

**Pathogenesis of Post-Tuberculosis Lung Disease: Defining Knowledge Gaps and Research Priorities  
at the 2<sup>nd</sup> International Post-Tuberculosis Symposium**

Sara C Auld MD, MS<sup>1\*</sup>, Amy K Barczak MD<sup>2</sup>, William Bishai MD, PhD<sup>3</sup>, Anna K Coussens PhD<sup>4</sup>, Intan MW Dewi MD, PhD<sup>5</sup>, Steven C Mitini-Nkhoma MBBS, MSc<sup>6</sup>, Caleb Muefong PhD<sup>7</sup>, Threnesan Naidoo MD, MSc, LLM<sup>8</sup>, Anil Pooran PhD<sup>9</sup>, Cari Stek MD, PhD<sup>10</sup>, Adrie JC Steyn PhD<sup>11</sup>, Liku Tezera MD, MSc, PhD<sup>12</sup>, Naomi F Walker MRCP, PhD<sup>13\*</sup>

1 Departments of Medicine, Epidemiology, and Global Health, Emory University School of Medicine and Rollins School of Public Health, Atlanta, Georgia, USA. ORCID 0000-0002-8972-3736

2 Massachusetts General Hospital, Division of Infectious Diseases, Boston, USA; The Ragon Institute of Mass General, MIT, and Harvard, Cambridge, USA; Harvard Medical School, Boston, Massachusetts, USA. ORCID 0000-0003-3806-2381

3 Division of Infectious Diseases, Department of Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland, USA. ORCID 0000-0002-8734-4118

4 Infectious Diseases and Immune Defence Division, Walter and Eliza Hall Institute of Medical Research (WEHI), Parkville, Victoria Australia and Department of Medical Biology, University of Melbourne, Parkville, Victoria Australia. ORCID 0000-0002-7086-2621

5 Microbiology Division, Department of Biomedical Sciences, Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia; Research Center for Care and Control of Infectious Diseases (RC3ID), Universitas Padjadjaran, Bandung, Indonesia. ORCID 0000-0002-4910-9651

6 Malawi Liverpool Wellcome Clinical Research Programme, Blantyre, Malawi. ORCID 0000-0002-3276-6890

7 Department of Microbiology, University of Chicago, Chicago, Illinois, USA. ORCID 0000-0001-8817-9188.

8 Departments of Forensic & Legal Medicine and Laboratory Medicine & Pathology, Faculty of Medicine & Health Sciences, Walter Sisulu University, Eastern Cape, South Africa; Africa Health Research Institute, KwaZulu Natal, South Africa. ORCID 0000-0002-1864-4301

9 Centre for Lung Infection and Immunity, Division of Pulmonology, Department of Medicine and University of Cape Town Lung Institute and MRC/UCT Centre for the Study of Antimicrobial Resistance, Cape Town, South Africa. ORCID 0000-0001-5808-433X

10 Wellcome Center for Infectious Diseases Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa. ORCID 0000-0001-8813-0819

11 Africa Health Research Institute, University of KwaZulu-Natal, Durban, South Africa. Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, USA. Centers for AIDS Research and Free Radical Biology, University of Alabama at Birmingham, Birmingham, AL, USA. ORCID 0000-0001-9177-8827

12 NIHR Biomedical Research Centre, School of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom. ORCID 0000-0002-7898-6709.

13 Department of Clinical Sciences and Centre for Tuberculosis Research, Liverpool School of Tropical Medicine, Liverpool, UK; Tropical and Infectious Diseases Unit, Liverpool University Hospitals NHS Foundation Trust, Liverpool, United Kingdom. ORCID 0000-0002-3345-7694

\*Co-corresponding authors:

Sara Auld, MD, MS, 615 Michael St NE Suite 200, Atlanta, GA 30322, USA; +1-404-727-9140;

[sauld@emory.edu](mailto:sauld@emory.edu)

Naomi Walker, MD, PhD, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, UK, L3

5QA; +44 (0) 151-705-3100; [naomi.walker@lstm.ac.uk](mailto:naomi.walker@lstm.ac.uk)

