In order of importance, list of ten best papers of the candidate, highlighting the important discoveries/contributions described in them briefly (Max 1.5 MB).

1. **Int J Biol Macromol** [IF 8.2] 14:2024:274. A novel strategy to elicit enduring anti-morphine immunity and relief from addiction by targetting Acr1 protein nano vaccine through TLR-2 to dendritic cells. Nanda S, Zafar MA, Lamba T, Malik JA, Khan MA, Bhardwaj P, Bisht B, Ghadi R, Kaur G, Bhalla V, Sehrawat S, Owais M, Jain S, **Agrewala JN***.

Highlights of the manuscript. Morphine addiction remains a formidable challenge in global healthcare, with existing opioid substitution therapies like buprenorphine, naloxone, and methadone often leading to secondary dependence. Recognizing the urgent need for innovative solutions, we have developed a groundbreaking vaccine that integrates morphine and Pam3Cys—a potent TLR-2 agonist—onto the surface of Acr1 nanoparticles. This vaccine is self-adjuvanting, specifically targeting TLR-2 receptors on antigen-presenting cells, particularly dendritic cells. This advanced vaccination strategy stimulates a robust immune response by promoting the proliferation and differentiation of morphine-specific B-cells and Acr1-reactive CD4 T-cells. Remarkably, the vaccine induces the production of high-affinity anti-morphine antibodies, which efficiently neutralize morphine in both the bloodstream and the brain in murine models. Additionally, it significantly downregulates the expression of addiction-related μ-opioid receptor and dopamine genes, directly addressing the neurobiological pathways of addiction. The vaccine also demonstrates the ability to substantially increase memory CD4 T-cells and B-cells, indicating the potential for long-lasting immunity against morphine. This novel approach not only offers a promising prophylactic measure but also represents a significant leap forward in the fight against opioid addiction, potentially transforming the landscape of addiction treatment and prevention.

2. **Aging Cell** 22:2023:13838 [IF: 11]. Age-mediated gut microbiota dysbiosis promotes loss of tolerogenic potential in dendritic cells. Bashir H, Singh S, Singh RP, **Agrewala JN***, Kumar R*.

Highlights of the manuscript. Aging-related loss of immune tolerance is a significant contributor to the onset of autoimmune and inflammatory diseases, posing a major challenge in elderly healthcare. Dendritic cells (DCs), which are crucial for maintaining immune tolerance through cytokine secretion and regulatory T-cell (Treg) generation, are particularly affected by the aging process. Aging disrupts the gut microbiota, leading to immune system dysregulation, but the specific impact of gut dysbiosis on DC-mediated tolerance has remained unclear—until now. Our research sheds light on the complex relationship between aging, gut dysbiosis and the loss of DC tolerance. We discovered that DCs derived from aged (DC^{Old}) or gut-dysbiotic young mice ($DC^{Dysbiotic}$)

displayed a significant loss of tolerance, unlike DCs from young healthy mice (DC^{Young}). This loss of tolerance was evident in the DC^{Old} and $DC^{Dysbiotic}$ cells' diminished ability to induce Treg generation and regulate CD4 T cell over-activation. Mechanistically, the loss of DC tolerance in these models was linked to the over-activation of NF-κB, reduced Treg frequency, and an imbalance in cytokine production—characterized by elevated levels of pro-inflammatory molecules (IL-6, IL-1β, TNF-α, IL-12, IFN-γ) and decreased anti-inflammatory factors (IL-10, TGF-β, IL-4, IDO, arginase, NO, IRF-4, IRF-8, PDL1, BTLA4, ALDH2). Notably, a significant decline in the beneficial *Lactobacillus* genus was observed in the gut microbiota of aged mice. Crucially, we found that replenishing the gut of aged mice with *Lactobacillus plantarum* restored DC tolerogenic function by modulating inflammatory and metabolic pathways. This groundbreaking discovery highlights the potential of targeting gut microbiota, specifically by administering *Lactobacillus plantarum*, as a novel therapeutic strategy for treating age-associated immune disorders. Our study offers a new perspective on the role of gut dysbiosis in immune aging. It opens up promising avenues for developing microbiota-based interventions to counteract the age-related loss of immune tolerance. This could lead to innovative treatments for autoimmune and inflammatory diseases, significantly enhancing the quality of life for the aging population.

3. **J Biol Chem**. 2022 Oct 15:102596. [IF: 5.49]. *Mycobacterium tuberculosis* epitope entrapped in nanoparticles expressing TLR-2 ligand targeted to dendritic cells elicit protective immunity. Das DK, Zafar MA, Nanda S, Singh S, Lamba T, Bashir H, Singh P, Maurya SK, Nadeem S, Sehrawat S, Bhalla V*, **Agrewala JN***.

