sensitive and non-MDR M. tuberculosis clinical isolates displaying a Haarlem spoligoprofile isolated during the last decade. The main objective of the training at IPG was to carry out phylogenetic and statistical analyses to understand the transmission dynamics of the Haarlem 3 genotype strains in this region. Phylogenetic inferences were explored using three applications: BioNumerics (version6.6, Applied Maths, Sint-Marteen-Latem,Belgium), MrBayes3 and PHYLIP. Back to IPT, N. Dekhil has completed all strain typing and is currently drafting a publication.

- Training at the Research Center Borstel (FZB)

TB accounts as the most important fields of research in Borstel. Key work has been done on drug resistance mechanisms, molecular typing and evolution of the M. tuberculosis complex and on the dissection of key molecules involved in the pathogenesis of the disease. A high throughput laboratory for phenotypic/molecular characterization of mycobacterial isolates is available (including state-of-the-art molecular methods such as MIRU-VNTR genotyping as well as DNA sequencing and gene expression analysis). Furthermore, an S3 laboratory for generation of and working with M. tuberculosis mutants is in place. The bio safety level 3 facilities also comprise space for M. tuberculosis infected mice, flow cytometry, and primary macrophage cultures.

FZB therefore responds perfectly to the expectations of MPC and its fecilities could allow them to gain access to hightroughput analysis and genetic manipulation of virulent mycobacteria.

FZB hosted two short trainings and a 9-month long training conducted in the context of a specific twinning action (3.4.2).

A PhD student from IPT (Ms Leila Jeljeli) performed a short 10-day training at FZB in Stefan Niemann's laboratory (June 14-24, 2011), as a preliminary to establish a future twinning action. Before traveling to Borstel, DNAs of MIRU typed Tunisian M. tuberculosis strains were sent to FZB. During her training, Miss Jeljeli was introduced to the automated 24 loci MIRU approach and confirmed the genotypes of the Tunisian strains. Ms Jeljeli got also acquainted with Genemapper modules and carried out phylogenetic analyses. During her training, Miss Jeljeli had also the opportunity to access the Biosafety level 3 (S3) Laboratory of the Department of Molecular Infection Biology and got a hands on practice on macrophage infection by virulent M. tuberculosis. These activities were particularly targeted to endow the MPC PhD student with some skills in high throughput analyses and working in a biosafety level 3 laboratory and animal facilities. These 10 days training also allowed the PhD student to discuss in details of what could be done and what should be prepared for a future collaborative twinning action. We also took advantage of the travel to Borstel to facilitate the participation of Miss Jeljeli to the 32nd Congress of the European Society of Mycobacteriology in Lübeck (June 26-29, 2011) where she presented an oral communication titled "High prevalence of Mycobacterium tuberculosis Latin American Mediterranean (LAM) strains harboring multiple PE/PPE deletions in Tunisia". Her participation in the ESM congress was an occasion to further discuss, with scientists from FZB, additional investigations in the context of a twinning action to delineate the molecular basis underlying the success of a particular LAM clonal complex, differentially deleted in three PE/PPE genomic loci. In this respect, a second long-term scientific stay has been planned during which the biological traits that might explain the apparent success of the LAM strains will be explored.

The second two-week training hosted by FZB involved a technician from IPA, Miss Fatma Zohra Gacem. Ms Gacem was trained in several aspect of molecular genotyping of clinical M. tuberculosis complex (MTBC) isolates. She performed 24-Loci-MIRU-VNTR typing, spoligotyping as well as sequence analysis using the newest 24 capillary ABI 3500xl sequencer. In addition, she was trained in bioinformatics analysis of genotyping data ranging from ABI Genotyper to Applied Maths Bionumerics software. Aside from establishing a tight collaboration between IPA and FZB, this training will allow IPA members to finalize publishing the population structure of M. tuberculosis circulating in Algeria.

- Research training at the Instituto Aragonés de Ciencias de la Salud (I+CS, University of Zaragoza)

The Research Group of Mycobacterial Genetics, headed by Carlos Martin, is based at Instituto Aragonés de Ciencias de la Salud (I+CS) and has been working since 1992 on molecular genetics of mycobacteria.