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## Abstract

Post-tuberculosis (TB) lung disease (PTLD) is increasingly recognized as a major contributor to the global burden of chronic lung disease, with recent estimates indicating that over half of TB survivors have impaired lung function after successful completion of TB treatment. However, the pathologic mechanisms that contribute to PTLD are not well understood, thus limiting the development of therapeutic interventions to improve long-term outcomes after TB. This report summarizes the work of the “Pathogenesis and Risk Factors Committee” for the Second International Post-Tuberculosis Symposium, which took place in Stellenbosch, South Africa in April 2023. The committee first identified six areas with high translational potential: (1) tissue matrix destruction, including the role of matrix metalloproteinase dysregulation and neutrophil activity, (2) fibroblasts and profibrotic activity, (3) granuloma fate and cell death pathways, (4) mycobacterial factors including pathogen burden, (5) animal models, and (6) the impact of key clinical risk factors including HIV, diabetes, smoking, malnutrition, and alcohol. We share here the key findings from a literature review of those areas, highlighting knowledge gaps and areas where further research is needed.

Keywords: Tuberculosis, post-Tuberculosis lung disease, pathogenesis, fibrosis, neutrophils, matrix metalloproteinases

## Introduction

With over 10 million new cases and 1.6 million deaths each year, tuberculosis (TB) remains a major public health priority (1). However, morbidity and mortality due to TB does not end after successful TB treatment. Rather, people treated for TB have a nearly three-fold increased risk of death compared to people who have never had TB (2) and over half of pulmonary TB survivors have evidence of ongoing respiratory morbidity after completion of TB treatment (3). With an estimated 155 million TB survivors alive in 2020 (4), post-TB lung disease (PTLD) is a major contributor to the global burden of chronic lung disease, estimated to account for 47% of the 122 million disability-adjusted life-years (DALYs) attributed to incident TB in 2019 (5). Despite increasing recognition of the substantial epidemiologic burden of PTLD and growing consensus on clinical manifestations and phenotypes, from disease involving the airways and parenchyma to pleural and pulmonary vascular disease (Box 1), there is a paucity of knowledge regarding the pathologic mechanisms underlying PTLD. A greater understanding of these diverse mechanisms and how they correspond to distinct post-TB sequelae would enable the identification of biological subtypes, or endotypes, of PTLD which could be therapeutically targeted with directed interventions (6).

In April 2023, a multidisciplinary group of international stakeholders gathered for the Second International Post-Tuberculosis Symposium in Stellenbosch, South Africa(7). This report summarizes the work of the Symposium's "Pathogenesis and Risk Factors Committee," which aimed to identify key research priorities around biological pathways and mechanisms important to PTLD. The committee, comprised 13 investigators invited to participate based on their clinical and scientific expertise, identified six areas with high translational potential for a focused review of the literature: (1) tissue matrix destruction, including the role of matrix metalloproteinase (MMP) dysregulation and neutrophil activity, (2) fibroblasts and profibrotic activity, (3) granuloma fate and cell death pathways, (4) mycobacterial factors including pathogen burden, (5) animal models (Figure 1), in addition to (6) the impact of key clinical risk factors including HIV, diabetes, smoking malnutrition

and alcohol. (See Supplemental Table 1 for ranked list of research areas considered for review.) Here we report the key findings from this work and highlight knowledge gaps, where further research focus is needed to inform and advance our understanding of the pathogenesis of PTLD. While there is growing recognition of the many long-term sequelae of TB disease, from epigenetic modifications and alterations in the host microbiome to increased risks of cardiovascular disease and cancer (8-12), this report focuses primarily on biological pathways and mechanisms relevant to the airways and lung parenchyma, which have been the most studied PTLD manifestations to date.

