In order of Importance, list of 10 best papers of the applicant highlighting the important discoveries/contributions described in them briefly (Max. 1 MB)

1. Dhiraj Kumar Singh, Ashima Bhaskar, Isha Pahuja, Aishwarya Shaji, Barnani Moitra, Yufang Shi, **Ved Prakash Dwivedi**, Gobardhan Das. Co-treatment with Clofazimine and Rapamycin eliminates drug-resistant tuberculosis by inducing polyfunctional central memory T cell responses. **J Infect Dis. 2023 Jun 8:jiad214. Online ahead of print.** **Co-Corresponding author**

   Importance: *Mycobacterium tuberculosis* (*M.tb*), the causative agent of tuberculosis (TB), is acquiring drug resistance at a faster rate than the discovery of new antibiotics. Therefore, alternate therapies that can limit the drug resistance and disease recurrence are urgently needed. Emerging evidences indicate that combined treatment with antibiotics and an immunomodulator provides superior treatment efficacy. Clofazimine (CFZ) enhances the generation of T central memory (T_{CM}) cells by blocking the $Kv1.3^{+}$ potassium channels. Rapamycin (Rapa) facilitates M.tb clearance by inducing autophagy. In this study, we observed that the co-treatment with CFZ and Rapa potently eliminates both multiple and extensively drug-resistant (MDR and XDR) clinical isolates of *M.tb* in a mouse model by inducing robust T cell memory and polyfunctional T_{CM} responses. Furthermore, co-treatment reduces the expression of latency-associated genes of M.tb in human macrophages. Therefore, CFZ and Rapa co-therapy holds promise for treating patients infected with MDR and XDR strains of *M.tb*.

2. Isha Pahuja, Akanksha Verma, Antara Ghoshal, Suparba Mukhopadhyay, Anjna Kumari, Aishwarya Shaji, Shivam Chaturvedi, **Ved Prakash Dwivedi**, Ashima Bhaskar. Biapenem, a Carbapenem Antibiotic, Elicits Mycobacteria Specific Immune Responses and Reduces the Recurrence of Tuberculosis. **Microbiol Spectr. 2023 Jun 5:e0085823.** **Co-Corresponding author**

   Importance: Tuberculosis (TB) still tops the list of global health burdens even after COVID-19. However, it will sooner transcend the current pandemic due to the prevailing risk of reactivation of latent TB in immunocompromised individuals. The indiscriminate misuse and overuse of antibiotics have resulted in the emergence of deadly drug-resistant variants of *M.tb*. This study aims to characterize the functionality of the carbapenem antibiotic-Biapenem (BPM) in generating long-lasting immunity against TB. BPM treatment significantly boosted the activation status of the innate immune arm-macrophages by augmenting p38 signaling. Macrophages further primed and activated the adaptive immune cells $CD4^{+}$ and $CD8^{+}$ T-cells in the lung and spleen of the infected mice model. Furthermore, BPM treatment significantly amplified the polarization of T lymphocytes toward inflammatory subsets, such as Th1 and Th17. The treatment also helped generate a long-lived central memory T-cell subset. The generation of central memory T lymphocyte subset upon BPM treatment in the murine model led to a significant curtailing in the recurrence of TB due to reactivation and reinfection. These results suggest the potentiality of BPM as a potent adjunct immunomodulator to improve host defense against M.tb by enriching long-term protective memory cells.

3. Ashima Bhaskar, Isha Pahuja, Kriti Negi, Akanksha Verma, Antara Ghoshal, Babu Mathew, Gaurav Tripathi, Jaswinder Singh Maras, Shivam Chaturvedi and **Ved Prakash**

Dwivedi. SIRT2 inhibition by AGK2 enhances mycobacteria- specific stem cell memory responses by modulating beta-catenin and glycolysis. **iScience. 2023 Apr 10;26(5):106644.**

