

d. Signed Details of the Research Work of Dr Sangita Mukhopadhyay for which the Sun Pharma Science Foundation Research Fellowships, 2024 (in Medical Sciences – Basic Research) is claimed

The major goals of Dr Sangita Mukhopadhyay's group are to decode the intricate cell signaling networks that are important for macrophage activation and subsequent regulation of T cell immune responses and to elucidate how expression of various genes are regulated/modulated leading to establishment of diseased states, mainly Inflammation and Tuberculosis. Another area where she has focused is to repurpose *Mycobacterium tuberculosis* (Mtb) protein/peptide as anti-inflammatory therapeutic in the treatment of diseases like sepsis and tissue injury/IBD that are associated with extreme inflammation.

She had contributed in detailed to understand the signaling cascades that play a crucial role in regulating infection (tuberculosis [TB]) and inflammatory responses. She is one of the pioneer scientists in the area of Immunology and Infection Biology. While working on tuberculosis to understand how various proteins of *Mycobacterium tuberculosis* (Mtb) modulate immune responses of the host, she identified various important signaling pathways that regulate immune responses that play crucial roles not only in the pathogenesis of tuberculosis but also in establishment of other various human disorders associated with inflammation like tissue injury, wound healing, sepsis and cancer. She designed strategies and host-directed therapeutics to inhibit or modulate these signaling pathways to tackle *M. tuberculosis* infection as well as to manipulate inflammatory signaling to cure these human disorders. She has worked especially on the Mtb intriguing PE/PPE family proteins of Mtb like PE11, PPE18 and PPE2 and ESAT-6 proteins to understand their virulence properties and established that these are potential drug targets (Figure 1). Importantly, based on the immunomodulatory properties of some of these proteins, Dr Mukhopadhyay has made seminal contributions in translational research leading to filing of several patents to use these proteins and synthetic peptide from these proteins as therapeutics to treat some inflammatory disorders like inflammation/tissue injury, wound healing and sepsis. **Thus, she identified a new area of repurposing Mtb proteins as new generation therapeutic agents towards biomedical applications (Granted Indian patent [2023]; Filed Indian Patent[2020]; Pal et al[2022]EMBO Molecular Medicine and Ahmed et al[2018]Journal of Immunology).** Data generated from her research work have important clues towards development/designing of novel drugs and/or immunomodulatory agents with therapeutic potential against tuberculosis and inflammatory diseases. [She is involved for commercialization of her innovations through Industry-Academy collaboration with NC TRAC and BCIL](#)

Her main research interests include

- [a. Cell signaling and Signal transduction in macrophages](#)
- [b. TLRs, Innate and Adaptive immunity and regulation of inflammatory signaling](#)
- [c. Host-pathogen interaction in tuberculosis and Identification of novel drug targets and Designing of therapeutic interventions against Mtb](#)
- [d. Repurposing Mtb proteins/peptides as new generation therapeutics towards biomedical applications, mainly to treat health disorders with extreme inflammation like sepsis, wound healing, inflammatory bowel disease and cancer.](#)

In this research direction, Dr Mukhopadhyay has published 73 research papers in high impact factor peer reviewed International Journals and one USA patent has been granted. She has filed 4 more Indian patents. This Scientific contribution is well recognized by the 'American Society of Hematology (ASH), USA, 2007' and 'The American Association of Immunologists (AAI), USA, 2011' by electing me as member of