### **Matrix destruction, MMPs and Neutrophils**

Destruction of the lung extracellular matrix during *Mycobacterium tuberculosis* (*Mtb*) infection often begins a considerable time prior to TB diagnosis and continues during TB treatment. The radiological hallmark of pulmonary TB is a cavity, radiologically defined as a lucency within a zone of pulmonary consolidation, a mass, or a nodule that is surrounded by a wall, classically affecting the lung apices (13). Cavitory TB is the major source of *Mtb* transmission and is associated with higher bacterial burden in respiratory secretions and longer time to microbiological cure (14); bronchiectasis often accompanies cavitation, and residual cavitation is a common feature of PTLD (15, 16). The absence of collagen and elastin, the major structural proteins of the lung, inside cavities points to the involvement of MMPs—a family of host enzymes that collectively degrade the extracellular matrix, including collagenases, gelatinases, and stromelysins. MMPs have roles in wound healing and are implicated both in tissue destruction and remodeling in TB (17). MMPs are physiologically tightly regulated but are upregulated in monocytes, macrophages, and neutrophils directly in response to *Mtb* infection and via cellular networks, including uninfected stromal cells, without compensatory upregulation of specific inhibitors, the Tissue Inhibitors of Matrix Metalloproteinases (TIMPs), described as a “matrix-degrading phenotype” (18-21). Elevated concentrations of MMPs, including MMP-1, -2, -3, and -8, have been detected in sputum from TB patients compared to respiratory symptomatic and healthy control patients, and have also been associated with cavitation and

sputum smear positivity (22-25). MMP-1 polymorphisms have also been associated with more extensive pulmonary fibrosis and increased risk of tracheobronchial stenosis after TB treatment completion, further implicating this collagenase in both tissue damage and pathological remodeling leading to PTLD, with pathological extracellular matrix remodeling considered an integral process in other fibrotic lung diseases, where MMP dysregulation has also been implicated, such as silicosis and idiopathic pulmonary fibrosis (see below) (26-30).

Although animal models are limited in their capacity to reproduce features of PTLD (see below section), they have provided specific mechanistic insights with respect to inflammation and matrix destruction during TB. For example, a longstanding tenet in TB pathology was that caseous necrosis led to tissue destruction and cavitation. However, a series of experiments leveraging transgenic MMP-1 expressing mice found that extracellular matrix destruction actually precedes caseous necrosis (31). This pathology, which is analogous to human TB, was not evident in wild-type or MMP-9 expressing transgenic mice (31). Animal models have also been used to study the effects of MMP inhibition in TB disease. The potent MMP-1 inhibitor cipemastat worsened TB pathology in the absence of concomitant TB treatment in a mouse model but did not impact cavitation in a rabbit model (32, 33). Combining MMP inhibition with TB treatment reduced tissue damage following *Mtb* infection in several other animal studies (reviewed in (34)). Administration of the broad MMP inhibitor, marimastat, promoted delivery and retention of rifampicin and isoniazid in the lungs of *Mtb*-infected mice due to an effect on vasculature, with increased pericyte coverage and decreased leakage of surrounding blood vessels (35). In humans, a randomized controlled trial of doxycycline, a broad MMP inhibitor, administered for two weeks alongside TB treatment demonstrated that doxycycline reduced sputum MMP levels, suppressed collagen and elastin destruction, and decreased pulmonary cavity volume on chest radiograph (36). These findings provide the most robust evidence to date that MMP inhibition can mitigate matrix destruction in pulmonary TB and potentially offer a strategy to reduce or prevent PTLD. However, these results should now be

replicated in larger cohorts with extended follow-up, including measurement of lung function.

Additional work is also needed to determine whether specific, more selective MMP inhibitors may be required, as has been seen in the cancer field (37, 38).

