Dated: 27/8/24

I, SHAILZA SINGH, certify that the research work under reference to SUNPHARMA under Pharmaceutical Sciences has not been given any fellowship.

Research Achievements:

IL-10 and IL-12 are reported as prime regulatory cytokines in visceral leishmaniasis model where epigenetic regulators such as HDAC11 and HDAC1 regulate IL-10/IL-12 expression ratio in macrophages. CD40 signaling through ERK and p38 has been known to play dual role in regulation of both cytokines. Although visceral leishmaniasis is prevalent in India, Cutaneous Leishmaniasis (CL) is prevalent globally accounting for 1 million cases every year. Hence, our work is focused on identifying IL-10 and IL-12 regulatory axis in *L.major* mediated CL, identifying prevalent pathways playing a role in their reciprocal expression, identification of principle components in the said network, identifying phenotypic changes in macrophages population to discover novel subsets and designing systematic model based synthetic immuno-modulatory circuit as a therapeutic thus making our study holistic. Through our systems based immuno-regulatory mathematical model and protein expression studies, we identified that IL-10 and IL-12 reciprocal regulation takes place post 6h of infection with *L.major* marking an early onset of reciprocity. TLR2 mediated MyD88 pathway, IFNγ signalling, PI3K pathway, ERK pathway and Brx mediated hypertonicity inducing pathway were key signalling in regulating reciprocity. We identified NFAT5 as epigenetic regulator which regulates the expression of both cytokines in early stage of infection. SHP-1 was identified as regulator of NFAT5 which is directly activated by *Leishmania* antigen GP63 to promote IL-10 expression over IL-12. We studied the interaction behaviour of both NFAT5 and SHP-1 and its impact on IL-10 and IL-12 expression through molecular dynamics simulations (MDS) and protein expression studies. Using artificial intelligence and we designed synthetic inhibitoy peptides to regulate SHP-1 activity. Conservation and stability of the peptides were analysed using position specific scoring matrix algorithms and MDS for its efficient delivery in vitro and in vivo and their effects on parasite elimination and cytokine expression was analysed. For expression of peptides *in vitro* and *in vivo*, we designed TET-ON based inducible synthetic circuit and we observed high efficiency in elimination of intracellular parasite in the ON state. In the OFF state, the circuit did not alter cytokine expression although in ON state with *L.major* infection, high pro-inflammatory cytokine expression and restoration of IL-12 expression was observed. For all experiments we had IL12p40-/- and IL10-/- as controls, high salt diet mice as NFAT5 positive control. Using adoptive transfer of engineered macrophages, CRISPR-Cas9 mediated Knock-IN of synthetic circuit in mice to generate synthetic circuit +/+ transgenic mice and through nano-particles mediated delivery of synthetic circuit in mice we aim to validate the efficiency of synthetic circuit *in vivo* as therapeutic and vaccine. This

work is executed in my lab culminating interdisciplinary science and leverages the new BioE3 policy of precision bio therapeutics through synthetic biology aiding biomanufacturing for biomedical devices. Our lab has also been granted Indian patent on 23rd July 2024 for an Immunomodulator in Infection model. European patent for the same is underway. I acknowledge the work of my graduate student Shweta Khandibharad for her dedication and sincerity in performing the tasks assigned to her.

This work carried out in my lab has its significant impact under Pharmaceutical Sciences category.