Importance: Bacille Calmette-Guerin (BCG) generates limited long-lasting adaptive memory responses leading to short-lived protection against adult pulmonary TB. Here, we show that host sirtuin 2 (SIRT2) inhibition by AGK2 significantly enhances the BCG vaccine efficacy during primary infection and TB recurrence through enhanced stem cell memory (T_{SCM}) responses. SIRT2 inhibition modulated the proteome landscape of CD4+ T cells affecting pathways involved in cellular metabolism and T-cell differentiation. Precisely, AGK2 treatment enriched the IFNγ-producing T_{SCM} cells by activating β-catenin and glycolysis. Furthermore, SIRT2 specifically targeted histone H3 and NF-κB p65 to induce proinflammatory responses. Finally, inhibition of the Wnt/β-catenin pathway abolished the protective effects of AGK2 treatment during BCG vaccination. Taken together, this study provides a direct link between BCG vaccination, epigenetics, and memory immune responses. We identify SIRT2 as a key regulator of memory T cells during BCG vaccination and project SIRT2 inhibitors as potential immunoprophylaxis against TB.

4. Anjna Kumari, Isha Pahuja, Kriti Negi, Antara Ghoshal, Suparba Mukhopadhyay, Meetu Agarwal, Babu Mathew, Jaswinder Singh Maras, Shivam Chaturvedi, Ashima Bhaskar and **Ved Prakash Dwivedi** (2023). Withaferin A protects against primary and recurrent tuberculosis by modulating mycobacteria-specific host immune responses. **Microbiol Spectr. 2023 Mar 14;11(2):e0058323.**

Importance: The fate of Mycobacterium tuberculosis infection is governed by immune signaling pathways that can either eliminate the pathogen or result in TB. Anti-TB therapy (ATT) is extensive and is efficacious only against active, drug-sensitive strains of *M.tb*. Due to severe side effects, ATT often causes impairment of host immunity, making it imperative to use novel immunotherapeutics for better clinical outcomes. In this study, we have explored the immunomodulatory potential of withaferin A (WA) as an immunotherapeutic against TB. Here, we demonstrate that WA can constrain intracellular drug-sensitive and -resistant strains of *M.tb* by augmenting host immune responses. We also established the potential of WA treatment in conjunction with isoniazid. We show that WA directs the host macrophages toward defensive M1 polarization and enhances T_H1 and T_H17 immune responses against *M.tb* infection. The reduced bacterial burden upon T cell adoptive transfer further corroborated the augmented T cell responses. Interestingly, WA stimulated the generation of T cell memory populations by instigating STAT signaling, thereby reducing the rate of TB recurrence due to reactivation and reinfection. We substantiate the prospects of WA as a potent adjunct immunomodulator that enriches protective memory cells by prompting STAT signaling and improves host defense against M. tuberculosis.

5. Isha Pahuja, Kriti Negi, Anjna Kumari, Meetu Agarwal, Suparba Mukhopadhyay, Babu Mathew, Jaswinder Singh Maras, Shivam Chaturvedi, Ashima Bhaskar and **Ved Prakash Dwivedi** (2023) Berberine governs NOTCH3/AKT signaling to enrich lung-resident memory T cells during tuberculosis. **PLoS Pathog. 2023 Mar 7;19(3):e1011165.**

Importance: Stimulation of naïve T cells during primary infection or vaccination drives the differentiation and expansion of effector and memory T cells that mediate immediate and long-term protection. Despite self-reliant rescue from infection, BCG vaccination, and treatment, long-term memory is rarely established against *M.tb* resulting in recurrent TB. Here, we show that berberine (BBR) enhances innate defense mechanisms against *M.tb* and stimulates the differentiation of Th1/Th17 specific effector memory (T_{EM}), central memory (T_{CM}), and tissue-resident memory (T_{RM}) responses leading to enhanced host protection against drug-sensitive and drug-resistant TB. Through whole proteome analysis of human PBMCs derived from PPD^{+} healthy individuals, we identify BBR modulated NOTCH3/PTEN/AKT/FOXO1 pathway as the central mechanism of elevated T_{EM} and T_{RM} responses in the human $CD4^{+}$ T cells. Moreover, BBR-induced glycolysis resulted in enhanced effector functions leading to superior Th1/Th17 responses in human and murine T cells. This regulation of T cell memory by BBR remarkably enhanced the BCG-induced anti-tubercular immunity and lowered the rate of TB recurrence due to relapse and re-infection. These results thus suggest tuning immunological memory as a feasible approach to augment host resistance against TB and unveil BBR as a potential adjunct immunotherapeutic and immunoprophylactic against TB.