The group is particularly interested in:
(i) construction of new vaccines against TB;
(ii) molecular epidemiology of TB,
(iii) molecular bases of drug resistance in TB, and
(iv) transposition and latency of M. tuberculosis.

The I+CS coordinators involved in EUMEDNETVSTB are Sofia Samper, who is in charge of the molecular epidemiology aspect, and María José Iglesias, an epidemiologist, involved in the health systems for control of tuberculosis.

In the context of EUMEDNETVSTB, I+CS hosted two trainings. The first training was performed by Chawki Ben Abdessalem, a permanent scientist recently recruited at IPT, working in the group of R. Barbouche. C. Ben Abdessalem first attended the second EUMEDNETVSTB international symposium at Madrid (New tuberculosis vaccines for the Future/TB Host-Pathogen Interaction: the New Paradigms), then traveled to I+CS Zaragoza, the venue of his training. C. Ben Abdessalem stayed two weeks within the group of Mycobacterial Genetics, starting October 19, 2011. The second training benefited to Nedra Meftahi, a PhD student, supervised by H. Mardassi at IPT. N. Meftahi works on the molecular basis of drug resistance by generating mycobacteria with defined mutations in drug resistance conferring genes. In her PhD work, she also develops mycobacteria harboring deletions in genes potentially involved in virulence. Therefore, her training in a laboratory involved in these aspects is a good opportunity to learn more and to establish contact with scientists in the same field. Indeed, the host laboratory is currently interested in developing a live attenuated vaccine, MTBVAC. This vaccine is based on an MTB that has two stable independent deletions of virulence genes PhoP and fadD26. C.

Both C. Ben abdessalem and N. Meftahi were introduced to good laboratory practices that must be followed in a P3 environment and performed several infection experiments using virulent strains of M. tuberculosis essentially H37Rv and a clinical strain (MTZ) that has adapted to the population of the region of Aragon in Spain. They also learned how to differentiate macrophages derived from stem cells from bone marrow of a mouse, and also handled MHS cell line of murine alveolar macrophages. The two types of cells were used for the infection in order to compare replication of strains H37Rv and MTZ. Infection was followed up by fluorescence microscopy, using a recombinant virulent strain of MTB expressing the GFP protein. During their stay in I+CS, both trainees also had the opportunity to visit the animal facility of the Faculty of Veterinary Medicine of Zaragoza, and accessed the P3 room where mice could be experimentally infected with virulent M. tuberculosis strains. They had the opportunity to handle a collection of lungs from mice infected with H37Rv and MTZ to calculate the bacterial load in these lungs.

For both trainees, the stay was an opportunity for meeting and discussing with researchers of the host laboratory, opening the way for potential future collaborations. Actually, N. Meftahi is applying for scholarships to be able to carry out long term training in the context of a collaborative work aiming at assessing the pathogenicity of a BCG strain harboring a deletion in a PE_PGRS gene, strongly suspected to be a major determinant of MTBC strains virulence.

4.2. Twinning actions

During the course of EUMEDNETVSTB, at least 5 twining actions have been established, three of which has reached reasonable level of progress.

- INH-IPG twinning action
Owing to the partial support of EUMEDNETVSTB, a collaborative work between IPG and INH has been fully completed. The work consists in the determination of the population structure of M. tuberculosis in Morocco and has been published in PloS One*. Such collaboration allowed thus Ouafae Lahlou, a EUMEDNETVSTB participant from INH to fulfill the requirements for her PhD thesis. This collaborative work provided a detailed snapshot of the genotypic diversity of M. tuberculosis strains in Morocco. It has been found that the population structure of M. tuberculosis

From Moroccan patients is very homogeneous consisting essentially of Euro-American lineages, where le LAM genotype is predominating. These findings confirm what has been previously published from Tunisia, and thus comfort the idea to adopt a regional plan to fight against TB in the region. The EUMEDNETVSTB network represents a good foundation to convince the three country authorities to reconcile their efforts with regard to TB surveillance.

