Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).

Development of Novel Self-Adjuvanting Vaccines Against Tuberculosis. Despite nearly a century of BCG vaccination, tuberculosis remains a significant global health challenge. Clinical trials have highlighted BCG's limited efficacy against pulmonary tuberculosis, particularly in endemic regions. Factors like non-tuberculous mycobacteria, environmental influences, and helminth infections may contribute to BCG's inefficacy. Recent research underscores that the presence of non-tuberculous mycobacteria and latent *Mtb* infections can hinder BCG's ability to induce lasting protective immunity due to antigen processing issues. This suggests that vaccines relying on extensive antigen processing might not work well in TB-endemic areas. As a result, innovative vaccination strategies are urgently needed in high TB-burden countries. To address this challenge, novel chimeric vaccines (L91, L4.8) have been developed by us, by incorporating promiscuous peptides from TB10.4 and 16 kDa antigens of *Mtb*, linked to a TLR-2 ligand (Pam3Cys). These lipidated vaccines do not require extensive antigen processing and generate enduring Th1 memory response. This is evidenced by the fact that vaccines significantly improved the activation, proliferation, and generation of protective T cells. Furthermore, vaccines surmount the barrier of major histocompatibility complex polymorphism and induce better protection than BCG. This peptide has self-adjuvanting properties and activates dendritic cells. Importantly, vaccines activate T cells isolated from purified protein derivative-positive healthy volunteers and TB patients.

This vaccine differs significantly from BCG and holds the potential for successful application in both TB-endemic and TB-non-endemic populations. The uniqueness of this vaccine lies in the following aspects:

a. It possesses self-adjuvanting properties owing to the inclusion of a TLR-2 ligand, eliminating the need for additional adjuvants in the immunization process.
b. The vaccine can be effectively targeted to DCs due to their high expression of TLR-2.
c. TB10.4 is prominently expressed during active TB, while Acr1 is prevalent during latency. This characteristic ensures the vaccine's effectiveness against both active and latent TB.
d. The vaccine does not necessitate extensive antigen processing as it can directly bind to both MHC I and MHC II.

The vaccine triggers a persistent Th1 memory response. This is demonstrated by its notable enhancement of T cell activation, proliferation, and the generation of protective T cells. Moreover, the vaccine overcomes the challenge of major histocompatibility complex polymorphism and provides better protection compared to BCG. Significantly, the vaccine robustly activates T cells from healthy volunteers who tested positive for purified protein derivative but did not respond to BCG. In essence, **this vaccine holds promising potential as a future vaccine candidate against tuberculosis, as indicated by various scientific publications and patents and ICMR involvement in supporting clinical trials** [**United States Patent No. 9340622, J Biol Chem. 2022, J Proteome Res. 2020, BMC Infect Dis. 2020, J Trans Med. 2017, 2018, Front Microbiol. 2017, Sci Rep 2016, Clin Exp Immunol. 2015, Crit Rev Microbiol. 2015, Amino Acids. 2014, Indian J Med Res. 2013, BMC Immunol. 2012, J Infect Dis. 2011, Trends Mol Medicine 2012, Crit Rev Microbiol. 2011, Amino Acids. 2010, J Proteome Res. 2008**].

Bolstering BCG Efficacy by Expressing Memory-Enhancing Cytokines IL-7 And IL-15 to Elicit Long-Lasting Memory T Cells and Protection against *Mycobacterium tuberculosis*. BCG provides childhood protection against TB but not in adulthood, suggesting it lacks the ability to induce lasting immune memory. IL-7 and IL-15 are recognized for enhancing T cell memory. We vaccinated mice with BCG supplemented with IL-7 + IL-15, later challenging them with *Mtb* after 240 days. The combination led to significantly increased CD4 and CD8 T cell memory and protective response compared to BCG alone. T cell proliferation and expansion of Th1 cells were boosted, along with an augmented pool of multifunctional *M. tuberculosis*–specific memory T cells. This approach led to reduced mycobacterial burden and lung pathology. These findings suggest that adding IL-7 and IL-15 to the BCG vaccine could enhance its effectiveness by bolstering T cell memory response. [**J Infect Dis.** 2010, **PLoS One** 2011]. **Currently, we have generated recombinant BCG by transfecting it with memory T cell-enhancing cytokines IL-7 and IL-15 and examining its protective efficiency against *Mtb***.