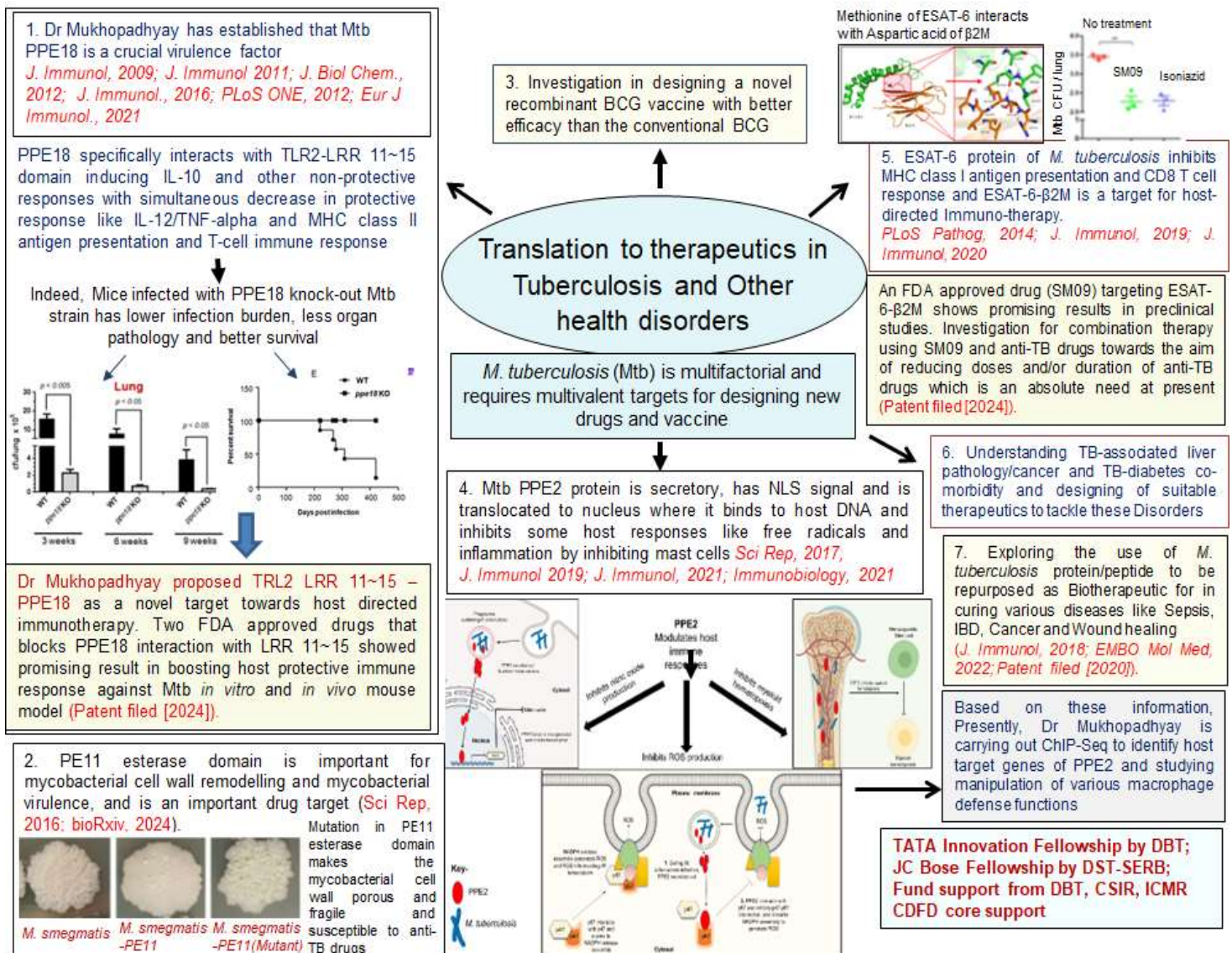
these prestigious societies and by ‘The National Academy of Sciences of India (NASI)’, 2010’; ‘The Indian Academy of Sciences, Bangalore, 2013’; ‘The Indian National Science Academy, New Delhi, 2016’ and ‘The Telangana Academy of Sciences, Telangana, 2016’ by electing me as a ‘Fellow’. Also, she is serving as member of various committees of India like 1) Member of the Subject Expert Committee (SEC) on Life Sciences (LS) of DST, Govt of India, 2) Member of Research Progress Committee of Nirma University, Ahmedabad, Gujarat; 3) Member of the DBT Task Force Committee (Infectious Disease); 4) Member of the Screening Committee Meeting of Twinning R&D program for NER (Medical Biotechnology, DBT); 5) M.Sc Biotechnology Advisory Committee of University of Hyderabad., 6) Member of the Scientific Advisory Committee of NIBMG, Kalyani, West Bengal, 7) DST-SERB PAC committee member, 8) Committee member of INSPIRE Faculty Fellowship Scheme, Life Sciences Biomedical 9) Member of Sectional Committee – IX, INSA, New Delhi, 10) Member of the CSIR Medical Sciences Research Committee, etc. She was also a Special Invitee of the RAP-SAC committee of NCCS, Pune, 2015 and 2016 and Chairperson of various Institutional (CDFS) committees. Also, she served as reviewer of several grant proposals of DBT, Govt. of India and DST, Govt. of India, UPE-II Research Project proposals of University of Hyderabad, Dr. D.S. Kothari Postdoctoral Fellowship, Pune. She also served as peer reviewer of several National and International journals like ‘Indian Journal of Medical Research’, ‘Current Science’, ‘PLoS ONE’, ‘Journal of Biosciences’, ‘Journal of Infectious Diseases’, ‘Genes to Cell’, ‘DNA’ and ‘Cell Biology’, ‘Journal of Medical Microbiology’, Scientific Reports, IUBMB Life etc. and examiner of PhD/M.Sc students of several Universities and Institutes of India and abroad. Dr Mukhopadhyay has been bestowed with many highly prestigious ‘National Awards’ and the TATA Innovation Fellowship, 2017-2018 of DBT and J. C. Bose Fellowship, 2022 and continuous funding supports from various funding agencies like TWAS, Italy, DBT, Govt of India, DST-SERB, Govt of India, CSIR, Govt of India and ICMR, Govt of India. She is involved for commercialization of our innovations through Industry-Academy collaboration with NC TRAC and BCIL. Her research has been highlighted in the Hindu and vigyanprasar Government Web portal (1.<https://www.thehindu.com/sci-tech/health/novel-mechanism-may-lead-to-better-tb-control/article6764393.ece> and 2. <http://vigyanprasar.gov.in/isw/A-new-boost-to-anti-tb-crusade.html>)