Highlights of the manuscript. The fight against tuberculosis (TB) is at a critical juncture, with the World Health Organization's "End TB Strategy" underscoring the urgency of novel vaccination approaches to curb this global health threat. The challenge is compounded by the rise of drug-resistant strains of *Mycobacterium tuberculosis (Mtb)*, particularly in patients coinfected with HIV-AIDS. To address this, we have developed an innovative vaccine formulation that leverages an immunodominant peptide derived from the Acr1 protein of *Mtb*, presented on nanoparticles that also display a toll-like receptor-2 (TLR-2) ligand for targeted delivery to dendritic cells (DCs). Our formulation is designed for efficient uptake by DCs via a TLR-2-dependent pathway, leading to the activation of these cells and the production of key immune mediators such as nitric oxide and proinflammatory cytokines (IL-6, IL-12, and TNF-α). Additionally, the vaccine upregulates the expression of major histocompatibility complex (MHC) class II molecules and costimulatory molecules like CD80 and CD86, priming the immune system for a robust response. *In vivo* studies have demonstrated that this vaccine induces a potent immune response, characterized by a significant

increase in effector and memory Th1 and Th17 cells. Importantly, this heightened immune response translates into a marked reduction in bacterial load in the lungs of animals challenged with aerosolized live *Mtb*. These findings suggest that our novel vaccine formulation not only enhances protective anti-*Mtb* immunity but also offers a powerful tool to combat the growing threat of TB, including drug-resistant strains. This breakthrough could be instrumental in advancing global efforts to end tuberculosis.

4. **Cell Mol Life Sci.** 79:2022:567 [IF: 9.2]. *Mycobacterium tuberculosis* exploits MPT64 to generate myeloid-derived suppressor cells to evade the immune system. Singh S, Maurya SK, Aqdas M, Bashir H, Arora A, Bhalla V, **Agrewala JN***.

Highlights of the manuscript. *Mycobacterium tuberculosis (Mtb)* is an adept and resilient pathogen capable of manipulating the host immune system to ensure its persistence. A key player in this process is the MPT64 (Rv1980c) protein, which is released in high quantities in patients with active tuberculosis (TB). Recognizing the potential role of MPT64 in immune evasion, we sought to investigate its impact on differentiating dendritic cells (DCs) and its contribution to *Mtb's* survival strategy. Our findings reveal that when differentiating DCs are pre-exposed to MPT64 (DC^{MPT64}), they transform into a phenotype resembling myeloid-derived suppressor cells (MDSCs). These DC^{MPT64} cells exhibit elevated expression of immunosuppressive molecules such as PD-L1, TIM-3, nitric oxide (NO), arginase 1, IDO-1, IL-10, and TGF-β, while simultaneously inhibiting the production of key pro-inflammatory cytokines including TNF-α, IL-6, and IL-12. Moreover, DC^{MPT64} cells demonstrate impaired chemotaxis due to reduced CCR7 expression, further compromising their immune functions. Notably, DC^{MPT64} cells facilitate the generation of regulatory T cells (Tregs) and suppress the differentiation of Th1 and Th17 cells, creating an immune environment that favors *Mtb* survival. Additionally, these cells exhibit metabolic quiescence, characterized by high lipid and methylglyoxal content, reduced glucose consumption, and diminished phagocytic capacity. This metabolic state not only hampers their ability to combat *Mtb* but also provides a safe haven for the intracellular survival of the bacterium. The underlying mechanism driving the impaired function of DC^{MPT64} cells is linked to the increased production and accumulation of methylglyoxal, a key factor in their metabolic reprogramming. For the first time, we have demonstrated that MPT64 plays a crucial role in converting DCs into MDSCs, thereby facilitating *Mtb's* immune evasion and persistence within the host. This discovery opens up new avenues for targeting MPT64 and its associated pathways, offering potential strategies for the development of novel therapeutic interventions aimed at disrupting Mtb's ability to evade the immune system and enhancing the effectiveness of TB treatments.

5. **Autophagy** 16:2020:1021 [IF: 16.01]. Induction of autophagy through Clec4e in combination with TLR-4: an innovative strategy to restrict the survival of *Mycobacterium tuberculosis.* Pahari S, Negi S, Aqdas M, Arnett E, Schlesinger LS, **Agrewala JN***.