Potent Role of Vaccines Prepared from Macrophages Infected with Live Bacteria in Protection Against *Mycobacterium tuberculosis* and *Salmonella typhimurium* Infections. Around a third

of the global population carries *Mtb*, yet only a small percentage (5%–10%) develop active tuberculosis, while the rest (90%–95%) acquire effective immunity. This indicates that those infected with *Mtb* develop stronger and longer-lasting protective immunity. An intriguing theory is that active bacilli might produce distinctive molecules within macrophages, fostering *Mtb*–specific effector and memory T cells crucial for protection. This suggests that *Mtb* protective antigens are secreted within macrophages by the bacterium. Using this concept, we have pursued vaccine development against TB and typhoid. We cultivated live *Mtb* and *Salmonella typhimurium* within macrophages, later eliminating bacteria through drug treatment and gamma irradiation. This approach worked successfully in eliciting protective T-cell immunity and significantly decreased mortality of mice challenged with live *Mtb* and S. *typhimurium* [**J Infect Dis.** 2004, **US Patent** *6783765*, 2004].

Deciphering the Structural Enigma of HLA Class-II Binding Peptides for Enhanced Immunoinformatics-based Prediction of Vaccine Epitopes. The available *in silico* predictors of human leukocyte antigen II (HLA-II) binding epitopes are sequence-based techniques, which ostensibly have balanced sensitivity and specificity. Structural analysis and understanding of the cognate peptide and HLA-II interactions are essential to empirically derive a successful peptide vaccine. However, the availability of structure-based epitope prediction algorithms is inadequate compared with sequence-based prediction methods. We have attempted to understand the structural aspects of HLA-II binders by analyzing the Protein Data Bank (PDB) complexes of pHLA-II. Furthermore, we mimic the peptide exchange mechanism and demonstrate the structural implication of an acidic environment on HLA-II binders. Finally, we discuss a structure-guided approach to decipher potential HLA-II binders within an antigenic protein. This strategy may accurately predict the peptide epitopes and thus aid in designing successful peptide vaccines [**J Proteome Res.** 2020, **J Proteome Res.** 2008, **Front Immunol. 2017, Amino Acids** 2014, **BMC Immunol**. 2012, **Expert Rev Proteomics** 2009, **Amino Acids** 2010].

A novel immunosuppressive role of Caerulomycin. Our group has been trying to identify the impact of microbes isolated from the environment of different niches of India and the gut and other organs of human beings for immunosuppressive, anti-TB and anti-cancer activities. In this connection, we isolated an immunosuppressive molecule secreted by the novel species of

actinomycetes *Actinoalloteichus spitiensis*. On structure elucidation, the molecules were found to be 'Caerulomycin A'. For the first time, we deciphered the immunosuppressive role of Caerulomycin A. It showed better immunosuppressive function than cyclosporin, rapamycin and FK506. Caerulomycin A treatment improved the acceptance of skin allografts in the experimental model of transplantation **[Transplantation** 2014, **PloS One** 2014]. Further, we demonstrated the therapeutic role of Caerulomycin A in the regression of asthma and arthritis symptoms. Regulatory T cells (Tregs) responsible for the generation of peripheral tolerance are under the tight regulation of the cytokine milieu. We reported a novel role of Caerulomycin A, in inducing the generation of Tregs. It was observed that Caerulomycin A considerably augmented the percentage of Tregs, as evidenced by an increased frequency of $CD4^+$ $Foxp3^+$ cells. In contrast, it significantly suppressed the number of Th1 cells and Th17 cells. The mechanism deciphered indicated that Caerulomycin A interfered with IFN-γ-induced STAT1 signaling by augmenting SOCS1 expression. An increase in the TGF-β-mediated Smad3 activity was also noted. Furthermore, Caerulomycin A rescued Tregs from IFN-γ-induced inhibition. These results were corroborated by blocking Smad3 activity, which abolished the Caerulomycin A -facilitated generation of Tregs. In essence, the results propose a novel role of Caerulomycin A in inducing the generation of Tregs. This finding suggests that Caerulomycin A has enough potential to be considered a potent future drug for the treatment of autoimmune diseases. [**J Biol Chem.** 2014, **Scientific Report** 2015, **Autoimmunity** 2017]. The findings have been patented [**United States Patent No. 8,114,895]** and the technology has been licensed for 3 million US dollars [INR 24 crore] to Nostrum, a USA-based Pharma Company**.**