Importance of the research work of Dr Mukhopadhyay for the benefit of mankind (Figure 1)

- i) Decoding for the first time the role of the PE/PPE family proteins (PE11, PPE2 and PPE18) and ESAT-6 in the modulation of macrophage immune functions and their involvement in the pathogenesis of *M. tuberculosis*. While the proteins are shown to play a crucial role in suppressing the protective immune responses of the host, PE11 is also shown to play a crucial role in bacterial cell wall remodeling and maintenance of the cell wall architecture. Two FDA approved drugs ESAT-6 and PPE18 are projected to be repurposed for treatment of TB – an approach of host-directed immunotherapy which was supported by TATA Innovation Fellowship [Two Indian patent filed [2024]]
- ii) Identification of a novel cell wall-targeting drug (PE11) for the treatment of TB including drug resistant *M. tuberculosis*.
- iii) The anti-oxidant N-acetyl-cysteine as a novel immunomodulator to boost immune response for control of tuberculosis
- iv) An approach to designing of a recombinant BCG vaccine with improved efficacy
- v) Identification for the first time a broad-spectrum non-steroidal anti-inflammatory biologic, (PPE2 protein /PPE2 peptide) that suppresses mast cell population. This invention provides an attractive therapeutic

alternative to steroid-based anti-inflammatory drugs for the treatment of mast cell-centric health problems as described below.

- A. Application of PPE2 protein and a synthetic peptide derived from PPE2 in the treatment of tissue injury and inflammation which is associated with mast cell activity (Filed Indian patent, 2020; Pal et al[2022]).
- B. Topical application to treat scar-less wound (Filed Indian 2020). The wound healing compound provides safe, non-toxic, non-irritant, pain-free, highly absorbent and cost-effective scar-free wound healing. Recalcitrant wounds like pressure ulcers and diabetic foot ulcers can be better clinically managed using this molecule and studies are underway in collaboration with NC TRAC, Bangalore. This molecule could be useful in plastic surgery or surgeries in non-covered areas like face and hands.
- C. Treatment to alleviate Inflammatory bowel disease (funded by CSIR [37WS(0020)/2023-24/EMR-II/ASPIRE]).
- D. PPE2 peptide to treat Melanoma cancer through Gel-based topical application (project supported by NC TRAC [2021] for commercialization to industry).



Research Contribution: Regulation of immune/inflammatory signaling and designing of therapeutics to tackle tuberculosis and other inflammatory disorders

Research contribution of Dr Mukhopadhyay is categorized in 2 sections as described below.

Section 1. Regulation of inflammatory signaling in macrophages during tuberculosis

Dr Mukhopadhyay for the first time hinted about the role of TLR2 LRR domain in regulation of inflammation and cytokine signaling leading to alteration of immune responses during infection. She observed that *Mycobacterium tuberculosis* (Mtb) PPE18 protein interacts with TLR2 LRR 11~15 domain, and specifically activates the non-protective IL-10 cytokine T-helper (Th) 2-type immune response that favors Mtb to persist successfully inside the host (*Nair et al.[2009]Journal of Immunology, 183:6269; Bhat et al.[2012]PLoS ONE, 7:e52601*). This interaction further results in inhibition of the protective cytokines like IL-12 and TNF-alpha (*Nair et al.[2011]Journal of Immunology, 186:5413*). PPE18 also inhibits MHC class II antigen presentation and the protective CD4 T cell activation (*Dolasia et al.[2020]European Journal of Immunology, 51:603*). Based on these hints, research was initiated to design FDA approved drug that specially block interaction of PPE18 with TLR2 LRR 11~15 domain to prevent induction of non-protective IL-10/Th2 with simultaneous activation of anti-mycobacterial Th1-type response for stimulation of protective immunity against Mtb. Based on this idea, Dr Mukhopadhyay demonstrated that TLR2-LRR 11~15 domain can be novel targets for host-directed therapy and designed two FDA approved drugs that showed promising effects against TB which was supported by TATA Innovation Fellowship by DBT (*Indian patent filed [2024] Application no 202441058271*). Also, she discovered another drug target ESAT6:β2M and identified FDA approved drug to be repurposed as anti-TB drug (*Indian patent filed [2024] Application no. 202441050958*).

While continuing studies on importance of TLR2 signaling in *M. tuberculosis* virulence, Dr Mukhopadhyay has decoded another important contribution that was published in the *Journal of Biological Chemistry* (*Parveen et al.[2013]Journal of Biological Chemistry, 288:24956*) to explain how interaction with TLRs can differently dictates the type of polarization and inflammatory signaling during the innate immune responses of macrophages which is likely to be useful in devising strategies to manipulate macrophage innate responses as well as inflammatory signaling to engineer a host protective immunity against *M. tuberculosis* as well as other TLR-associated diseases targeting the TLR2/4.