-Ouafae Lahlou, Julie Millet, Imane Chaoui, Radia Sabouni, Abdelkarim Filali-Maltouf, Mohammed Akrim, Mohammed El Mzibri, Nalin Rastogi2, Rajae El Aouad. (2012) The Genotypic Population Structure of Mycobacterium tuberculosis Complex from Moroccan Patients Reveals a Predominance of Euro-American Lineages. PloS One, vol7, Issue 10, E47113.

- IPT-IP twinning action
The Mycobacterial Genetics Unit at IP, headed by Brigitte Gicquel, belongs to the department of Genomes and Genetics. It has an undisputed renown worldwide in terms of mycobacterial genetics. This unit has developed PCR tests for the identification of M. tuberculosis and isolated the first DNA sequence that was used as a marker for strain genotyping (IS6110). This unit was amongst the first which developed mycobacterial genetic tools, shuttle vectors, transposons, systems for gene inactivation and studied host-pathogen interactions at the level of the pathogen and of the host. This unit discovered several virulence factors and identified the major receptor of M. tuberculosis on Dendritic cells (DC-SIGN). It has identified in M. tuberculosis horizontal genetic transfer and important polymorphisms in DNA repair genes. Recently, the unit got involved in comparative genomics to better understand the evolution of MTBC and the molecular bases of virulence and drug resistance. Taking advantage of this huge expertise, a specific twinning action was soon established and discussed during the visit of H. Mardassi at B. Gicquel's Lab in August 2010. The objective of the twinning action consists in the sequencing of the genomes of a set of naturally isogenic M. tuberculosis strains involved in the MDR-TB outbreak that emerged in

Tunisia a decade ago. Furthermore, this twinning action benefited of the fact that the Postdoctoral trainee who is in charge of this aspect originated from IPT, and has already worked on this aspect while he was a PhD at IPT. Overall, 7 genomes have been sequenced and compared, one of which represents a drug susceptible strain that seems to be a close ancestor of MDR-TB outbreak strain. The data indicate an important rate of genetic rearrangement, which is in contrast what has been published for drug sensitive strains, and which may reinforce the idea that drug resistant strains have a hypermutable phenotype. We also identified a deletion restricted to the outbreak strain, in a particular gene that might contribute to drug resistance. The comparative and confirmatory analyses are under way and a publication will be drafted soon. In this particular context, EUMEDNETVSTB was critical as it allowed an MPC TB research entity to benefit of Genomics.

- ITP-FZB twinning action

As mentioned earlier, the 10-day training of L. Jeljeli at FZB (section 3.4.1) was a preliminary to a long-term training in the context of a research twinning action, whose objectives had been previously discussed between H. Mardassi and S. Niemann, during the international symposium of Tunis, and later in the ESM congress held in Lübeck (June 26-29, 2011). The twinning action consists to adopt a pathogenomics approach to unravel the molecular basis underlying the success of a Tunisian Latin-American and Mediterranean (LAM) genotype of M. tuberculosis harboring several deletions in PE/PPE loci.

Previously, in our laboratory at the Institut Pasteur de Tunis, we found that the LAM family of M. tuberculosis represents the most predominant lineage in Tunisia, with 37.9% of the strains showing spoligoprofiles harboring a typical LAM signature. Strikingly, almost all LAM strains could be divided into two major subpopulations, one characterized by the presence of two large deletion events within PE/PPE loci (LSPTUN4 and LSPTUN6) and the second, which is significantly more prevalent (66.4% vs. 28.8%), featured an additional large deletion (LSPTUN2) mapping to the PE/PPE genes of the secretion system ESX-5. By performing MIRU-VNTR typing (15 loci), we confirmed that the Tunisian M. tuberculosis LAM population consists of two distantly related clades that perfectly matched the two subpopulations disclosed by the LSP analysis suggesting that the LAM strains characterized by the concomitant presence of LSPTUN2, LSPTUN4 and LSPTUN6 define a new branch of the LAM family.