6. Mona Singh, Santosh Kumar, Baldeep Singh, Preeti Jain, Anjna Kumari, Isha Pahuja, Shivam Chaturvedi, **Ved Prakash Dwivedi** and Gobardhan Das. The 1, 2-ethylenediamine SQ109 provides host protection against tuberculosis by promoting M1 macrophage polarization through the p38 MAPK pathway. **Commun Biol. 2022 Jul 28;5(1):759.**
Co-Corresponding author

Importance: Directly Observed Treatment Short-course (DOTs), is an effective and widely recommended treatment for TB. The antibiotics used in DOTs, are immunotoxic and impair effector T cells, increasing the risk of re-infections and reactivation. Multiple reports suggest that addition of immune-modulators along with antibiotics improves the effectiveness of TB treatment. Therefore, drugs with both antimicrobial and immunomodulatory properties are desirable. N1-(Adamantan-2-yl)-N2-[(2E)-3,7-dimethylocta-2,6-dien-1-yl]ethane-1,2-diamine (SQ109) is an asymmetric diamine derivative of adamantane, that targets Mycobacterial membrane protein Large 3 (MmpL3). SQ109 dissipates the transmembrane electrochemical proton-gradient necessary for cell-wall biosynthesis and bacterial activity. Here, we examined the effects of SQ109 on host-immune responses using a murine TB model. Our results suggest the pro-inflammatory nature of SQ109, which instigates M1-macrophage polarization and induces protective pro-inflammatory cytokines through the p38-MAPK pathway. SQ109 also promotes Th1 and Th17-immune responses that inhibit the bacillary burden in a murine model of TB. These findings put forth SQ109 as a potential-adjunct to TB antibiotic therapy.

7. Santosh Kumar, Ashima Bhaskar, Gautam Patnaik, Chetan Sharma, Dhiraj Kumar Singh, Sandeep Rai Kaushik, Shivam Chaturvedi, Gobardhan Das and Ved Prakash Dwivedi. Intranasal Immunization with Peptide-based Immunogenic Complex Enhances BCG Vaccine Efficacy in murine model of Tuberculosis. **JCI Insight. 2021 Jan 14;145228. doi: 10.1172/jci.insight.145228.**

Importance: Prime-boost immunization strategies are required to control the global TB pandemic, which claims approximately 3 lives every minute. Here, we have generated an immunogenic complex against *M.tb*, consisting of promiscuous T cell epitopes (*M.tb* peptides) and TLR ligands assembled in liposomes. Interestingly, this complex (peptide-TLR agonist-liposomes; PTL) induced significant activation of $CD4^+$ T cells and IFN-γ production in the PBMCs derived from PPD^+ healthy individuals as compared with PPD-controls. Furthermore, intranasal delivery of PTL significantly reduced the bacterial burden in the infected mice by inducing *M.tb*-specific polyfunctional ($IFN\text{-}\gamma^+IL\text{-}17^+TNF\text{-}\alpha^+IL\text{-}2^+$) immune responses and long-lasting central memory responses, thereby reducing the risk of TB recurrence in DOTS-treated infected animals. The transcriptome analysis of peptide-stimulated immune cells unveiled the molecular basis of enhanced protection. Furthermore, PTL immunization significantly boosted the Bacillus Calmette-Guerin-primed (BCG-primed) immune responses against TB. The greatly enhanced efficacy of the BCG-PTL vaccine model in controlling pulmonary TB projects PTL as an adjunct vaccine against TB.

8. Ashima Bhaskar, Anjna Kumari, Mona Singh, Santosh Kumar, Shivam Chaturvedi, Vinod Yadav and **Ved Prakash Dwivedi**. [6]-Gingerol exhibits potent anti-mycobacterial and immunomodulatory activity against tuberculosis. **Int Immunopharmacol. 2020 Oct;87:106809.**

