List of 10 best papers

1. **Reena Bharti**, Trisha Roy, Sonia Verma, D. V. Siva Reddy, Hasham Shafi, Khushboo Verma, Sunil K Raman, Sampita Pal, Lubna Azmi, Amit K Singh, Lipika Ray, Madhav N Mugale, Amit Misra* Transient transfection of the respiratory epithelium with gamma interferon for host-directed therapy in pulmonary tuberculosis. Molecular Therapy-Nucleic Acids. 2020 Dec 4;22:1121-8

   Nebulized gamma interferon (IFN-γ) protein has been studied for clinical safety and efficacy against pulmonary tuberculosis (TB). The protein is expensive, requires a cold chain, and is difficult to deploy in limited-resource, high-incidence settings. We generated a preclinical proof of concept (PoC) for a dry powder inhalation (DPI) containing DNA constructs to transiently transfect the lung and airway epithelium of mice with murine IFN-γ. Bacterial colony-forming units (CFU) in the lungs of mice infected with *Mycobacterium tuberculosis* (Mtb) reduced from about 10^6/g of tissue to ~10^4 after four doses given once a week. Nodular inflammatory lesions in the lungs reduced significantly in number. Immunohistochemistry of infected lung sections for LC3-1 and LAMP-1 indicated autophagy induction between 18 and 48 h after inhalation. ELISA on bronchoalveolar lavage (BAL) fluid showed differences in kinetics of IFN-γ concentrations in the epithelial lining fluid of healthy versus infected mice. Uninfected mice receiving DNA constructs expressing a fluorescent protein were live-imaged. The fluorescence signals from the intracellular protein peaked at about 36 h after inhalation and declined by 48 h. These results establish preclinical PoC of the efficacy of a DPI and dosing regimen as a host-directed and transient gene therapy of experimental pulmonary TB in mice, justifying preclinical development

2. .**Bharti R**, Roy T, Verma S, Reddy DS, Shafi H, Verma K, Raman SK, Pal S, Azmi L, Singh AK, Ray L. Transient, inhaled gene therapy with gamma interferon mitigates pathology induced by host response in a mouse model of tuberculosis. Tuberculosis. 2022 May 1;134:102198.

   A dry powder inhalation formulation was prepared with plasmids expressing gamma interferon or fluorescent proteins. The formulation was optimized and characterized for inhalation. Stability of plasmid DNA was checked in prepared formulation and MMAD of particles was checked through Cascade impactor. *In- vitro* and *in-vivo* studies were done to investigate the kinetics of expression of IFN-γ and/or reporter genes. Immunohistochemistry was done to investigate the kinetics of induction of autophagy as a consequence of expression of functional IFN-γ in the lungs of *Mtb* infected mice. Efficacy of DPI in terms of reduction of bacterial burden in lungs and spleen of *Mtb* infected mice were shown.

Our study has shown that it is possible to transiently transfect the lung and airway epithelium with gamma interferon for an optimal period of time. Condos et al. reported the clinical efficacy of nebulized gamma interferon (IFN- γ) protein in patients with pulmonary TB, but the prohibitive cost of IFN- γ protein is a hurdle in developing this as a host-directed therapy (HDT) for TB. Our study addressed the transient transfection of the lung and airway epithelium with IFN-γ. A prototype dry powder inhalation (DPI) product was developed for transient gene therapy over an optimal period of gene expression. The formulation is potentially affordable, because it is not a sterile product, does not require cold chain storage, and can be self-administered by outpatients. Transient transfection with IFN-γ did not induce pathological inflammation of the lung and airways. The long story cut to short is that IFN-γ, as expected of host-directed therapy, 'heals the host;' but does not 'kill the bug.

3. **R Bharti,** N Greenland, J Santos, SJ Cleary, JR Greenland, DR Calabrese" CCR5 Drives NK Cell Mediated Airway Damage in an Endotoxin-induced Model of Acute Lung Injury" American Thoracic Society, A6837-A6837

   Acute respiratory distress syndrome is a clinical severe acute lung injury (ALI) pathology, which may result from a responses to bacterial endotoxin. CCR5-dependent natural killer (NK) cell responses mediate ALI in pulmonary ischemiareperfusion injury, but the involvement of CCR5 and NK cell responses in ALI due to other causes is poorly understood. Innate immune responses to bacterial lipopolysaccharides (LPS) are implicated in ALI due to lung infections and lung storage prior to transplantation. Here, we test the hypothesis that CCR5-dependent NK cell responses mediate LPS-induced ALI.

