**Progression of* M. tuberculosis* from Latency to Disease: *In vitro* and *in vivo* Tracking of Iron Content in Alveolar Macrophages**

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**Section I. The Idea**

| The basic idea of the proposed research is to understand the progression of *M. tuberculosis* from latency to disease by tracking the *in vivo* iron content of the alveolar macrophages (AMs). |

**Exposure to pollutants**  
There are conclusive evidences that support the hypothesis that exposure to respirable pollutants from combustion of tobacco and biomass fuels increases the risk of both tuberculosis (TB) infection and TB disease [1]. Smoke also impairs the function of pulmonary AMs, which are not only the cellular target of *M. tuberculosis* infection but also constitute an important early defense mechanism against the bacteria.

**Iron content of AMs**  
Investigators [2,3] noted that smoke-exposed AMs have a markedly elevated iron content. This iron overload impairs defense against intracellular microorganisms because of reduced production of both tumor necrosis factor-α and nitric oxide. The impairment of the iron-laden macrophages could be attributed to increased incidences of TB, with a progression from latency to disease. It is hypothesized that iron content and its temporal variations in AMs can provide leads to understand the TB latency and assist in a quick detection of state of the latency.

**Tracking iron content: technologies**  
A novel method of *in vivo* tracking of the iron content in the AMs will be developed and utilized (see next section), which may provide insight on the host-bacillus interactions. Temporal data from a large population (see paragraph below) would be used to develop associative models between iron content and state of latency. As *in vivo* testing is inconvenient and not feasible on a long run, the *in vivo* data/models would be used to design more amenable and less expensive *in vitro* sensors. These *in vitro* sensors would be used for subsequent tracking of the state of latency.

**Study area**  
The study area for this research is the city of Kanpur (longitude 88° 22′E and latitude 26° 26′N, population 4 million), India. Kanpur is an industrial city, having high fine particulate levels in air (seasonal average PM$_{2.5}$ levels ~ 100-400 µg/m$^3$ [4]). In addition to industrial and automobile pollution, there is a significant smoke exposure from domestic cooking using cheap fuels like wood, coal and biomass (as a large portion of the population is in the economically lower strata). Prevalent tobacco smoking further aggravates the ill effects of smoke exposures. The unusually large cases of TB infection (40%) [5] and high air pollution exposure in Kanpur makes it an appropriate study area.

**Uniqueness of this study**

- Human subjects for the study (as animal models are not representative) *
- *In vivo* studies
- Testing the hypothesis of association between iron content in AMs with latency
- *In vitro* sensing to study state of latency

(* all studies will be conducted under the ethics guidelines of the Indian Institute of Technology Kanpur and the Indian Council of Medical Research, India.*)
Section II. Project Design and Implementation Plan

The very fact that almost 40% of the population of the study area has TB infection provides an opportunity to have a large cohort of human population (> 400 persons; healthy people, infected people, early stage cases, patients undergoing treatments) with different stages of latency and disease progression. The results from the study can establish causal link between latency and disease. The large sample size and development of novel techniques for sensing in vivo iron content in AMs are important features of this study. The work plan is presented below in a schematic and accompanying brief description.

(1) Assess integrated exposure of air pollutants, PM\textsubscript{10}, PM\textsubscript{2.5}, elemental & organic carbons, metals (including Fe), CO, SO\textsubscript{2} and NO\textsubscript{2} along with physical parameters like humidity, temperature etc.

(2) Design a sensor mechanism, deliverable through the inhalation route, e.g. based on fluorescence resonance energy transfer (FRET), targeted delivery mechanisms, etc.

(3) Temporal \textit{in vivo} tracking of iron content in AMs. The study is proposed for a sample size > 400 over a time period of 1 (+1) year.

(4) Formulate models based on \textit{in vivo} sensor results with samples collected by bronchoscopy/sputum etc.

(5) Realization of an \textit{in vitro} sensory mechanism based principles of immunological, electrochemical, mass absorption from the \textit{in vivo} data.

Support from two institutions in the city of Kanpur, (i) GSVM Medical College with medical expertise in respiratory diseases with large historical record of patient history (name, address, age, sex, area of residence, admissions (hospital and intensive care units), cause of death etc.) and (ii) Central Pollution Control Board, Kanpur having expertise and data on emission inventory and air quality would aid to this effort.

Timeline and budget

In the first year, the \textit{in vivo} sensors would be developed, iron contents in the AMs would be measured, and appropriate models would be formulated, all within the specified budget.

Follow up/Future work

In the second year, these models would be used to develop the \textit{in vitro} sensors.

References