Importance: The currently available ATT comprises exclusively of anti-bacterial drugs, is very lengthy, has adverse side effects on the host and leads to the generation of drug-resistant variants. Therefore, a combination therapy directed against the pathogen and the host is required to counter TB. Here we demonstrate that [6]-Gingerol, one of the most potent and pharmacologically active ingredients of ginger restricted mycobacterial growth inside the lungs, spleen and liver of mice infected with *M.tb*. The spleen of [6]-Gingerol treated mice displayed increased expression of pro-inflammatory cytokines and enhanced Th1/Th17 responses confirming the immunomodulatory action of [6]-Gingerol. Finally, [6]-Gingerol displayed an excellent potential as an adjunct drug, along with front line anti-TB drug isoniazid. Interestingly, [6]-Gingerol displayed stark anti-tubercular activity against dormant/starved bacilli and drug-resistant variants of *M.tb*. Taken together, these results indicate strong prospects of [6]-Gingerol as an adjunct anti-mycobacterial and immunomodulatory drug for the treatment of drug-susceptible and drug-resistant strains of TB.

9. Ashima Bhaskar, Santosh Kumar, Mehak Zahoor Khan, Amit Singh, **Ved Prakash Dwivedi** and Vinay Kumar Nandicoori. Host Sirtuin 2 as an Immunotherapeutic Target against Tuberculosis. **Elife. 2020 Jul 22;9:e55415.**

Importance: *M.tb* employs plethora of mechanisms to hijack the host defence machinery for its successful survival, proliferation and persistence. Here, we show that *M.tb* upregulates one of the key epigenetic modulators, NAD^+ dependent histone deacetylase Sirtuin 2 (SIRT2), which upon infection translocate to the nucleus and deacetylates histone H3K18, thus modulating the host transcriptome leading to enhanced macrophage activation. Furthermore, in Mtb specific T cells, SIRT2 deacetylates NFκB-p65 at K310 to modulate T helper cell differentiation. Pharmacological inhibition of SIRT2 restricts the intracellular growth of both drug-sensitive and resistant strains of *M.tb* and enhances

the efficacy of front line anti-TB drug Isoniazid in the murine model of infection. SIRT2 inhibitor-treated mice display reduced bacillary load, decreased disease pathology and increased *M.tb*-specific protective immune responses. Overall, this study provides a link between *M.tb* infection, epigenetics and host immune response, which can be exploited to achieve therapeutic benefits.

10. Santosh Kumar, Chetan Sharma, Sandeep Rai Kaushik, Ankur Kulshreshta, Shivam Chaturvedi, Ranjan Kumar Nanda, Ashima Bhaskar, Debprasad Chattopadhyay, Gobardhan Das and **Ved Prakash Dwivedi**. The phytochemical bergenin as an adjunct immunotherapy for tuberculosis in mice. **J Biol Chem. 2019 May 24;294(21):8555-8563.**

Importance: The widespread availability and use of modern synthetic therapeutic agents have led to a massive decline in ethnomedical therapies. However, these synthetic agents often possess toxicity leading to various adverse effects. For instance, ATT is toxic, lengthy, and severely impairs host immunity, resulting in posttreatment vulnerability to reinfection and reactivation of TB. Incomplete ATT enhances the risk for the generation of multidrug- or extensively drug-resistant (MDR or XDR, respectively) variants of *M.tb*, the TB-causing microbe. Therefore, a new therapeutic approach that minimizes these risks is urgently needed to combat this deadly disease and prevent future TB epidemics. Previously, we have shown that the phytochemical bergenin induces T helper 1 (Th1)- and Th17 cell-based protective immune responses and potently inhibits mycobacterial growth in a murine model of *M.tb* infection, suggesting bergenin as a potential adjunct agent to TB therapy. Here, we combined ATT therapy with bergenin and found that this combination reduces immune impairment and the length of treatment in mice. We observed that co-treatment with the anti-TB drug isoniazid and bergenin produces additive effects and significantly reduces bacterial loads compared with isoniazid treatment alone. The bergenin co-treatment also reduced isoniazid-induced immune impairment; promoted long-lasting, antigen-specific central memory T cell responses; and acted as a self-propelled vaccine. Of note, bergenin treatment significantly reduced the bacterial burden of a multidrug-resistant TB strain. These observations suggest that bergenin is a potent immunomodulatory agent that could be further explored as a potential adjunct to TB therapy.

Place: New Delhi

Signature of the Applicant

Date: 29th August 2023