सीएसआईआर-केन्द्रीय औषधि अनुसंधान संस्थान
CSIR-CENTRAL DRUG RESEARCH INSTITUTE

ACADEMY OF SCIENTIFIC AND INNOVATIVE RESEARCH
AcSIR

Amit Misra Chief Scientist, Division of Pharmaceutics and Pharmacokinetics Professor, Academy of Scientific and Innovative Research

To the Sun Pharma Science Foundation Fellowship Committee,

I am pleased to nominate my former Ph.D. student, Reena Bharti, for the Sun Pharma Science Foundation Research Fellowship - 2024. This nomination is endorsed by Dr. Radha Rangarajan, Director, CSIR-Central Drug Research Institute who is the Head of my Institution.

Reena is currently working as a post-doctoral fellow at the University of San Francisco, and has expressed keen interest in returning to India to continue research on high-risk, high-reward problems. I believe Reena's research embodies the spirit of innovation and has significant potential to contribute to the potential therapeutics of respiratory diseases through her innovative ideation. Reena has consistently demonstrated exceptional dedication and scientific insight in her research, particularly in the development of an innovative dry powder inhalation (DPI) formulation during the course of her PhD research. This formulation successfully achieved transient transfection of the lung and airway epithelium with gamma interferon (IFN-γ), showing promising potential as a host-directed, transient gene therapy against respiratory diseases; and can be a strong competitor of Actimmune®. The active pharmaceutical ingredient (API) in the proposed formulation is a plasmid (DNA), whereas Actimmune® uses the recombinant protein. This level of differentiation also opens up opportunities for producing intellectual property. Other competitive advantages of the DPI include its cheap manufacturing cost compared to Actimmune®.

Reena's significant contributions include the preparation of a DPI containing DNA constructs of gamma interferon polyplexed with poly(ethyleneamine) (PEI). Notably, the DNA incorporated in the particles remained intact during processing, as confirmed by various characterization techniques including electron microscopy, micromeritics, and cascade impaction. The DPI was engineered to ensure deep lung deposition, with the median Martin's diameter of the particles measured at 1.2µm, and a mass median aerodynamic diameter (MMAD) of 2.85µm ± 1.8 µm GSD. Reena exposed A549 cells to particles incorporating the gene for Green Fluorescence Protein (GFP) and used fluorescence microscopy to check the expression kinetics of the translated protein. She observed the expression of GFP starting 6 hours after exposure. At 24 hours post-transfection, the GFP co-localized with lysosomes, indicating the onset of protein degradation. These findings were pivotal in understanding the temporal expression and localization of the therapeutic protein. She then conducted *in-vivo* experiments in mice, where inhalations of the DPI resulted in detectable expression of the RFP gene as early as 6 hours post-administration, peaking at 24 hours. The kinetics of gene expression and subsequent protein activity were rigorously evaluated through bronchoalveolar lavage (BAL) and ELISA, revealing a peak IFN-γ secretion of approximately 800 pg/ml at 33 hours post-inhalation. Very remarkably, IFN-γ 'pharmacokinetics' were very different between healthy animals and animals infected with *Mycobacterium tuberculosis.* The onset and Tmax of IFN-γ was delayed in infected animals. This enabled us to conclude that whereas healthy animals were able to amplify the cytokine response in an autocrine/paracrine manner, infected animals were 'immunosuppressed'—hower the gene therapy intervention overcame and surpassed this immunosuppression. This research demonstrated the feasibility of using a DPI formulation to deliver the IFN-γ gene for transient

transfection in the lungs, offering a promising approach for gene therapy applications where the gene product is required only transiently—respiratory intracellular bacterial and viral infections and lung and airway cancers. The findings suggest that this prototype product is ready for detailed preclinical investigation of its safety and efficacy, particularly as an adjunct therapy to prevent severe immunopathology in respiratory diseases. Such a product has additional advantages:

1. *Non-Sterile Product*: Unlike Actimmune®, which requires a sterile liquid formulation for injection, DPI formulations do not need to be sterile. This considerably reduces production costs because DPI does not require the strict and costly asceptic processing or terminal sterilization processes that injectable formulations do.

2. *No Cold-Chain Logistics*: The DPI formulation remains stable at ambient temperature, removing the need for cold-chain logistics. Actimmune, on the other hand, requires refrigeration during storage and transportation, increasing the complexity and cost of distribution. The DPI formulation's room-temperature stability makes it more inexpensive and accessible, particularly in areas with inadequate infrastructure.

3. *Self-Administration*: Patients can self-administer the DPI formulation using a simple inhaler device, minimizing the need for frequent healthcare visits. In contrast, Actimmune® requires subcutaneous injections, which must be delivered by a healthcare practitioner. The ability to self-administer the DPI lowers both direct healthcare expenditures and indirect costs such as time off work or travel for medical appointments.

These variables together make the DPI formulation a more cost-effective choice for delivering genes of interest for transiently transfecting the lung and airway epithelium, potentially saving patients and healthcare providers money while also providing a return on investment for the innovator. The essay written out by her for this nomination outlines how she will implement product development them if she is funded for the current proposal.

Please feel free to contact me if you require any additional information.

Thank you for considering this nomination,

Amit Misra
Chief Scientist, Professor (AcSIR)