Immunomodulation and Host-Directed Therapies (HDT). Despite of tremendous scientific efforts to control infectious diseases, they continue to inflict one in four deaths. Consequently, novel treatment strategies with improved outcomes are urgently required to reduce the high morbidity and mortality. Moreover, drug therapy always has a high risk of side effects on the host and the emergence of drug-resistant strains of the bacteria.

Recent insights into host-pathogen interactions are leading to the identification and development of a wide range of host-directed therapies, which are becoming viable adjuncts to standard antimicrobial treatment. Host-directed therapies involve the modulation of specific host immune pathways to bolster immunity against pathogens (**Crit Rev Microbiol. 2017**). Consequently, we are studying the host molecules that play a cardinal role in reinforcing

innate and adaptive immunity against pathogens. The C-type lectin receptor CLEC4E and Toll-like receptor TLR-4 expressed by host cells are among the first line of defense in encountering pathogens. Therefore, we exploited signaling of macrophages through CLEC4E in association with TLR-4 agonists (C4.T4) to control the growth of *Mycobacterium tuberculosis (Mtb)*. We observed significant improvement in host immunity and reduced bacterial load in the lungs of *Mtb*-infected mice and guinea pigs treated with C4.T4 agonists. Further, intracellular killing of *Mtb* was achieved with a 10-fold lower dose of isoniazid or rifampicin in conjunction with C4.T4 than the drugs alone. This study suggests a unique host-directed-immunotherapeutic approach involving CLEC4E in conjunction with TLR4 to restrict the survival of *Mtb* through autophagy (**Autophagy 2020, Autophagy 2021**).

Chronic infections result in T-cell exhaustion, a state of functional unresponsiveness. To control the infection, it is important to salvage the exhausted T cells. In this study, we delivered signals through Toll-like receptor 2 (TLR-2) to reinvigorate functionality in chronically activated Th1 cells. TLR-2 signaling bolstered the ability of chronically stimulated Th1 cells to activate B cells, and reduced lung pathology in the chronic infection model of tuberculosis. These data demonstrated the importance of TLR-2 in rescuing chronically activated Th1 cells from undergoing exhaustion. This study will pave the way for targeting TLR-2 in developing therapeutic strategies to treat chronic diseases involving loss of Th1 cell function (**J Infect Dis. 2015; Crit Rev Microbiol. 2017**).

Tuberculosis (TB) treatment is lengthy and inflicted with severe side effects. Here, we attempted a novel strategy to reinforce host immunity through NOD-like receptor (NOD-2) and Toll-like receptor (TLR-4) signaling in the murine model of TB. Intriguingly, we noticed that it not only bolstered immunity but also reduced the dose and duration of rifampicin and isoniazid therapy increased the intracellular killing of *Mycobacterium tuberculosis (Mtb)*. Additionally, NOD-2 and TLR-4 signaling reinforces the efficacy of dendritic cells and reduces the dose of TB drugs against *Mycobacterium tuberculosis*. We infer that the signaling through NOD-2 and TLR-4 may be an important approach to reducing the dose and duration of the drugs to treat TB. (**ACS Infect Dis. 2021, J Innate Immunity 2016**).

