Signed details of the excellence in research work for which the Sun Pharma Research Fellowship is claimed, including references & illustrations (Max. 2.5 MB). The candidate should duly sign on the details.

Development of vaccine against substances of abuse. Substance abuse, especially opioid addiction, is a growing crisis in Punjab, with even children falling victim to drug use. This problem is not confined to any region; it is a global issue affecting every country. Opioid Use Disorder (OUD) is a widespread condition characterized by compulsive opioid consumption despite harmful consequences, affecting approximately 35 million people worldwide. The COVID-19 pandemic has exacerbated the issue, with a notable rise in alcohol and drug abuse. Morphine is a potent opioid that is often used for pain management but poses a high risk for addiction. Prolonged morphine use impairs immune responses, including T-cell activation and antibody production. It reduces the effectiveness of vaccines by compromising the function of immune cells like macrophages and dendritic cells. Existing opioid addiction treatments, such as methadone and buprenorphine, can also lead to dependency, highlighting the need for new approaches.

Vaccines have successfully eradicated diseases like smallpox and polio, demonstrating their potential to combat various health challenges. Given this, researchers are exploring vaccines as a solution for drug addiction. Recent studies have shown that anti-heroin vaccines can prevent withdrawal symptoms in non-human primates, marking progress toward a viable vaccine for addiction.

However, current vaccines face limitations in neutralizing opioids in the bloodstream and often require external adjuvants, which can affect the immune response. Nanoparticle-based vaccines offer significant advantages over traditional methods, such as increased antigen stability, targeted delivery, and enhanced immunogenicity without additional adjuvants. These nanoparticles can effectively present antigens to immune cells, making them highly efficient in generating a strong immune response. We have developed a novel vaccine against morphine addiction using nanoparticles that display morphine and Pam3Cys (a TLR-2 agonist) on their surface. This innovative vaccine harnesses the immune-stimulating properties of carrier protein Acr1, derived from *Mycobacterium tuberculosis*, to activate T-cells to help B cells to produce high-affinity anti-morphine antibodies. In experimental models, the vaccine effectively neutralizes morphine, reduces addiction-related gene expression, and increases memory T-cells

and B-cells, suggesting its potential to provide long-lasting immunity against morphine addiction [**Int J Biol Macromol. 2024, Eur J Pharmacol. 2024, Int Immunopharmacol. 2023, J Biosci. 2023, Patent application No. 202311081474** dated 30/11/2023]. Given the rising drug addiction rates and the limitations of current treatments, our vaccine represents a promising new strategy to combat opioid addiction. It offers a potential prophylactic measure that could significantly impact public health by reducing dependency on opioids and improving the quality of life for affected individuals.

Age-related gut microbiota dysbiosis disrupts dendritic cell tolerance. Age-related loss of immune tolerance predisposes individuals to various autoimmune and inflammatory diseases. Dendritic cells (DCs) are critical immune system sentinels, maintaining immune tolerance by promoting regulatory T-cell (Treg) generation and cytokine balance. However, aging disrupts the gut microbiota, leading to immune dysregulation, yet the direct impact of gut dysbiosis on DC tolerance remains largely unexplored. In our study, we investigated the influence of aging on gut dysbiosis and its subsequent effect on DC tolerance. We found that DCs derived from aged mice (DC^{old}) or young, gut-dysbiotic mice ($DC^{dysbiotic}$) showed a marked loss of tolerance compared to DCs from young, healthy mice (DC^{young}). This loss of tolerance was evidenced by a reduced ability to induce Treg generation and an inability to control $CD4^+$ T cell over-activation. Mechanistically, the diminished tolerance of DC^{old} and $DC^{dysbiotic}$ was associated with NF-κB over activation, reduced Treg frequency, increased levels of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α, IL-12, IFN-γ), and decreased anti-inflammatory mediators (IL-10, TGF-β, IL-4, IDO, arginase, NO, IRF-4, IRF-8, PDL1, BTLA4, ALDH2). Notably, there was a significant reduction in the frequency of Lactobacillus in the gut of aged mice. Remarkably, supplementation with *Lactobacillus plantarum* restored the tolerogenic function of DCs by modulating inflammatory and metabolic pathways. **Our findings, for the first time, highlight the impact of age-related gut dysbiosis on the loss of DC tolerance, suggesting a novel therapeutic avenue for age-associated disorders through the use of *Lactobacillus plantarum* (Aging Cell, 2023, Gut Microbes 2023)**.

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 show that the intestinal microbiota may be a

crucial determinant in 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 potency of INH. Further, we observed that the gut dysbiosis thwarts the efficacy of vaccine against *Mycobacterium tuberculosis.* Gut microbiota plays 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 key 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 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 that gut microbiota shapes lung immunity against *Mtb* via mincle (**Front Immunol. 2016; Front Immunol. 2019**).