L. Jeljeli, during her second 9-month stay in FZB, carried out an automated MIRU-VNTR typing using the set of 24 loci on the 132 clinical isolates of the Tunisian LAM strain collection and further confirmed the 15-format MIRU-VNTR typing. Sequencing of the deletion boundaries of LSPTUN2, LSPTUN4 and LSPTUN6 showed that these genomic events were identical in the two LAM subpopulations, strongly indicating that they are clonally derived from each other. Next, the biological traits that might explain the apparent success of the LAM clinical strains was investigated by infecting human monocyte-derived macrophages (HMDM) with the PE/PPE differentially deleted LAM strains. All the experiments were done in class III cabinets in a Bio-safety level III facility available at the Research Center Borstel. The Results from seven independent experiments showed that the intracellular growth of the strain carrying the ESX-5 PE/PPE deletion was significantly higher after a one-week incubation period, a finding in favor with its apparent success. However, there was no difference in the cytokine released profile of MDMs.

During the same training, L. Jeljeli attempted to better decipher the genomic variations between the tested LAM strains and in the whole population of LAM strains in Tunisia. Towards this end, five genomes of strains selected from each subpopulation were sequenced using the Illumina MiSeq system. Comparative deletion-based analyses are actually in progress to confirm the critical role of the PE/PPE deletion in the natural success of the LAM genotype in Tunisia.

Part of the results obtained during this second 9-month internship in FZB was presented in the 33rd Congress of the European Society of Mycobacteriology (ESM) in Brasov (Romania). Furthermore, 2 publications* are actually under preparation as a result of this collaborative action. Importantly, the progress made in the objectives set for this twinning action prompted Ms L. Jeljeli for the German Academic Exchange Service (DAAD) scholarship. Such application was approved by the selection committee and L. Jelejeli will benefit of a financial support for a one-year stay in Borstel. During this DAAD-supported collaborative interaction, we will expand upon our initial EUMEDNETVSTB-funded twinning action, to deeply explore the molecular mechanism underlying the higher rates of infectivity and sucess of the M. tuberculosis clone harboring the ESX-5 PE/PPE deletions. Given that the genome sequence of 7 strains have been determined thanks to a tripartite collaboration between the Institut Pasteur de Tunis, the Institut Pasteur of Paris, and the Center of Borstel, we have sufficient data to realistically adopt a pathogenomic approach. We anticipate that this will result in high standard research outputs, thus achieving one of the most expected outputs sought by REGPOT funding, which is increased visibility of MPC TB research entities. The adressed research theme is of particular interest, since we still do not know what are the factors governing the success of M. tuberculosis under natural infection conditions. Indeed, most of the studies carried thus far used animal models which proved completely different and do not reflect the mechanismss operating in humans. Recently, another research group, reported that an ESX-5 PE/PPE deletion similar to the one identified in our sucessful and virulent clone, produced an attenuating phenotype in mouse. This finding completely contrast with our and stresses out the fact that conclusions drawn using animal models could not be exptrapolated to humans. It also ampasize the importance to explore naturally occuring phenotypes to better address the criticial mechanisms implicated in host-pathogen interaction.

Furthermore, with regard to the MPC PhD student, the DAAD scholarship, will not only allow her to work in an internationally renowned center, but will provide her with more competitveness for the search of post doctoral trainings.

-Predominance of a Latin American Mediterranean (LAM) Mycobacterium tuberculosis clone harboring an ESX-5 PE/PPE deletion that correlates with increased replication in human macrophages.

-Comparative genomics of a successful Latin American Mediterranean (LAM) Mycobacterium tuberculosis clone harboring a PE/PPE deletion mapping to the ESX-5 secretion locus

5. MPCs technical platform upgrading

Upgrading the technical platform of MPC TB research entities is a major objective that is critical to ensure effective S&T transfer and depending on which the research activities could be sustained. A separate workshop

(WP3) was dedicated to the equipment platform, in which three main objectives were sought:

- Establishment of an equipment list according to the specific needs of each MPC research entity
- Achievement of all administrative and ordering steps
- Implementation and use of the equipments

At the project term the three above objectives have been entirely achieved by the three MPCs. Indeed, each institution devised an equipment list, defined the technical characteristics, which is necessary to issue a call for tender and, examined the suppliers' submissions for technical and financial conformity. The three MPC countries achieved these steps according to their respective rules and law.