Neutrophils are innate phagocytes which may release MMPs and a range of inflammatory mediators in response to *Mtb*. Neutrophils have emerged as key players in TB immunity and immunopathology. Excessive neutrophil activity has been associated with the extent of inflammatory pathology and cavitation on chest radiograph, with a positive association reported between the peripheral blood neutrophil count and the extent of lung disease in TB patients, and increased concentrations of neutrophil-derived MMP-8, MMP-9 and S100A8 in patients with greater lung involvement on chest radiograph at baseline (39-41). In one study evaluating TB severity in humans, pulmonary destruction on chest radiograph, sputum *Mtb* load, disseminated TB, and death, were strongly associated with a relative expansion of circulating neutrophils, particularly subtypes of segmented and banded neutrophils, and a decrease in lymphocyte:neutrophil ratios (42). Although chest radiograph severity at the time of diagnosis cannot be considered a reflection of PTLD, natural history studies indicate that the extent of radiographic disease at diagnosis is a strong predictor of the risk of PTLD and baseline chest radiograph has been used as a screening tool to identify individuals at high risk of PTLD in clinical trials (43, 44). One study has investigated neutrophil function by extent of radiographic recovery at TB treatment completion, finding baseline granulocyte proportions post-stimulation (with ESAT-6/CFP-10 fusion protein and *Mtb* lysate in a whole blood assay) were increased in those with poor compared to good recovery (39).

In addition to the MMPs, evidence from patients and mouse studies suggests that neutrophil activity and products, including myeloperoxidase (MPO), neutrophil elastase (45), cytokines and chemokines (46), and even interferon (IFN)-induced neutrophil extracellular trap (NET) formation (47) may contribute to lung damage in TB disease. *Mtb*-infected neutrophils were determined to produce

NETs and co-localize with the collagenase MMP-8 *in vitro* (25). In TB patients, NETs were noted in sputum, and MMP8 was associated with cavitary disease on chest radiograph (25). Increased NET concentrations were also found in sputum from TB patients as compared to healthy controls (25). Moreover, neutrophil-specific inflammatory mediators like S100A8/9 and IL-8 have been associated with increased lung damage severity, with high *Mtb* loads and cavitation, in TB patients pre-treatment (39). In *Mtb*-infected C57BL/6 mice, IFN- $\gamma$  neutralization resulted in exacerbated inflammation and increased accumulation of S100A8-producing Gr1+ neutrophils (48). S100A8/A9 production, which was induced by IL-17 overexpression, further promoted neutrophil accumulation by inducing production of proinflammatory chemokines and cytokines and influencing leukocyte trafficking. In contrast, IFN- $\gamma$  neutralization in *Mtb*-infected S100A9 knockout mice resulted in loss of lung inflammation and reversed neutrophil accumulation, without impacting bacterial burdens. A separate study demonstrated dysregulated granulocytic influx promotes disease by creating a permissive long-lived intracellular niche for mycobacterial growth and persistence (49). These studies suggest a disconnect between tissue damage and bacterial burden, with TB disease driven by inflammatory host factors rather directly by the *Mtb* pathogen burden in the tissue. The complex relationship between *Mtb* pathogen burden and PTLD is discussed further below.

### **Fibroblasts and profibrotic activity**

Pulmonary fibrosis is likely a substantial contributor to multiple clinical manifestations of PTLD, including reduced exercise capacity and chronic hypoxia (50-52). In a small cohort study of 30 people with pulmonary TB in India, elevated concentrations of the profibrotic cytokine transforming growth factor- $\beta$  (TGF $\beta$ ) and IL-6 were associated with worse lung function and greater respiratory symptoms, respectively, after TB treatment completion (53). Given the prevalence of TB globally and the prevalence of PTLD among TB survivors, TB is arguably the single leading cause of pulmonary fibrosis around the world. However, to date there has been strikingly little investigation into TB-specific mechanisms of fibrogenesis. Rather, any understanding of TB-associated fibrosis is primarily

inferred from studies of fibrosis in other pulmonary diseases. Idiopathic pulmonary fibrosis (IPF) is the most intensively studied pulmonary fibrotic process and serves as the basis for comparative understanding of fibrosis in other disease processes, including TB, however the underlying pathologic drivers are likely quite different. The cells associated with fibrosis and molecules that contribute to fibrogenesis are of particular interest, as both offer potential therapeutic targets. In IPF, defined subsets of myofibroblasts have been associated with fibrogenesis, and endothelial cell injury and apoptosis have been implicated as early causative events (reviewed in (29)). A role for epithelial cell-derived secreted factors, including MMPs, in driving migration, activation and proliferation of myofibroblasts and extracellular matrix remodelling has been proposed in IPF (29, 54). The technical approaches used to advance an understanding of molecular and cellular contributors to IPF pathogenesis, including single-cell RNA sequencing and complementary immunofluorescence to define spatial relationships, hold significant promise for similarly advancing our understanding of TB-associated fibrosis (55, 56).