Further, it is known that reactive oxygen species (ROS), generated during the innate immune response and inflammation are antimicrobial agents. However, overproduction of ROS ‘per se’ do not kill many pathogens including Mtb, rather are involved in exacerbating disease, which hint that ROS may actually favor pathogen survival by directly suppressing host’s immune responses. Dr. Mukhopadhyay’s research establishes that excess ROS could directly inhibit IL-12 induction as well as Th1 immune response to induce immunosuppression (*Khan et al.[2006]Blood, 107:1513; Khan et al.[2007]Free Radical Biology and Medicine, 42:686*). These may augur well to understand the basis of immunosuppression observed in related diseases like cancer and various diseases associated with excess inflammation like inflammatory bowel disease, wound healing or various intracellular infections where IL-12/Th1-dominated immune response is important for protection. Thus, Dr. Mukhopadhyay's work provides novel cues for cure and prevention of a large spectrum of disorders including infectious, metabolic, and neoplastic diseases. This manuscript highlights the importance of anti-oxidants to boost the immune system to fight against these health disorders (*Alam et al.[2010]Journal of Immunology, 184:2918*).

Section 2. Therapeutic use of PPE2 protein of *Mycobacterium tuberculosis* in treating Inflammation/Tissue injury, Wound healing, Inflammatory bowel disease and Melanoma cancer

2a. PPE2 protein/peptide reduces Inflammation and tissue injury

During the research work of 7-8 years, Dr Mukhopadhyay very specifically has shown that one of the PE/PPE (proline glutamic acid/proline proline glutamic acid) family proteins of *Mycobacterium tuberculosis* (Mtb), PPE2 is a secretory protein having a Nuclear localization signal and DNA binding property (*Bhat et al.[2013]Annals of the New York Academy of Sciences, 1283:97; Bhat et al.[2017]Scientific Reports, 7:39706*). She showed that during infection, PPE2 is secreted by the bacterium (*Bisht et al.[2023]Tuberculosis, 143:102421*) and localizes to the macrophage nucleus by exploiting the classical importin- α/β -dependent import system. Once inside the nucleus, it binds to the promoter region of *inos* (inducible nitric oxide synthase) gene to inhibit transcription from the *inos* promoter by physically masking the GATA-1-binding sites critical for *inos* transcription (*Bhat et al., [2017]Scientific Reports, 7:39706*). iNOS is responsible for production of nitric oxide (NO) which is known to be cytotoxic against the microbes (Chan et al., 2001). Expectedly, PPE2-null mutants caused higher production of nitric oxide in infected macrophages indicating a direct role of PPE2 in inhibiting NO production (*Bhat et al., 2013; Bisht et al., 2023*). In addition to NO, during infection, activated macrophages also generate reactive oxygen species (ROS) which are shown to be cytotoxic against *M. tuberculosis* (Hussain Bhat and Mukhopadhyay. 2015; Pizzolla et al., 2012) and *M. tuberculosis* employs strategies to inhibit ROS production in addition to inhibition of NO to safely persist and multiply inside macrophages. Dr Mukhopadhyay found a novel mechanism by which PPE2 can also directly inhibit ROS production by destabilizing NADPH-oxidase complex in the phagosome. During infection, PPE2 is secreted into the cytoplasm and binds to p67^{phox} subunit of NADPH-oxidase complex via its SRC Homology 3 (SH3) domain. The PPE2-p67^{phox} interaction results in inhibition of translocation of p67^{phox} molecule from cytosol to the membrane leading to reduced NADPH activity and ROS production (*Srivastava et al.[2019]J. Immunology 203:1218*). This results in higher mycobacterial burden in macrophages. Further, in an interesting study, Dr Mukhopadhyay has shown that PPE2 acts in a pleiotropic manner inhibiting mast cell population also (*Pal and Mukhopadhyay, [2021]Immunobiology 226:152051*). Thus, *M. tuberculosis* exploits PPE2 to its own advantage by inhibiting both NO and ROS as well as mast cells and favors survival of the bacilli (*Bisht et al.[2023]*).

NO is known to play a key role in the pathogenesis of inflammation. Large amount of NO is produced at sites of inflammation through the action of *inos* present in both infiltrating leucocytes and activated, resident tissue cells. Nitric oxide and its oxidation products are known to cause tissue injury (Tripathi et al., 2007). Again, ROS generation by polymorphonuclear neutrophils at the site of inflammation is known to cause endothelial dysfunction and tissue injury (Mittal et al., 2014). Also, mast cell plays a crucial role in innate immunity and the role of mast cells is eminent in tissue inflammation (Lee et al., 2002).

Proteins from pathogens have been used as therapeutic for many years. More recently, focus has shifted to exploiting virulence protein factors from bacteria and viruses for therapy of immune related disorders, coronary syndromes etc (Lucas et al., 2009; Ruter and Hardwidge, 2014). Recently, FDA has approved large number of recombinant protein therapeutics to treat clinical problems including autoimmunity/inflammation, infection, cancer and genetic disorders. In comparison with typical small-molecule drugs, proteins are highly specific with lesser side effects and decreased toxicity and is in high demand.