Development of novel 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) [**J Biol Chem. 2022, J Trans Med. 2017, 2018, J Infect Dis. 2011, Trends Mol Medicine 2012, Crit Rev Microbiol. 2015**].

This vaccine differs significantly from BCG and holds 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 require extensive antigen processing as it can directly bind to 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 than 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 (J Biol Chem. 2022, J Trans Med. 2017, J Trans Med. 2018, J Infect Dis. 2011, Ind J Med Res. 2013, Sci Rep 2016, United States Patent No. 9340622).**

BCG provides childhood protection against TB but not in adulthood, suggesting it cannot 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*.

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 successfully elicited protective T-cell immunity and significantly decreased mortality of mice challenged with live *Mtb* and S. *typhimurium* [**US Patent** *6783765*].

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].

<u>*Exploiting microbes for human welfare*</u>. Our group has been trying to identify the impact of microbes isolated from the environment of different niches of India and 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 was found to be

'Caerulomycin A'. For the first time we deciphered the immunosuppressive role of Caerulomycin A. It showed better immunosuppressive function than the 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 generating 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 a potent future drug for treating autoimmune diseases. [**J Biol Chem.** 2014, **Scientific Report** 2015, **Autoimmunity** 2017]. The findings have been patented [**United States Patent No. 8,114,895]**. **The technology was licensed for 3 million US dollars [INR 24 crore] to Nostrum, a USA-based Pharma Company.**

Immunomodulation and Host-Directed Therapies (HDT). Despite 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 identification and development of a wide range of host-directed therapies, which are becoming viable adjuncts to standard antimicrobial treatment. Host-directed therapies involves modulation of specific host immune pathways to bolster the immunity against the pathogens (**Crit Rev Microbiol. 2017**). Consequently, we are studying the host molecules that play a cardinal role in reinforcing the innate and adaptive immunity against the 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, Eur J Pharmacol. 2024**).

Chronic infections result in T-cell exhaustion, a state of functional unresponsiveness. It is important to salvage the exhausted T cells to control the infection. 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, Int J Biol Macromol. 2023**).

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, increasing 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 reduce the dose and duration of the drugs to treat TB. (**ACS Infect Dis. 2021, J Innate Immun. 2016**).

We have elegantly demonstrated that signalling through NOD-2 receptor can differentiates bone marrow precursors to 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 costimulatory molecules can enhance antigen presentation cells activation, proliferation and differentiation. Costimulatory molecules expressed on the surface of various cells play a decisive role in the initiation and sustenance of immunity. Exploitation of the "code of conduct" of costimulation pathways provides evolutionary incentive to the pathogens and thereby abates the functioning of the immune system. Mtb, HIV, Leishmania sp., and other pathogens manipulate costimulatory molecules to establish chronic infection. Impairment by pathogens in the signaling events delivered by costimulatory molecules may be responsible for defective T-cell responses; consequently organisms grow unhindered in the host cells. The pathogens employ to tune and tame the immune system using costimulatory molecules. Studying host-pathogen interaction in context with costimulatory signals unveil the molecular mechanism that will help in understanding the survival/death of the pathogens. We emphasize that the very same pathways can potentially be exploited to develop immunotherapeutic strategies to eliminate intracellular pathogens. [**PLoS Pathogens** 2012].

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 a 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* utilizes 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 to MDSCs and limiting their effectiveness against the pathogen (**Cell Mol Life Sci 2022**). Furthermore, truncated hemoglobin, 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**).

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 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 a lower frequency of M2 macrophages and a higher percentage of M1 macrophages had better survival. The ratio of M1 and M2 macrophages could indicate 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 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**).

Local recurrence after surgery for early-stage triple-negative breast cancer is a significant challenge. To prevent the regrowth of residual tumors, we developed an autologous therapeutic hybrid fibrin glue for intra-operative use. This glue utilizes autologous serum proteins to stabilize high drug-loaded lapatinib-NanoSera (Lap-NS; ~66% L.C.) and imiquimod-

MicroSera (IMQ-MS; ~92% L.C.). Additionally, plasmonic nanosera (PNS) with a ~67% photothermal conversion efficiency was created for enhanced treatment. While Lap-NS or PNS alone reduced tumor regrowth, combining them with IMQ-MS boosted immunogenic cell death and immune cell infiltration at the surgical site. This localized combination immunotherapy using the Nano-MicroSera-based fibrin implant demonstrated superior tumor control and survival, showing strong potential for clinical use **(Nanoscale 2024)**.

Total citations: 16050

H-index: 40

i-index: 98

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3.	Nanoscale	2024	5.8
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5.	Int J Biol Macromol	2023	8.05
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