Focused studies in cellular and animal models of TB have offered some insights into TB-associated fibrogenesis. While few cellular models are amenable to studying complex processes such as TB-associated fibrosis, exposure of human fibroblasts to *Mtb* results in the production of the secreted pro-fibrotic connective tissue growth factor (CTGF) in a Toll-like receptor (TLR2)-dependent fashion (57). To date, no small animal models of TB have been applied to study the mechanisms of TB-associated fibrosis within tissue, but a few studies have used non-human primate (NHP) models. In one study, NHP granulomas were stratified based on histopathologic staining for collagen to probe potential molecular associations with fibrosis. TGF $\beta$  and  $\alpha$ -smooth muscle actin ( $\alpha$ SMA), two molecules implicated in fibrosis were found to be associated with fibrotic TB granulomas (58). In a subsequent study, computational models validated with NHP specimens pointed to macrophage-to-fibroblast transformation as a key mechanism underlying granuloma-associated fibrosis in TB (59). Extension of that computational work suggested that the local cytokine milieu may contribute to the

distinct patterns of fibrosis observed within granulomas with different spatial patterns of collagen deposition (60). The development of small animal models that reflect human-like lung fibrotic pathology will enable mechanistic studies that identify candidate targets for anti-fibrosis therapies and pre-clinical studies of efficacy.

Ultimately, a full understanding of TB-associated fibrogenesis will depend upon tissue-centric studies in human lung samples. Studies taking advantage of more accessible compartments can offer insight into circulation of molecules known to be associated with fibrosis (61); however, understanding the spatial molecular and cellular relationships that drive fibrosis is necessary to developing future interventions. While animal models can suggest what the cells, molecules, and relationships that drive TB-associated fibrosis might be, human tissue-based studies will be necessary to confirm or refute the relevance of predicted associations in human disease. Further, such investigations must be carried out using tissues samples from multiple time points during and after treatment of TB disease, to enable an understanding of both early and late events in fibrogenesis and lung remodeling.

### **Granuloma fate and cell death pathways**

TB granulomas embody a spectrum of disease states, featuring dynamic and spatially organized structures comprising macrophages, granulocytes, fibroblasts, and lymphocytes, among others. This intricate composition gives rise to a diverse spectrum of lesions, encompassing necrotic, non-necrotic, caseous, fibrotic, cavitory, and calcified manifestations, whose fate is directly intertwined with the structural changes observed in PTLD. While the necrotic granuloma is commonly perceived as the quintessential histopathological feature of *Mtb* infection, it is noteworthy that necrotic granulomas can also be induced by nontuberculous mycobacteria (NTM), sarcoidosis, fungal infections (e.g., histoplasmosis, cryptococcus, blastomycosis, coccidiomycosis), and certain vasculitides (e.g., granulomatosis with polyangiitis). The ultimate trajectory of the TB granuloma

toward either effective healing or lung immunopathology associated with PTLD will depend on the type and extent of the host immune response, *Mtb* strain, and their interaction. A granuloma can effectively contain bacteria to prevent dissemination, as for those rich in inducible Bronchus-Associated Lymphoid Tissue (62). However, it can also serve as a reservoir for uncontrolled replication, as seen in neutrophil-rich granulomas (63).

Meanwhile, historical studies suggest that caseation leads to bacillary destruction, a proposition substantiated by the absence of acid-fast bacilli within caseum as demonstrated by Canetti in 1955, and routinely observed in modern-day pathology laboratories (64). Although new, more sensitive techniques including RNAscope, have been shown to detect *Mtb* RNA in lesions that are absent of stained acid-fast bacilli (65). As a hallmark of primary TB, caseating granulomas have been of considerable focus in animals models investigating the outcome of primary infection. However, post-primary TB, the phenotype of disease that reflects most adult pulmonary TB, is characterized by obstructive lobular pneumonia with abundant neutrophil infiltration (66). Secondary granulomas form as a consequence, rather than cause of cavitation in post-primary TB.