PPE2 was shown to act as an important anti-inflammatory molecule inhibiting both NO and ROS and mast cells that are helpful for the *M. tuberculosis* to create a favorable niche for the bacilli to survive and multiply inside the host, Dr Mukhopadhyay opined that the same properties of PPE2 can be exploited to use PPE2 protein or a synthetic peptide derived from PPE2 as a therapeutic to treat inflammatory disorders like acute and chronic inflammation/tissue injury, wound healing and inflammatory bowel disease and melanoma cancer (*Wound healing and Melanoma cancer projects are supported by NC TRAC, Bangalore for commercialization to industry*).

Dr Mukhopadhyay demonstrated that mice injected intraperitoneally with a single dose of recombinantly purified PPE2 (rPPE2) had significant reduction in formalin induced paw inflammation within 3 hours of formalin injection as compared to paw inflammation in mice treated with PBS alone (Figure 2). Administration of single dosage of rPPE2 also prevented inflammation and tissue damage for later time points (21 days). PPE2 showed its beneficial effect even when injected 48 hours post tissue injury. Levels of various mast-mediators and inflammatory molecules like TNF- α , IL-6, and MPO activity were found to be lower in the paw-tissue of mice treated with PPE2 when compared to the untreated mice with inflammation. rPPE2 at 3 mg/kg showed a better and faster healing than the commercial anti-inflammatory drug, Diclophenac which was unable to heal tissue injury at 3 mg/kg. Higher dose of Diclophenac at 10 mg/kg dose showed liver and kidney toxicity, but PPE2 did not show any liver and kidney toxicity.

PPE2 exerts its anti-inflammatory activity by affecting Fibroblast-mast cell communication. It mainly induces its anti-inflammatory activity by suppressing the mast cell population in the injured tissue and inhibits mast cell degranulation. Interestingly, through PPE2 inhibited mast cells, the commercial anti-inflammatory drug Diclophenac did not show any effect on inhibition of mast cells. The levels of various mast cell mediators like β -hexosaminidase, MCP-3 and Mcpt4 are lower in injured tissue in PPE2 injected mice as compared to PBS control. Bone-marrow derived mast cells (BDMCs) transplantation experiments clearly demonstrated that PPE2 specifically targets mast cells for its anti-inflammatory properties. The stem cell factor (SCF) is required for mast cell proliferation, maintenance, and migration at the site of injury. PPE2 was found to localize to the nucleus of fibroblasts, binds to the SCF promoter, and inhibits SCF transcription (Figure 2). Thus, PPE2 inhibits mast cells by directly inhibiting SCF transcription.

For easy synthesis, better stability and easy cellular delivery, Dr Mukhopadhyay, next designed a synthetic peptide derived from PPE2 which is a 36 amino acid long peptide. Based on the nuclear migration and DNA binding property of PPE2, the PPE2-derived peptide was designed. The PPE2 synthetic peptide showed similar anti-inflammatory property and was able to suppress formalin-induced paw inflammation, redness and swelling in mice. The peptide also suppressed SCF and mast cells population in paw tissue. Mast cells play an important role in pathology caused by inflammation. There are drugs available in the market that suppress mast cells activity by neutralizing one or more mast cell mediators like anti-histamine to lower inflammation but, at present there is no drug available to limit mast cell population. Focusing on mast cell as a whole seems a better solution rather than focusing on its mediators, as the previous strategy will not only be more efficient in curbing inflammation but also be effective for a longer duration. Thus, PPE2 protein or the peptide may be an important non-steroidal biological molecule to be used successfully in the treatment of tissue inflammation. **This work has recently been published in EMBO Molecular Medicine (Pal et al., [2022] EMBO Molecular Medicine, e1489). Patent has also been filed based on this study [Therapeutic composition for Inflammation/Tissue Injury", 2020, Mukhopadhyay S, Pal R and Battu MB. Indian Patent No. 201941000876]).**