4. Tsao T, Qiu L, **Bharti R**, Shemesh A, Hernandez AM, Cleary SJ, Greenland NY, Santos J, Shi R, Bai L, Richardson J. CD94+ Natural Killer cells potentiate pulmonary ischemia-reperfusion injury. European Respiratory Journal. 2024 Jan 1

   Pulmonary ischemia-reperfusion injury (IRI) is a major contributor to poor lung transplant outcomes. We recently demonstrated a central role of airway-centered NK cells in mediating IRI; however, there are no existing effective therapies for directly targeting NK cells in humans. We hypothesized that a depleting anti-CD94 monoclonal antibody (mAb) would provide therapeutic benefit in mouse and human models of IRI based on high levels of *KLRD1* (CD94) transcripts in bronchoalveolar lavage samples from lung transplant patients. We found that CD94 is highly expressed on mouse and human NK cells, with increased expression during IRI. Anti-mouse and anti-human mAbs against CD94 showed effective NK

cell depletion in mouse and human models and blunted lung damage and airway epithelial killing. In two different allogeneic orthotopic lung transplant mouse models, anti-CD94 treatment during induction reduced early lung injury and chronic inflammation relative to control therapies. Anti-CD94 did not increase donor antigen-presenting cells that could alter long-term graft acceptance. Lung transplant induction regimens incorporating anti-CD94 treatment may safely improve early clinical outcomes.

5. Tsao T, Qiu L, Shemesh A, Hernandez AM, Shi K, Richardson J, **Bharti R**, Santos J, Lanier L, Looney M, Greenland J. Novel Anti-CD94 Treatment Reduces Mouse and Human Experimental Pulmonary Ischemia-Reperfusion Injury. The Journal of Heart and Lung Transplantation. 2024 Apr 1;43(4):S119.

Primary graft dysfunction from ischemia-reperfusion injury (IRI) is a major contributor to poor lung transplant outcomes. We recently demonstrated a central role for airway natural killer (NK) cells during IRI; however, there are no targeted therapies for pathologic NK cells in humans. We hypothesized that a monoclonal antibody against the NK cell co-receptor CD94 (αCD94) would reduce NK cells and lung damage in mouse and human experimental models of IRI and we conclude that selective CD94-based treatment blunts damage following IRI through NK cell depletion without promoting rejection. Including anti-CD94 in induction regimens may be a promising strategy to prevent primary graft dysfunction.

6. Reddy DS, Shafi H, **Bharti R**, Roy T, Verma S, Raman SK, Verma K, Azmi L, Ray L, Singh J, Singh AK. Preparation and evaluation of low-dose calcitriol dry powder inhalation as host-directed adjunct therapy for tuberculosis. Pharmaceutical Research. 2022 Oct;39(10):2621-33.

It is unclear whether Vitamin D is efficacious as a host-directed therapy (HDT) for patients of tuberculosis (TB). We investigated pulmonary delivery of the active metabolite of Vitamin D3, i.e., 1, 25-dihydroxy vitamin D3 (calcitriol) in a mouse model of infection with Mycobacterium tuberculosis (Mtb). We optimized a spray drying process to prepare a dry powder inhalation (DPI) of calcitriol using a Quality by Design (QbD) approach. We then compared outcomes when Mtb-infected mice were treated with inhaled calcitriol at 5 ng/kg as a stand-alone intervention versus DPI as adjunct to standard oral anti-tuberculosis therapy (ATT). The DPI with or without concomitant ATT markedly improved the morphology of the lungs and mitigated histopathology in both the lungs and the spleens. The number of nodular lesions on the lung surface decreased from 43.7 ± 3.1 to 22.5 ± 3.9 with the DPI alone and to 9.8 ± 2.5 with DPI + ATT. However, no statistically significant induction of host antimicrobial peptide cathelicidin or reduction in bacterial burden was seen with the DPI alone. DPI + ATT did not

significantly reduce the bacterial burden in the lungs compared to ATT alone. We concluded that HDT using the low dose calcitriol DPI contributed markedly to mitigation of pathology, but higher dose may be required to evoke significant induction of bactericidal host response and bactericidal activity in the lung.

7. Rajeev Ranjan, Ashish Shrivastava, **Reena Bharti**, Lipika Ray, Jyotsna Singh, Amit Misra; Preparation and optimization of a dry powder for inhalation of second-line anti-tuberculosis drugs, *International Journal of Pharmaceutics*.,547(1-2):150-157,2018

A spray drying process was standardized to prepare an inhalable powder comprising d-cycloserine and ethionamide, two "second line" drugs employed for treating multi-drug resistant (MDR) tuberculosis (TB). The aim of the process development effort was to maximize product yield. Contour plots were generated using a small central composite design (CCD) with face centered ($\alpha = 1$) to maximize the process yield as the response criterion. The design space was experimentally validated. Powder was prepared and characterized for drug content (HPLC), geometric size (laser scattering), surface morphology (scanning electron microscopy) aerosol behaviour (cascade impaction) and powder flow properties. The optimized process yielded a powder with a median mass aerodynamic diameter (MMAD) of 1.76 μ ± 3.1 geometric standard deviation (GSD). Mass balance indicated that the major proportion of the particles produced by spray drying are lost to the outlet filter. The process represents a best-case compromise of spray-drying conditions to minimize loss during droplet drying, collection and process air discharge.