Technological advances in the last several years have allowed unbiased approaches combining transcriptomic, proteomic, and imaging techniques to perform landscape analyses of diverse granulomas with a high degree of resolution. Techniques such as laser microdissection coupled with mass spectrometry (67, 68), immunofluorescence detection (69), multiplexed ion beam imaging by time of flight (MIBI-TOF) (70), single cell RNAseq (71), and micro/nano-computed tomography (72, 73) have revealed tissue microenvironments within and between granulomas with distinct immunological signatures. A recent study in cynomolgus macaques investigating longitudinal granuloma development, which would be difficult to perform in humans, showed that early-appearing granulomas with a high bacterial burden were characterized by a type 2 anti-inflammatory immune response whereas late-appearing granulomas with a low bacterial burden had

a Th1/Th17-mediated pro-inflammatory response (71). These studies also revealed extensive inter- and intra-lesion heterogeneity of granulomas within a single animal, which was highly influenced by the balance between pro-inflammatory and anti-inflammatory/regulatory immune responses within the different tissue microenvironments. The ultimate course of the granuloma, whether it leads to successful healing or lung immunopathology and PTLD, hinges upon factors such as the nature and intensity of the host immune response to bacillary burden and antigens, and the intricate interplay between the host and the pathogen. Further investigation with longitudinal sampling of granulomas during the course of disease is needed to determine the timing and molecular mechanisms that drive distinct granulomas and lung microenvironments towards these different PTLD manifestations.

The timing and triggering of different cell death pathways across cell types also influences initial granuloma fate and the phenotype and resolving or non-resolving nature of disease that develops (74). Apoptosis, primarily considered a protective non-inflammatory response will cause the infected cell to die and be cleared by neighbouring phagocytes and at the same time kill and degrade the intracellular bacilli trapped in the apoptotic cell, enhancing antigen presentation to T and B cells. *Mtb* has developed apoptosis-evasion mechanisms, tipping the cell to trigger various necrotic inflammatory cell death pathways that benefit the bacterium (75, 76). Inflammatory cell death releases alarmins, endogenous immunomodulatory molecules, to coordinate immune cell help, but rupture of the cell membrane also enables the release of both *Mtb* and cellular proteases that degrade tissue matrix.

While *Mtb* has been shown to trigger a variety of inflammatory cell death programs, how these relate to the phenotypes of TB disease and their long-term impact on PTLD are relatively unknown. For example, ferroptosis, a cell death pathway associated with increased iron and lipid peroxidation, is induced only with high multiplicity of infection. Its relevance to human TB is not well established, but it may be that ferroptosis is a late response at states of high *Mtb* load and contributes to local

tissue necrosis (77). Differential regulation of the inflammasome pathway that leads to pyroptosis has also been identified between clinical *Mtb* strains from patients with different degrees of involvement on chest radiograph, again raising the complex question of how mycobacterial factors may impact the risk of PTLD (78).

### **Mycobacterial factors and pathogen burden**

Although it is recognized that PTLD arises from intricate interactions between host, pathogen, and the environment, mycobacterial factors that may contribute to the development of PTLD have not been well studied. Rather than directly driving tissue injury, bacterial factors are likely to be contributing primarily through their interactions with the host. Nevertheless, multiple studies have reported associations between TB-associated lung injury, pathogen burden, the presence of drug resistance, and *Mtb* strain diversity.