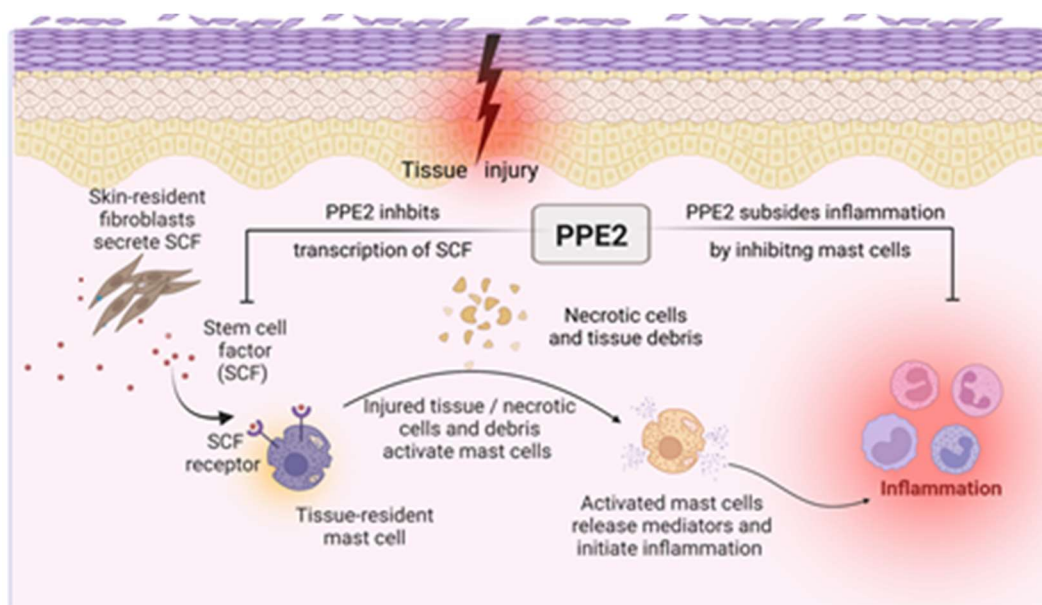


Figure 2. PPE2 reduces injury induced inflammation. PPE2 protein inhibits mast cell population in the site of injury through inhibiting SCF transcription factor from fibroblast, and thus inhibits production of mast cell-induced inflammatory mediators. This results in reduced inflammation in the site of tissue injury.

2b. PPE2 peptide induces faster and scar less wound healing

Next the efficacy of PPE2-peptide to act as a novel therapeutic for wound healing was tested in Dr Mukhopadhyay's laboratory. For creation of dermal wound, 6-8 weeks old male mice were anesthetized and a dermal wound was created using an 8 mm biopsy punch. After creation of wound, PPE2-derived synthetic peptide (*Pal et al., EMBO Molecular Medicine [2022] e14891*) was topically applied to the wound area in formulations of PPE2-GEL (prepared as 1 mg peptide per 1 gram of GEL). After that the wound area were examined for next 18 days. Digital photographs were taken every day and diameter of wounds were measured using vernier caliper. It was observed that, topical application of PPE2 peptide-GEL in external exposed wound yielded an early closure of the wound with no visible scar as compared to vehicle control or standard commercially available ointment (EGF-GEL) (**Figure 3A**). Very interestingly, Histopathological analysis of the healed skin has shown development of new dermal glands and blood vessels in PPE2-GEL (**Figure 3B**) unlike vehicle control and EGF-GEL implicating a regenerative healing of the wounds mainly in PPE2-GEL treated mice. Thus, development of the dermal glands and new blood vessels were more pronounced in the PPE2 peptide-GEL group, whereas none of these structures were observed in the vehicle or EGF-GEL (commercially available GEL) treated group. Same skin tissue samples were next stained with toluidine blue for estimation of the mast cell population. PPE2-GEL group showed a significant reduction in the mast cell population than vehicle and EGF-GEL (**Figure 3C**). Same skin samples were also tested for collagen deposition by Immunofluorescence and it was found that there was a lesser deposition of collagen type 1 in epidermis and dermis in PPE2-GEL group unlike vehicle and EGF-GEL group (**Figure 3D**) and therefore have minimum visible scar (*Filed Indian patent [2020] Patent No. 201941000876*).