IPT ordered and received a first equipment list that has been first used in the training workshop that was organized in September 24- October 1, 2010. The equipments list reflects the need of IPT to reinforce its ability to carry out typing and sequencing activities for both the pathogen and the host. Indeed, several studies conducted by the teams of H. Mardassi and R. Barbouche have reached a certain level of achievement which requires increased sequencing capacity. Such a need prompted Pr. Brigitte Gicquel to generously donate her 16-capillary automated sequencer (ABI Prism 3100) to support IPT's TB research activities. The availability of the 16-capillary sequencer was instrumental to foster sequencing of genes conferring drug resistance. Actually, the ABI Prism 3100 has been integrated to the institutional technical platform, and aside the TB activity, all other researchers could access to the facility. Furthermore, owing to the reinforcement of the equipment platform of IPT TB research entity, the following research activities were fully completed:

- 625 Tunisian isolates were spoligotyped243 Tunisian LAM strains were MIRU typed
- 243 Tunisian LAM strains were characterized by PE/PPE deletion analysis
- 252 Tunisian Haarlem Have been MIRU typed
- More than 1000 sequencing reaction of drug resistance conferring genes were processed
- A mycobacterial shuttle vector suitable for efficient allelic exchange was constructed

The completed work on LAM strains typed by MIRU and PE/PPE deletion analysis was presented in the 32nd Annual congress of the European Society of Mycobacteriology (27 June 2011, Lübeck, Germnay).

A second list of equipments has been ordered and implemented in IPT TB research entities (H. Mardassi & R. Barbouche labs), after obtaining the approval of the EC to use funds initially allocated to upgrading the P3 facility. Indeed, a 50 000 Euros have been booked to upgrade the P3 facility of IPT so that it could be used to host M. tuberculosis manipulation. For this purpose, and thanks to the contribution of I+CS, a contact has been made with LUWA ESPANOLA from Spain to evaluate the capacity of IPT's P3 facility to harbor experiments involving virulent M. tuberculosis. LUWA ESPANOLA has previously implemented the Glaxo Smith Kline's P3 facility in Spain. A report has been issued, which confirmed our expectations. Indeed, the IPT's P3 facility in its actual state could not allow manipulating pathogenic mycobacteria. According to the expert's report, it is even not recommended to upgrade the P3 but simply proceed

with the construction of a new facility, which would require a minimal sum of 500 000 Euros. Hence we proposed to reallocate the IPT's EUMEDNETVSTB budget for additional equipments.

Both INH and IPA received and implemented their equipments. As for IPT, they are currently being used to carry out molecular typing and/or host-pathogen interaction related techniques, particularly those initiated in the context of twinning actions. The equipments received by IPA were also used in the practical training organized therein in June 2012, including the GeneXpert machine.

6. Development of human resources

EUMEDNETVSTB also provided support to hire two MPC young researchers or to provide complement salary for one MPC Postdoctoral training in a European TB laboratory.

6.1. Recruitment of young MPC researchers:

A one-year contract under EUMEDNEvsTB has been established with Meriem Ben Ali, a young scientist recruited in the group of R. Barbouche. M. Ben Ali is a former R. Barbouche's PhD student. After performing a 2-year Post-Doctoral training in Lluis Quintana's research unit at IP, she came back to IPT in a hope to be permanently recruited. While she was waiting for a position, EUMEDNETVSTB offered her the opportunity to join the laboratory of R. Barbouche and implement a new research activity. M. Ben Ali started her work by September 1st 2010. She developed a research activity that expanded upon her PhD work, but using new techniques and approaches gained from her Postdoctoral training in L. Quintana's Lab. Briefly, M Ben Ali explored the role of TLR-associated polymorphism in resistance/susceptibility to TB in a Tunisian cohort and validation of their functional impact. In addition, M. Ben Ali looks at the haplotype contribution, including TLR1-6-10, in response (or non response) to BCG immunostimulating therapy in bladder cancer. In March 2011, M. Ben Ali successfully participated to a recruitment competition for a permanent position at IPT. She is now a permanent researcher of IPT. Hence, EUMEDNETVSTB funding was critical to attract and recruit a young scientist at IPT.