One key question is precisely how host inflammatory responses to the bacterium contribute to acute lung injury and pathologic remodeling. Multiple *Mtb* products, including cell wall and envelope components trehalose-6,6' dimycolate (TDM) and lipomannan (LM), are recognized by TLRs on host cells, with resulting release of pro-inflammatory cytokines (79). Further, intracellular components such as heat shock proteins and bacterial nucleic acids interact with intracellular TLRs, further propagating inflammation (79). The *Mtb* ESX-1 secretion system is required for activation of multiple components of the host response to *Mtb* infection, including the type I IFN response and activation of AIM2 and NLRP3 inflammasomes with subsequent release of the pro-inflammatory cytokines IL-1 and IL-18 (80-82). Further, secretion of ESX-1-dependent effectors Early Secreted Antigenic Target 6 (ESAT-6) and Culture Filtrate Protein 10 (CFP-10) inhibit macrophage phagolysosome fusion, induce IFN- $\gamma$  production, activate T cells and promote granuloma formation (79). ESX-1 proteins and the cell membrane component phthiocerol dimycocerosates (PDIM) also damage host cell membranes and promote cellular necrosis (83).

Recent work has identified a secreted *Mtb* effector, protein tyrosine phosphatase A (PtpA), that translocates to the infected macrophage nucleus and suppresses expression of a ferroptosis and pyroptosis inhibitor, glutathione peroxidase-4 (GPX4), by regulating macrophage DNA methylation via protein arginine methyltransferase 6 (PRMT6) (84). Another study found PtpB blocks pyroptosis by dephosphorylating host membrane lipids to prevent the pyroptosis effector cleaved gasdermin D (GSDMD) from inserting into the plasma membrane (85). By limiting host clearance of *Mtb*, this inhibition of pyroptosis may contribute to prolonged inflammation, potentially increasing the risk of PTLD.

Exactly how each of these components of the inflammatory response then contributes to acute tissue injury and/or pathologic tissue remodeling is an important area for future investigation. The capacity of the bacterium to evade sterilization by the host immune system, resulting in progressive immune cell recruitment and inflammatory signaling cascades at sites of infection is central to the TB disease process. *Mtb* factors that drive host cell death and enable bacterial survival in host cells, including PDIM and ESX-1, might thus be similarly considered to contribute to PTLD. However, despite the critical role for these proteins and lipids in *Mtb* infection and pathogenesis, their role in PTLD has not been studied. Animal models that enable quantification of individual components of PTLD are necessary to systematically and rigorously determine the contribution of individual bacterial factors to post-treatment features such as fibrosis, bronchiectasis, and residual cavitation.

There are conflicting data on the association between *Mtb* strain lineage and patterns of lung injury in TB patients. For example, while the *Mtb* Lineage 2 was associated with cavitary TB in one study from Russia, another study from Singapore found that Lineage 2 actually had a lower propensity to cause cavitation than other strains (86, 87). These conflicting reports highlight the complexity of the interactions between *Mtb* and the host immune response, including whether these interactions may

be impacted by different sublineages, different host populations and genomics, and different TB treatment regimens. Using a C3HeB/FeJ mouse model, Verma et al demonstrated that “high-transmission *Mtb* strains,” which were collected from individuals where a high proportion ( $\geq 70\%$ ) of TB household contacts had a positive tuberculin skin test (TST), had a lower bacterial burden but developed caseating granulomas with high potential to cavitate (88). In contrast, mice infected with “low-transmission *Mtb* strains,” collected from individuals where a low proportion ( $\leq 40\%$ ) of household contacts had a positive TST, exhibited significantly higher total lung bacterial burden with higher intracellular *Mtb* replication, developed diffuse inflammatory lung pathology, high neutrophil influx, and induced significantly higher lipid droplet formation in macrophages (88). During the chronic stages of infection, mice infected with low-transmission strains showed widespread tissue destruction while those infected with high-transmission strains had expanding, well-formed granulomas and higher extracellular numbers of *Mtb* bacilli despite a lower total *Mtb* burden (88). These data suggest that strains with different transmission potential may also induce distinct pulmonary pathology. Further studies are needed to determine whether strain-level factors, and how those factors elicit particular aspects of the host immune response, impact the development of specific post-TB pathologic sequelae.