We have elegantly demonstrated that signalling through the NOD-2 receptor can differentiate bone marrow precursors into dendritic cells with potent bactericidal activity. In addition, signaling through NOD-2 and TLR-4 bolsters the T-cell priming capability of dendritic cells

by inducing autophagy. (**Sci Rep 2016**; **Front Microbiol. 2016; Sci Rep. 2016**). We noticed that Curdlan Limits *Mycobacterium tuberculosis* survival through STAT-1 regulated nitric oxide production (**Front Microbiol. 2019, Sci Rep. 2019, Front Immunol. 2018, Front Immunol. 2017**). We showed that the stimulation through CD40 and TLR-4 is an effective host-directed therapy against *Mycobacterium tuberculosis* (**Front Immunol. 2016**).

We have discovered that signalling through CD86 can enhance the activation, proliferation and differentiation of B cells [**J Biol Chem**. 2002, **Expert Opin Ther Targets** 2008, **Curr Immunol Rev.** 2007, **PLoS Pathogens** 2012]. We also demonstrated, for the first time, that a distinct regulatory mechanism operates in macrophages and B cells for delivering costimulatory signals to T cells [**J Immunol. 1994, 1998**]. Our work has ascertained the potential role of B7-1 and CD28 costimulatory molecules in immunosuppression in leprosy patients [**Clin Exp Immunol.** 1998]. Our work revealed that resveratrol and curcumin suppress immune response through CD28/CTLA-4 and CD80 costimulatory pathway [**Clin Exp Immunol.** 2007]. Our study also infers that immunization with antigen along with costimulatory molecules may significantly reduce the dose of antigen and can generate a better immune response than antigen alone [**BMC Immunol.** 2006].

Mycobacterium tuberculosis (M. tuberculosis) in latently infected individuals survives and thwarts the attempts of eradication by the immune system. During latency, the Acr1 protein is predominantly expressed by the bacterium. However, whether *M. tuberculosis* exploits its Acr1 in impairing the host immunity remains widely unexplored. Hence, we have investigated the role of Acr1 in influencing the differentiation and function of dendritic cells (DCs), which play a cardinal role in innate and adaptive immunity. Therefore, for the first time, we have revealed a novel mechanism of Acr1 in inhibiting the maturation and differentiation of DCs by inducing tolerogenic phenotype. These DCs displayed decline in their antigen uptake capacity and reduced ability to help T cells. Interestingly, *M. tuberculosis* exhibited better survival in Acr1-treated DCs. Thus, this study provides a crucial insight into a strategy adopted by *M. tuberculosis* to survive in the host by impairing the function of DCs (**J Infect Dis. 2014; Front Immunol. 2017; Clin Exp Immunol**. **2015**).

We demonstrated that *Mtb* utilises its protein MPT64 to manipulate the immune system. When MPT64 interacts with differentiating dendritic cells, they transform into myeloid-derived suppressor cells (MDSCs), hindering immune response. These MDSCs suppress

inflammation and immune activity while promoting regulatory T cells (Tregs). Metabolically altered, MDSCs become less effective at engulfing *Mtb*, providing a safe haven for the pathogen. This mechanism involves increased methylglyoxal production by MPT64 and aids *Mtb* survival by differentiating DCs from MDSCs and limiting their effectiveness against the pathogen (**Cell Mol Life Sci 2022**). Furthermore, truncated haemoglobin, HbN, is post-translationally modified in *Mycobacterium tuberculosis* and modulates host-pathogen interactions during intracellular infection (**J Biol. Chem. 2013**). Furthermore, Mycobacterium tuberculosis modulates macrophage lipid-sensing nuclear receptors PPARγ and TR4 for survival (**J Immunol. 2012**).

