Two specific publications/research papers of the applicant relevant to the research work

1. **Reena Bharti**, Trisha Roy, Sonia Verma, D. V. Siva Reddy, Hasham Shafi, Khushboo Verma, Sunil K Raman, Sampita Pal, Lubna Azmi, Amit K Singh, Lipika Ray, Madhav N Mugale, Amit Misra* Transient transfection of the respiratory epithelium with gamma interferon for host-directed therapy in pulmonary tuberculosis. Molecular Therapy-Nucleic Acids. 2020 Dec 4;22:1121-8

   Nebulized gamma interferon (IFN-γ) protein has been studied for clinical safety and efficacy against pulmonary tuberculosis (TB). The protein is expensive, requires a cold chain, and is difficult to deploy in limited-resource, high-incidence settings. We generated a preclinical proof of concept (PoC) for a dry powder inhalation (DPI) containing DNA constructs to transiently transfect the lung and airway epithelium of mice with murine IFN-γ. Bacterial colony-forming units (CFU) in the lungs of mice infected with *Mycobacterium tuberculosis* (Mtb) reduced from about 10^6/g of tissue to ~10^4 after four doses given once a week. Nodular inflammatory lesions in the lungs reduced significantly in number. Immunohistochemistry of infected lung sections for LC3-1 and LAMP-1 indicated autophagy induction between 18 and 48 h after inhalation. ELISA on bronchoalveolar lavage (BAL) fluid showed differences in kinetics of IFN-γ concentrations in the epithelial lining fluid of healthy versus infected mice. Uninfected mice receiving DNA constructs expressing a fluorescent protein were live-imaged. The fluorescence signals from the intracellular protein peaked at about 36 h after inhalation and declined by 48 h. These results establish preclinical PoC of the efficacy of a DPI and dosing regimen as a host-directed and transient gene therapy of experimental pulmonary TB in mice, justifying preclinical development

2. .**Bharti R**, Roy T, Verma S, Reddy DS, Shafi H, Verma K, Raman SK, Pal S, Azmi L, Singh AK, Ray L. Transient, inhaled gene therapy with gamma interferon mitigates pathology induced by host response in a mouse model of tuberculosis. Tuberculosis. 2022 May 1;134:102198.

   A dry powder inhalation formulation was prepared with plasmids expressing gamma interferon or fluorescent proteins. The formulation was optimized and characterized for inhalation. Stability of plasmid DNA was checked in prepared formulation and MMAD of particles was checked through Cascade impactor. *In- vitro* and *in-vivo* studies were done to investigate the kinetics of expression of IFN-ɣ and/or reporter genes. Immunohistochemistry was done to investigate the kinetics of induction of autophagy as a consequence of expression of functional IFN-ɣ in the lungs of *Mtb* infected mice. Efficacy of DPI in terms of reduction of bacterial burden in lungs and spleen of *Mtb* infected mice were shown.