In INH, a two-year contract was signed with Ms. Ikram Morjane, beginning September 1st, 2011. She was committed to monitor the recruitment of patients, updating the database, and ensuring the follow-up with health centers. She was also in charge of dispatching samples to the investigation centers. All these tasks have been performed in the context of the INH TB cohort, which will be further explored to evaluate the Moroccan host immune response to TB.

6.2. EUMEDNETVSTB-supported postdoctoral training

EUMEDNETVSTB provided complement salary (equal to 5 months, 1st November 2012 to 28th February 2013) to support the postdoctoral training at the Institut Pasteur of Amine Namouchi, an MPC PhD. Amine Namouchi completed his Ph.D degree in Microbiology in the Unit of Typing and Genetics of Mycobacteria at the Institut Pasteur de Tunis, under the supervision of H. Mardassi. During his PhD work, A. Namouchi characterized in detail the population structure of M. tuberculosis in Tunisia, and focused on an MDR-TB outbreak, describing its emergence history based on IS6110 transposition events. During his PhD, A. Namouchi benefited of a 4-month

informatics course (Informatics in Biology) which allowed him to be skilled in several informatics languages and programming. In 2009, A. Namouchi started his postdoctoral training at the mycobacterial Genetics unit of IP, under the supervision of B. Gicquel, where he exploited his knowledge in molecular genetics of mycobacteria and bioinformatics, focusing mainly on genomics. During his postdoctoral training, A. Namouchi contributed by serving as a facilitator in the practical training course of Tunis, and worked in the context of the twinning action developed with IP and which consists in in analysing and comparing the genomes of MDR-TB strains involved in the outbreak he already characterized during his PhD. The genomic DNA of these strains was isolated in the Unit of Typing and Genetics of Mycobacteria and sent for whole genome sequencing using the illumina/Solexa technology. The genomic data was then analysed by Dr. Amine Namouchi at IP.
Comparative analyses are under way, upon which we aim to unravel critical genetic variations associated with the emergence of the epidemic phenotype and drug resistance of these MDR strains.

Potential Impact:

1. Impact on MPC TB research

Undoubtedly, the impact of EUMEDNETVSTB on TB research in the region is considerable. As a capacity building project, it addressed several requirements will all converge for an optimized research environment. Full achievement of EUMEDNETVSTB workpackages strengthened and upgraded the overall TB research potential of MPCs by as witnessed by the following measurable outputs:

Set up of a Euro Mediterranean network, allowing the TB MPC laboratories to be an active partner of the global scientific effort aimed at increasing knowledge on TB. MPC are therefore less isolated and could more easily get access, when needed, to advices, trainings, and emerging technologies.

- Updated MPC knowledge and brain gaining on current TB themes garnered by attending the organized international symposia and meetings. During the project period, MPC researchers have been infused with emerging themes in TB research and the state of the art methodologies

- Upgraded technical and equipment facilities, thus facilitating the advancement of ongoing research themes and fostering the application of molecular tools for tight TB surveillance

- Enhanced skills and knowhow of MPC young researchers, owing to trainings and involvement in twinning actions with European TB centers of excellence, thus promoting S&T transfer. This has a particular impact for sustained capacity building.

- Established research twinning actions between MPC and European partners to deepen and increase the research quality, thus contributing to increased visibility and competitiveness, as witnessed by the number of scientific papers that will be published (at least 8, one of which has been published in PloS One) and the DAAD scholarship awarded to an MPC PhD student.

- Increased awareness of decision makers which will facilitate future support and local funding. Thanks to EUMEDNETVSTB activities, decision makers, who have been invited to the meetings, are more akin to support research laboratories which will facilitate S&T transfer, especially molecular biology-based tools.

- Consolidated role of MPC research entities in their respective NTPs. MPC research laboratories will no more be perceived as acting on their own, motivated by basic research aspects only, but will contribute by alerting and guiding the NTP program based on their genotyping activities.

The aforementioned outputs allow us to be optimistic that the impact of EUMEDNETVSTB is not only immediate, but will benefit to the community at the long term.