8. Ranjan R, Srivastava A, **Bharti R**, Roy T, Verma S, Ray L, Misra A. Preclinical Development of Inhalable D-Cycloserine and Ethionamide To Overcome Pharmacokinetic Interaction and Enhance Efficacy against *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. 2019 May 24;63(6):00099-19. doi: 10.1128/AAC.00099-19. PMID: 30962335; PMCID:PMC6535545

We compared the pharmacokinetics and efficacy of a combination of d-cycloserine (DCS) and ethionamide (ETO) via oral and inhalation routes in mice. The plasma half-life ($t_{1/2}$) of oral ETO at a human-equivalent dose decreased from 4.63 ± 0.61 h to 1.64 ± 0.40 h when DCS was coadministered. The area under the concentration-time curve from 0 h to time t ($AUC_{0\text{-}t}$) was reduced to one-third. Inhalation overcame the interaction. Inhalation, but not oral doses, reduced the lung CFU/g of *Mycobacterium tuberculosis* H37Rv from 6 to 3 $\log_{10}$ in 4 weeks, indicating bactericidal activity.

9. Verma S, Dal NJ, Srivastava A, **Bharti R**, Siva Reddy DV, Sofi HS, Roy T, Verma K, Raman SK, Azmi L, Ray L. Inhaled Adjunct therapy with second-line drug candidates for dose reduction in chemotherapeutic regimens for multi-drug-resistant tuberculosis. AAPS PharmSciTech. 2023 Jun 8;24(5):130.

Chemotherapy of multi-drug-resistant tuberculosis (TB) requires prolonged administration of multiple drugs. We investigated whether pulmonary delivery of minute doses of drugs, along with reduced oral doses of the same agents, would affect preclinical efficacy. We prepared dry powder inhalation (DPI) formulations comprising sutezolid (SUT), the second-generation pretomanid analog TBA-354 (TBA), or a fluorinated derivative of TBA-354 (32,625) in a matrix of the biodegradable polymer poly(L-lactide). We established formulation characteristics, doses inhaled by healthy mice, and preclinical efficacy in a mouse model of TB. Oral doses of 100 mg/kg/day or DPI doses of 0.25-0.5 mg/kg/day of drugs SUT, TBA-354, or 32,625 administered over 28 days were sub-optimally effective in reducing lung and spleen burden of Mycobacterium tuberculosis (Mtb) in infected mice. The addition of 0.25-0.5 mg/kg/day of SUT, TBA-354, or 32,625 as DPI to oral doses of 50 mg/kg/day was non-inferior in clearing Mtb from the lungs of infected mice. We concluded that adjunct therapy with inhaled second-line agents has the potential to reduce the efficacious oral dose.

10. Roy T, Seth A, Shafi H, Reddy DS, Raman SK, Chakradhar JV, Verma S, **Bharti R**, Azmi L, Ray L, Misra A. Transcriptional regulation of suppressors of cytokine signaling during infection with Mycobacterium tuberculosis in human THP-1-derived macrophages and in mice. Microbes and Infection. 2024 Mar 1;26(3):105282.

Mycobacterium tuberculosis (Mtb) infection leads to upregulation of Suppressors of Cytokine signaling (SOCS) expression in host macrophages (Mϕ). SOCS proteins inhibit cytokine signaling by negatively regulating JAK/STAT. We investigated this host-pathogen dialectic at the level of transcription. We used phorbol-differentiated THP-1 Mϕ infected with Mtb to investigate preferential upregulation of some SOCS isoforms that are known to inhibit signaling by IFN-γ, IL-12, and IL-6. We examined time kinetics of likely transcription factors and signaling molecules upstream of SOCS transcription, and survival of intracellular Mtb following SOCS upregulation. Our results suggest a plausible mechanism that involves PGE2 secretion during infection to induce the PKA/CREB axis, culminating in nuclear translocation of C/EBPβ to induce expression of SOCS1. Mtb-infected Mϕ secreted IL-10, suggesting a mechanism of induction of STAT3, which may subsequently induce SOCS3. We provide evidence of temporal variation in SOCS isoform exspression and decay. Small-interfering RNA-mediated knockdown of SOCS1 and

SOCS3 restored the pro-inflammatory milieu and reduced Mtb viability. In mice infected with Mtb, SOCS isoforms persisted across Days 28-85 post infection. Our results suggest that differential temporal regulation of SOCS isoforms by Mtb drives the host immune response towards a phenotype that facilitates the pathogen's survival.