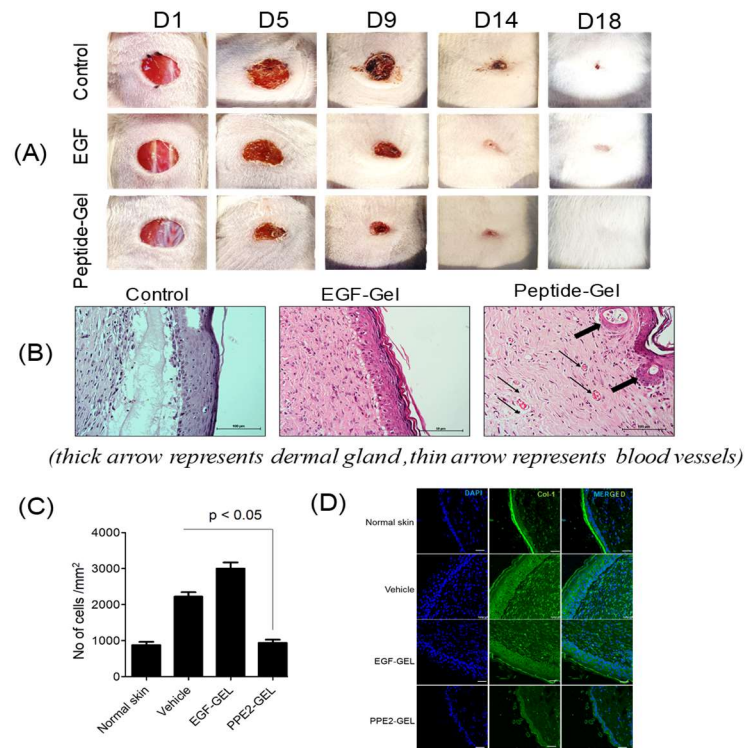


Figure 3. PPE2-GEL formulation promotes an early and scar less wound healing. (A-E) Wounds were created in the dorsal skin under sterile conditions using 8 mm biopsy punch. After creation of wounds, BALB/c mice were either left untreated or treated with either EGF-GEL (commercial GEL) or PPE2-peptide-GEL via topical application. (A) Representative digital pictures were taken at day 1, day 5, day 9, day 14 and day 18 post creation of wound. (B) After 18 days, mice were sacrificed and section of healed skin were prepared. These skin samples were stained with hematoxylin and eosin. And photographs of representative sections were visualized at 40X magnification. (C) The skin samples were stained with toluidine blue and counting of mast cells was performed in toluidine blue stained skin samples. Data shown are mean \pm SD of 5 mice per group. (D) Representative images of the skin samples stained for Collagen-1 visualized at 40X in a Leica multicolor SP8 confocal microscope.

2c. PPE2-peptide alleviates the severity of Inflammatory Bowel disease symptom

In order to test whether PPE2 protein-derived synthetic peptide could protect mice from colitis pathology, next, mice were injected with PPE2 peptide in PBS (8 mg/kg, intraperitoneally) at alternative days for four days while mice were under 3-5% dextran sulphate sodium (DSS) in drinking water as the only source of water. Mice administered with DSS showed exacerbated colitis pathology at day 11 characterized by reduced body weight as well as shortening of the colon (Figure 4), and loss of integrity of intestinal lining (histological examination) as well as crypt loss (Figure 4). The H & E staining of intestinal tissue indicated that DSS-treated mice showed decreased goblet cells, aberrant crypts, and edema. Also, the DSS-treated mice had blood in their stools and had diarrhea. Administration of PPE2-derived peptide (intraperitoneal injection for alternate days for 4 times) could rescue these pathologies in DSS-treated mice almost to the control level (Figure 4). PPE2 peptide inhibited inflammatory markers like IL-6, IL-1 β , TNF- α and inflammatory molecules and mast cells. All the DSS-injected mice died within 9 days whereas all mice administered with PPE2 peptide survived for a long time indicating that PPE2-peptide treatment induced marked protection from the DSS-mediated colitis pathology.

2d. PPE2 peptide had anti-tumorigenic effects in melanoma tumor

Tumors were induced in C57BL/6 mice of 6-8 weeks using B16-F10 melanoma cells. When tumor reached 75 mm³, topical application of PPE2-Gel was carried out every day for three weeks. The control group

received Gel alone. Animals were sacrificed and tumors were collected. The data indicate that the total tumor volume and weights were significantly reduced in the PPE2 peptide-treated mice in comparison to control mice left untreated (Figure 4). It could be observed that PPE2 could penetrate inside the skin when applied topically as PPE2-Gel. Dr Mukhopadhyay has shown that PPE2 peptide specifically inhibited cell cycle progression of melanoma cancer cells like B16-F10 (mice) and A375 and SK-Mel-2B (Human) without affecting normal cells like MEF. RNA-Seq data indicated that most of the genes that promote cell cycle progression (like Ki67, Top2a) were downregulated which could be due to the ability of PPE2 peptide to bind to specific gene promoter due to the presence of NLS signal and DNA-binding epitope in PPE2 peptide. Also, mast cells were low in PPE2-treated group. Histo-morphological examination of tumor tissues showed that interstitial space between the tumor cells in the untreated mice were less in comparison to PPE2-treated mice. Also, the tumor cells in the untreated mice were poorly differentiated in comparison the treated mice that were well differentiated. Based on the promising results obtained in Melanoma cancer, Dr Mukhopadhyay is also approaching application of PPE2 peptide in the treatment of other cancers like colon cancer and lung cancer.