2. Impact on TB control in the Euro Mediterranean region

As mentioned earlier, effective TB control and surveillance should combine classic tools with the new nucleic acid-based approaches to

rapidly detect new cases and emergence of drug resistance amongst them. Through EUMEDNETVSTB, the huge potential of molecular tools, for a better TB detection and surveillance has brought to the attention of the professionals, decision makers, and stakeholders. We are more than convinced that this will impact the way they will reorganize their TB program. They will work towards increasing the collaboration between reference diagnostic laboratories research entities, to promote efficient implementation of TB molecular detection tools.

3. Social impact

In the particular case of TB, the effectiveness of all forms of medical and organizational measures is dependent on the active implication of the society. Breaking TB transmission chains necessitates being aware of how it is transmitted and how drug resistance could develop. The public at large must be conscious of the difficulties encountered by TB patients. Various surveys have been conducted around the world to understand the knowledge, attitudes, behaviours, and practices regarding TB. In most cases (more than 90%), and this also applies for the Maghreb countries, people had heard of TB but only around 20% know how it is transmitted. Very few are aware of dug resistance and how it develops. Most people do not know to which extent TB, and particularly its drug resistant form, affects life-styles of patients and their family members. There are also several misconceptions about TB. In the Maghreb, many people, especially of the urban area, are surprised that TB still represents an important public issue. For many of them, TB could not affect a person that has received the BCG vaccine, and are convinced that it is readily eliminated with antibiotics. Others are profoundly convinced that TB is a disease of poor people only. In the Maghreb where water pipe smoking (nargile) is a frequent practice, only very few people know about the risk of TB due to water pipes sharing in street cafés. With these long standing and profound misconceptions, health care authorities may have spent lot of means and efforts to apply control measures, but in vain, as the tubercle bacillus will exploit every man's mistake and unconscious behaviour.

EUMEDNETVSTB, provided the opportunity to raise the consciousness of the Meghrebian community on TB issues, through the large diffusion of the international and regional scientific events. Furthermore, decision makers and stakeholders, were sensitised of the new opportunities for a better TB control and surveillance. This would have an impact on their future action plan and their willingness to contribute to the introduction in routine of TB detection molecular tools. On the other hands, professionals involved in TB control will be more prone to rely on M. tuberculosis genotyping for outbreaks tracking and surveillance.

4. Use and dissemination of foreground

- Section A

EUMEDNETVSTB, though primarily intended to increase the research capacity of MPCs, took advantage of the network and the organized international and regional meetings, to raise awareness, at all levels, including the public at large. EUMEDNETVSTB provided a unique opportunity to bring TB under the spotlight in the region, particularly the drug resistance issue.

EUMEDNETVSTB through its workpackage 5 (dissemination of the project outcomes) planned for several actions aimed at increasing stakeholders

consciousness and social awareness. In this respect, a web site (see http://www.eumednet-tb.com online) was created and updated twice (last time in December 2012). A CD and a leaflet introducing the EUMEDNETVSTB network and its objective were prepared and largely distributed in international events of 2010-2011 (TB International Symposium, Tunis, September 2010; European Society of Mycobacteriology, 2011). Two flyers have been prepared and will soon be electronically distributed to all governmental and nongovernmental organizations involved in TB and infectious diseases control and surveillance (EC, WHO, WHO-TDR, WHO-EMRO, Stop TB, Global fund, IUTLD, Institut Pasteur International Network, MPC ministries...). One flyer describes all EUMEDNETVSTB-supported events (2 international symposia, three regional workshops, and two training workshops) and the themes covered thereof, thus demonstrating the effectiveness of the finding in brain gaining and knowledge updating. The other flyer shows how EUMEDNETVSTB helped MPC TB research activities to build their capacities by benefiting of trainings and gaining access to new technologies. A booklet, dealing with TB basic facts in the region and the overall project outputs will be prepared.