Highlights of the manuscript. Host-directed therapies are emerging as a pivotal strategy in the fight against drug-resistant pathogens, addressing the limitations of traditional antibiotic treatments. The increasing antibiotic resistance accentuates the urgent need for innovative approaches that target host pathways to effectively control infections. Two critical components of the host immune response, the C-type lectin receptor CLEC4E and Toll-like receptor 4 (TLR-4) are key in the initial defence against pathogens. Our research has focused on enhancing the immune response by leveraging the signaling pathways of macrophages through CLEC4E in combination with TLR4 agonists (C4.T4). This novel approach demonstrated a significant boost in host immunity and a substantial reduction in bacterial load in the lungs of *Mtb*-infected mice and guinea pigs treated with C4.T4 agonists. Remarkably, the intracellular eradication of *Mtb* was achieved with a 10-fold lower dose of isoniazid or rifampicin when combined with C4.T4 compared to the standard drug regimens. At the molecular level, C4.T4 activation initiated a robust immune response, activating key signaling molecules such as MYD88, PtdIns3K, STAT1, and RELA/NFKB. This activation increased lysosome biogenesis, suppressing pro-inflammatory cytokines (IL-10, IL-4) and significantly enhancing autophagy—a critical process in pathogen clearance. Notably, macrophages deficient in autophagy (Atg5 knockout or Becn1 knockdown) exhibited higher survival rates of *Mtb*, highlighting the essential role of autophagy in controlling *Mtb* infection. This study unveils, for the first time, the pivotal role of CLEC4E in driving autophagy via the MYD88 pathway, a mechanism crucial for limiting *Mtb* survival. Our findings suggest a promising immunotherapeutic strategy that harnesses CLEC4E in conjunction with TLR-4 to enhance autophagy and combat *Mtb*. This approach offers a potential breakthrough in the development of new treatments for tuberculosis and other drug-resistant infections, aligning with the growing need for host-directed therapies in the pharmaceutical landscape.

6. **J Proteome Res**. [IF: 5.4]. 19:2020:4655. Deciphering the structural enigma of HLA class-II binding peptides for enhanced immunoinformatics-based prediction of vaccine epitopes. Chatterjee D, Priyadarshini P, Das DK, Mushtaq K, Singh B, **Agrewala JN**.

Highlights of the manuscript. Vaccines are the cornerstone of public health, offering the most effective means to prevent and eliminate diseases associated with high morbidity and mortality. Recent advancements in clinical trials emphasize the growing potential of peptide-based vaccines, particularly for diseases where effective treatments remain elusive. CD4 T-cell-based peptide vaccines, in particular, hold promise by utilizing antigenic determinants from pathogens or tumor cells to elicit a strong T helper cell response, thereby activating multiple arms of the immune system. Current *in silico* tools for predicting human leukocyte antigen II (HLA-II) binding peptides are predominantly sequence-based, offering a balance between sensitivity and specificity. However, the structural analysis and understanding of peptide-HLA-II interactions are crucial for the empirical design of successful peptide vaccines. Despite their importance, structure-based epitope prediction algorithms are significantly underdeveloped compared to their sequence-based counterparts. This study addresses this gap by providing a comprehensive structural analysis of HLA-II binders, leveraging Protein Data Bank (PDB) complexes of peptide-HLA-II (pHLA-II) interactions. We further explore the peptide exchange mechanism, demonstrating the structural implications of an acidic environment on HLA-II binding. Our research culminates in a structure-guided approach to identify potential HLA-II binders within antigenic proteins, offering a more accurate method for predicting peptide epitopes. This innovative strategy holds the potential to significantly enhance the design of peptide vaccines, paving the way for more effective immunotherapies and prophylactic treatments. By integrating structural insights with epitope prediction, we can advance the development of peptide-based vaccines, providing a powerful tool in the fight against challenging diseases.

7. **Eur J Immunol.** 16:2020:10 [IF: 6.8]. Intestinal microbiota disruption limits the isoniazid-mediated clearance of *Mycobacterium tuberculosis* in mice. Negi S, Pahari S, Bashir H, **Agrewala JN***.