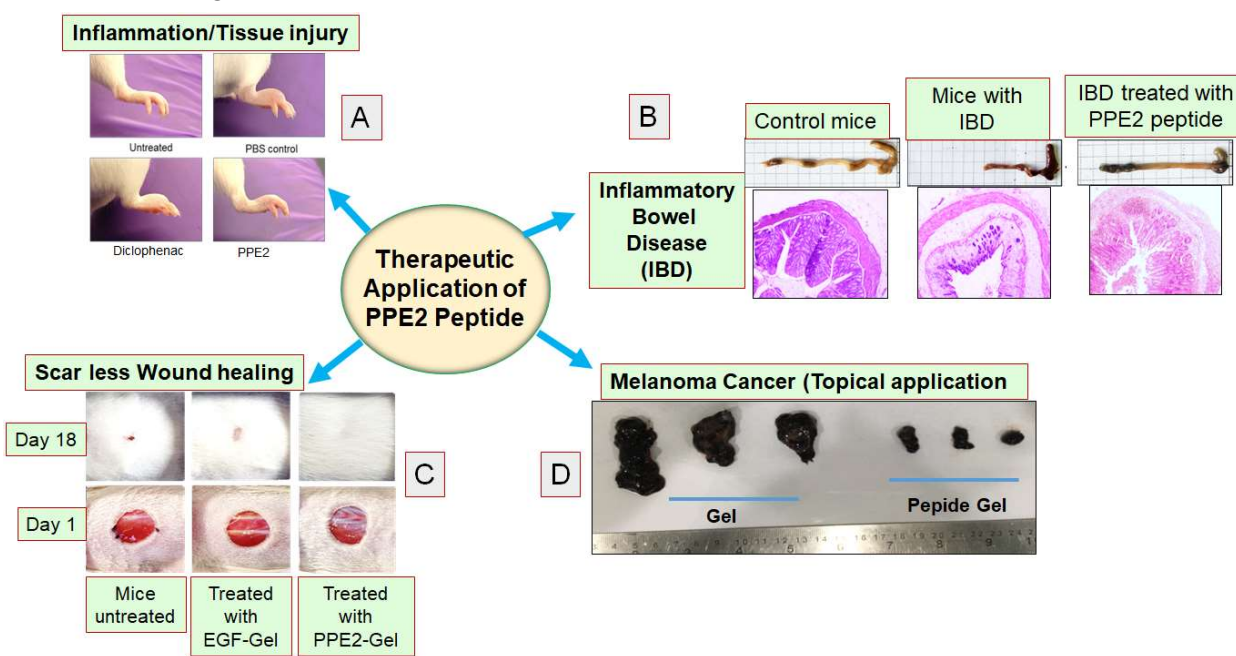


Figure 4. Therapeutic application of PPE2 peptide in the treatment of health disorders associated with extreme inflammation observed in situations such as Inflammation/Tissue injury, Wound healing, Inflammatory bowel disease and Melanoma cancer. A. PPE2 peptide injected intraperitoneally at a single dose reduces chemical-induced tissue inflammation and injury in mice. B. Also when injected intraperitoneally, PPE2 reduces IBD symptoms in mice induced by DSS like rescuing of i) colon length shortening (upper panel) and ii) other pathological parameters like decreased goblet cells, aberrant crypts, and edema (lower panel). C. Topical application of PPE2-Gel for alternate days causes scar less wound healing min mice by day 18. D. Again topical application of PPE2-Gel causes reduced tumor growth in melanoma cancer. The anti-inflammatory and inhibition of mast cell properties of PPE2 peptide are responsible for its pleiotropic therapeutic effects in management of diseases associated with inflammation.

Importance of Research Contributions: There are no commercial drug/molecule to inhibit mast cell population. Commercial anti-inflammatory drugs are often associated with undesirable side effects when used for a longer duration. Dr Mukhopadhyay has identified a novel biologic from Mtb that reduces mast cell population and the mast cell-driven inflammatory response in chemically injured tissues as well as in wound healing (when applied topically) (*Filed Indian patent[2020]*). Since mast cells are one of the sentinel cells that sense tissue aberration, their depletion would prevent mast cell-associated aggressive inflammation and is likely to stop associated damage. Efficacy of drugs available for neutralization of one

or a few mast cell mediators are often limited due to a lack of cell specificity and selective suppression of mast cells can provide better and broad-spectrum relief of excessive inflammation. Dr Mukhopadhyay discovered PPE2 protein/peptide as a broad-spectrum non-steroidal anti-inflammatory drug which is mainly a mast cell inhibitor as well as inhibitor of NO and ROS that play crucial role in triggering various diseases like inflammation/tissue injury, wounds/ulcer, inflammatory bowel disease and melanoma cancer. This invention provides a therapeutic composition for the treatment of Inflammation/tissue Injury, Wound healing, Inflammatory bowel disease and Melanoma cancer. **Though recent studies highlight application of microbial proteins/peptides in biomedical research (Invited review submitted to Trends in Molecular Medicine), there are not promising clues to repurpose Mtb proteins/peptides. Dr Mukhopadhyay for the first time highlight two Mtb proteins, PPE18 (for sepsis, Indian patent granted, 2023 and Ahmed et al[2018]J. Immunol) and PPE2 to be repurposed in therapy against inflammation. Dr Mukhopadhyay published this promising invention on PPE2 in *EMBO Molecular Medicine* as well as filed Indian patent. Also, approach is initiated with NC TRAC, Bangalore and BCIL, New Delhi for commercialization of PPE2 peptide for its therapeutic application (Gel-based topical application) to treat Wound healing and Melanoma cancer. PPE2 peptide-GEL will also be useful in plastic surgery or surgeries in non-covered areas like face and hands. Research is planned to use PPE2 peptide in management of recalcitrant wounds like Pressure ulcers and Diabetic foot ulcers.**

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Date 23.08.2024; Place: Hyderabad


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