Furthermore, EUMEDNETVSTB through its organized international symposia and regional meetings, allowed direct, face-to-face, interaction with stakeholders, policy/decision makers, health care professionals, representative of the civil society, and NGOs,. The international symposium of Tunis (20-22 September, 2010) for instance, attracted more than 120 persons (decision/policy makers, physicians, technicians, PhD and Master students, NGOs...) originating from 25 countries (Syria, Lebanon, Egypt, Palestine, Morocco, Algeria, Tunisia, Bulgaria, Cameroun, Cambodia, Republic of Central Africa, Guadeloupe, Madagascar, Nigeria, Sudan, Zambia, South Africa, Ghana, and Burkina Faso, France, England, South Africa, Canada, USA, and Switzerland). This event was largely covered by the local Tunisian press, and transmitted to the general population through the main national TV and radio channels. Interviews with organizers were also transmitted through national radio channels. Furthermore, and apart from TB specialized websites, eumednet-tb.org and molecular-tb.org, this symposium was highlighted in other web sites (WHO-TDR, Institut Pasteur International network). Likewise, the second EUMEDNETVSTB international symposium, held in Madrid (17-18 October 2011), was largely diffused as it took place with the summit event "TB vaccine for the future", organized by the TBVI with the support of the Ramon Areces Foundation. This events attracted TB experts from all over the world and was largely covered by TV channels, newspapers and electronic media. To further introduce the EUMEDNETVSTB network to larger audience worldwide, a brief talk on EUMEDNETVSTB was delivered to the audience.

At the MPC level, in addition to the international symposium organized in Tunis, both Algeria and Morocco held a workshop, and ensured large coverage of the events.

Finally, a booklet will be edited soon, highlighting all the outputs of EUMEDNETVSTB in terms of building capacities in MPCs and the existing potential which needs to be exploited and reinforced to move to a more efficient strategy to fight TB and its resistance forms.

- Section B

EUMEDNETVSTB intends to develop, with the help of European centres of excellence, the research potential of MPC countries in order to be better

integrated in the surveillance of TB, both regionally and globally. Given the consistent population migration flow between MPCs and the European countries, there is no doubt that the EUMEDNETVSTB initiative benefits to both parts of the Mediterranean Sea.

With regard to TB epidemiology and surveillance in the region, the available data mostly generated owing to EUMEDNETVSTB funding, show that MPCs harbour the same M. tuberculosis lineages (Euro American) prevailing in Europe. Given the host-pathogen compatibility that characterizes M. tuberculosis infection, the risk that clones originating from MPC expand in Europe is real. Therefore, a Euro Mediterranean regional concerted action against TB is more than necessary for a tight control of the disease. EUMEDNETVSTB has laid the foundation on which such concerted action could be built upon.

The EUMEDNETVSTB network has created a favourable context of scientific exchanges which is likely to benefit to both MPC and European countries. Indeed, a better understanding of TB epidemiology and host-pathogen interaction must rely on clinical findings and involve different settings around the world. The Collaborative research activities established between MPCs and European centres under EUMEDNETVSTB, will not only benefit to MPCs but will also raise several questions pertaining to the European situation, inasmuch as both regions share the same lineages of the tubercle bacillus. As a concrete example, the collaboration between IPT and FZB regarding the expansion in Tunisia of a Latin American Mediterranean (LAM) M. tuberculosis clone harbouring specific deletions in PE/PPE genes, showed, such deletions exist in the German population. Likewise, parallels in TB epidemiology between Morocco and Spain must exist given their proximity. Now that the population structure of M. tuberculosis in Morocco has been described, it could be of interest to establish a common surveillance strategy using data garnered from molecular studies conducted in the context of EUMEDNETVSTB. On the other hand, the genomic sequence data generated in the context of EUMEDNETVSTB, will not only be exploited by MPCs, but will also be integrated in publicly available databases and will enrich comparative analyses at the global level. Furthermore, the Moroccan TB cohort, whose establishment was reinforced thanks to EUMEDNETVSTB funding, will contribute to assess several key principles in immunology through collaborative projects with European laboratories.

All these exploitable results were highlighted during the project final meeting of EUMEDNETVSTB, and the question pertaining to their exploitation was raised. Of course, aside from soliciting international funding, it has been proposed that research efforts must be continued to be ready to submit joint collaborative research proposal once the EC horizon 2020 funding program will launched. In the meantime, publishing all the findings that resulted from the EUMEDNETVSTB-supported twinning action was strongly recommended to increase the chance to be favourably considered in the next EC funding calls.

List of Websites:

http://www.eumednet-tb.org