Highlights of the manuscript. Tuberculosis (TB) continues to remain a global threat due to the emergence of drug-resistant *Mycobacterium tuberculosis (Mtb)* strains and toxicity associated with TB drugs. 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. Here, we examined the impact of gut microbial dysbiosis on INH efficiency in killing *Mtb*. In this study, we employed an *in vivo* mouse model, pretreated with broad-spectrum antibiotics (Abx) cocktail to disrupt their intestinal microbial population prior to *Mtb* infection and subsequent INH therapy. We demonstrated that

microbiota disruption results in the impairment of INH-mediated *Mtb* clearance and aggravated TB-associated tissue pathology. Further, it suppressed the innate immunity and reduced CD4 T-cell response against *Mtb*. Interestingly, a distinct shift of gut microbial profile was noted with an abundance of Enterococcus and a reduction of Lactobacillus and *Bifidobacterium* populations. Our results show that the intestinal microbiota is a crucial determinant in the efficacy of INH in killing *Mtb* and impacts the host immune response against infection. This work provides an intriguing insight into the potential links between host gut microbiota and the potency of INH.

8. **J Infect Dis**. 211:2015: 486-96 [IF: 8.8]. Triggering through TLR-2 limits chronically stimulated Th1 cells from undergoing exhaustion. Chodisetti SB, Gowthaman U, Rai PK, Vidyarthi A, Khan N, **Agrewala JN***.

Highlights of the manuscript. Chronic infections result in T-cell exhaustion, a state of functional unresponsiveness. It is important to salvage the exhausted T cells to control the disease. In this study, we delivered Toll-like receptor 2 (TLR-2) signals to reinvigorate functionality in chronically activated T-helper type 1 (Th1) cells. This process significantly augmented T-bet expression, IFN-γ, IL-2, and the anti-apoptotic molecule Bcl-2. In contrast, it dampened the display of the exhaustion markers programmed death receptor 1 (PD-1) and lymphocyte activation gene 3 (Lag-3). Additionally, TLR-2 signaling bolstered the ability of chronically stimulated Th1 cells to activate B cells. Finally, the results were substantiated by observing reduced lung pathology upon administration of TLR-2 agonist in the chronic infection model of tuberculosis. This study demonstrated the novel role 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.

9. **J Biol Chem.** 289:2014:17515-28 [IF: 5.5]. Caerulomycin A enhances the TGF-β-Smad3 signalling by suppressing IFN-γ-STAT1 signalling to expand Tregs. Gurram RK, Kujur W, Maurya SK, **Agrewala JN***.

Highlights of the manuscript. Cytokines play a very important role in the regulation of immune homeostasis. Regulatory T cells (Tregs) responsible for the generation of peripheral tolerance are under the tight regulation of the cytokine milieu. In this study, we report a novel role of a bipyridyl compound, Caerulomycin A (CaeA), in inducing the generation of

regulatory T cells (Tregs). It was observed that CaeA substantially up-regulated the pool of Tregs, as evidenced by an increased frequency of CD4(+) Foxp3(+) cells. In addition, CaeA significantly suppressed the number of Th1 cells and Th17 cells, as supported by a decreased percentage of $CD4^+$/IFN-γ^+ and $CD4^+$/IL-17^+ cells, respectively. Furthermore, we established the mechanism and observed that CaeA interfered with IFN-γ-induced STAT1 signaling by augmenting SOCS1 expression. An increase in the TGF-β-mediated Smad3 activity was also noted. Furthermore, CaeA rescued Tregs from IFN-γ-induced inhibition. These results were corroborated by blocking Smad3 activity, which abolished the CaeA-facilitated generation of Tregs. This study indicates a novel role of CaeA in inducing the generation of Tregs. This finding suggests that CaeA has enough potential to be considered a potent future drug for treating autoimmunity.

10. **J Infect Dis.** 209:2014:1436-45 [IF: 7.8]. Latency Associated Protein Acr1 Impairs Dendritic Cells Maturation and Functionality: A Possible Mechanism of Immune Evasion by *Mycobacterium tuberculosis.* Siddiqui KF, Amir M, Gurram RK, Khan N, Arora A, K Rajagopal, **Agrewala JN***.

Highlights of the manuscript. *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 *M. tuberculosis*. However, whether *M. tuberculosis* exploits its Acr1 in impairing the host immunity remains unexplored. In this study, 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 mycobacterial Acr1 in inhibiting the maturation and differentiation of DCs by inducing tolerogenic phenotype by modulating the expression of PD-L1; Tim-3; indoleamine 2, 3-dioxygenase (IDO); and interleukin 10. Furthermore, Acr1 interferes with the differentiation of DCs by targeting STAT-6 and STAT-3 pathways. Continuous activation of STAT-3 inhibited the translocation of NF-κB in Acr1-treated DCs. Furthermore, Acr1 augmented the induction of regulatory T cells. 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 for the first time provides a crucial insight into a strategy adopted by *M. tuberculosis* to survive in the host by impairing the function of DCs.