Gut Microbiota and Immunomodulation. The old age-related loss of immune tolerance inflicts a person with a wide range of autoimmune and inflammatory diseases. Dendritic cells (DCs) are the sentinels of the immune system that maintain immune tolerance through cytokines and regulatory T-cells generation. Aging disturbs the microbial composition of the gut, causing immune system dysregulation. However, the vis-à-vis role of gut dysbiosis on DCs tolerance remains highly elusive. Consequently, we studied the influence of aging on gut dysbiosis and its impact on the loss of DC tolerance. We show that DCs generated from either the aged (DCOld) or gut-dysbiotic young ($DC^{Dysbiotic}$) but not young (DC^{Young}) mice exhibited loss of tolerance, as evidenced by their failure to optimally induce the generation of Tregs and control the overactivation of $CD4^+$ T cells. The mechanism deciphered for the loss of DCOld and DCDysbiotic tolerance was chiefly through the overactivation of NF-κB, impaired frequency of Tregs, upregulation in the level of pro-inflammatory molecules (IL-6, IL-1β, TNF-α, IL-12, IFN-γ), and decline in the anti-inflammatory moieties (IL-10, TGF-β, IL-4, IDO, arginase, NO, IRF-4, IRF-8, PDL1, BTLA4, ALDH2). Importantly, a significant decline in the frequency of the Lactobacillus genus was noticed in the gut. Replenishing the gut of old mice with the Lactobacillus plantarum reinvigorated the tolerogenic function of DCs through the rewiring of inflammatory and metabolic pathways. Thus, for the first time, we demonstrate the impact of age-related gut dysbiosis on the loss of DC tolerance. **This finding may open avenues for therapeutic intervention for treating age-associated disorders with the *Lactobacillus plantarum*** (**Aging Cell 2023**).

The intestinal microbiota has been reported to affect the host response to immunotherapy and drugs. However, how it affects the potency of first-line TB drug isoniazid (INH) is largely unknown. We demonstrated that gut microbiota dysbiosis results in the impairment of INH-mediated *Mtb* clearance aggravated TB-associated tissue pathology by suppressing the innate immunity and CD4 T-cell response against *Mtb*. This study shows that the intestinal microbiota may be a crucial determinant in the efficacy of INH to kill *Mtb* and impacts the host immune response against infection. Hence, this work provides an intriguing insight into the potential links between host gut microbiota and the potency of INH. Further, we observed that gut dysbiosis thwarts the efficacy of the vaccine against *Mycobacterium tuberculosis.* Gut microbiota plays a potential role in the induction and regulation of innate immune memory **(Eur J Immunol. 2020; Front Immunol. 2020; Front Immunol. 2019; Crit Rev Microbiol. 2014)**.

Gut microbes interact with immune cells through pattern recognition receptors (PRRs), impacting host immunity. DCs play a crucial role in responding to *Mtb* infection. The gut-lung axis, a potential target in tuberculosis treatment, remains incompletely understood. We found that changes in gut microbes increase susceptibility to tuberculosis. Antibiotic-induced gut imbalance reduced lung mincle receptor expression, aiding *Mtb* survival. The decline in effector T cells and expanded the frequency of Tregs in the lungs. Dysbiotic mice showed low mincle expression on lung DCs, impairing T cell activation and *Mtb* control. Treatment with mincle ligand TDB restored immune function, while Lactobacillus supplementation revived mincle expression, enhancing anti-*Mtb* response. This study suggests gut microbiota shapes lung immunity against *Mtb* via mincle (**Front Immunol. 2016; Front Immunol. 2019**).

Cancer Immunology. Glioblastoma is a highly prevalent and aggressive form of primary brain tumor. Macrophages are one of the major constituents of tumor-infiltrating immune cells in the human gliomas. The role of immunosuppressive macrophages is very well documented in correlation with the poor prognosis of patients suffering from breast, prostate, bladder and cervical cancers. The current study highlighted the correlation between the tumor-associated macrophage phenotypes and glioma progression. We observed an increase in the pool of M2 macrophages in high-grade gliomas. The glioma patients with lower frequency of M2 macrophages and higher percentage of M1 macrophages had better survival. The ratio of M1

and M2 macrophages could be an indicator of the early diagnosis and prognosis of the disease. Furthermore, TLR-3 stimulation skews M2 macrophages to M1 through IFN-αβ signaling and restricts tumor progression (**Cancer Immunol Immunother. 2019; Front immunol. 2018**). Further, we demonstrated that the low prevalence of anti-xenobiotic antibodies among the occupationally exposed individuals is associated with a high risk of cancer (**Cancer Medicine 2019**). Furthermore, we revealed for the first time that the signaling through CD80 can induce apoptosis in B cell lymphomas by augmenting the expression of anti-apoptotic molecules (**J Biol Chem. 2022**; **Expert Opin Ther Targets 2008**).

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