Insight into the impacts of mycobacterial strain on the risk of PTLD may also be garnered from a comparison of *Mtb* and *Mycobacterium africanum* (*M. africanum*), an important cause of TB disease in parts of West Africa. Although there are no published studies specifically focused on post-TB sequelae after *M. africanum* disease, there are conflicting data as to whether the extent of radiographic disease and cavitation at the time of diagnosis is higher or lower with *M. africanum* as compared to *Mtb* (89, 90). In addition, both the response to standard TB treatment regimens and the host immune response may differ for *M. africanum*, both of which could plausibly influence the risk of PTLD (91-93). Studies directly comparing PTLD rates and features between *Mtb* and *M. africanum* could yield important insights into how strain and lineage contribute to PTLD.

The causative relationship between *Mtb* burden and PTLD remains uncertain. While there are limited data to shed light on this question, several studies have found associations between *Mtb* burden and histologic and radiologic changes in TB patients and animal models, including positive associations between bacterial burden and fibrosis within granulomas in both macaques and humans (71, 94). *Mtb* burden detected in sputum in human disease may more likely reflect the extent of lung damage facilitating the translocation of *Mtb* bacilli into the airways, and *Mtb* growth in the wall of the cavity (95), than the total tissue *Mtb* burden. Thus, it remains unclear if high *Mtb* burden is a cause, or a consequence, of fibrotic or cavitary lung lesions and the status of the host immune response is likely central to this relationship (see Figure 2).

There are also limited data on the association between drug resistance and the risk of PTLD. A recent cross-sectional study from Peru reported that survivors of multidrug-resistant TB (MDR-TB) are more likely to have a restrictive pattern on spirometry testing than survivors of drug-susceptible TB (96). However, such findings likely reflect diagnostic delays for drug-resistant disease and/or the prolonged *Mtb* survival in tissues in the setting of less effective drug regimens, both of which would prolong the local inflammatory response and ongoing tissue damage, rather than the drug resistance itself. The development of preclinical models for PTLD, discussed below, will enable investigation of whether differences in mycobacterial strain and early bactericidal activity of antimycobacterial regimens impact the risk of PTLD. Adding measures of pulmonary impairment (e.g., chest radiography, spirometry) to ongoing clinical trials of novel TB treatment regimens will also provide critical human data as to the role of ongoing bacterial replication in driving the risk of PTLD.

### **Experimental models of PTLD**

Animal models can play a crucial role in enhancing our understanding of PTLD. Mechanistic and pre-clinical studies will ultimately require validated pre-clinical models of PTLD. While PTLD has multiple

component pathologies, to date animal models have mostly been used to study tissue destruction, as described for MMPs and neutrophils above, and granuloma formation and fate. Although mice are commonly used in studies of TB pathogenesis and offer logistical advantages for mechanistic studies, wild-type mice do not naturally develop necrotic tuberculous lesions, limiting their use in studying PTLD. Nonetheless, the C3HeB/FeJ and B6.Sst1<sup>S</sup> models develop necrotic granulomas resembling human TB lesions (97). While C3HeB/FeJ mice harbor multiple *Mtb* susceptibility loci, one of which is the Sst1<sup>S</sup> locus, the B6.Sst1<sup>S</sup> mouse model possesses only the Sst1<sup>S</sup> locus, which contains mutations in the SP110 and SP140 genes, backcrossed onto the C57BL/6 genetic background (98, 99). Macrophages from B6.Sst1<sup>S</sup> mice release high levels of Type 1 interferon (IFN-I) in response to TNF, leading to a proteotoxic stress response and up-regulation of the integrated stress response (ISR) (100). Inhibiting the ISR pathway with the small molecule inhibitor ISRIB reduces bacterial load and necrotic lung pathology in B6.Sst1<sup>S</sup> mice (101). Similarly, the IL-13 overexpressing transgenic mouse develops human-like necrotic granulomas following *Mtb* infection (102, 103). Granulomas in both of these models behave similarly to human lesions, including their ability to restrict access to certain anti-TB drugs. These mouse models provide valuable insights into causal mechanisms that may underlie lung tissue damage leading PTLD but require further validation to determine which pathologic features of PTLD are reliably reproduced in each model.

Other animal models have also been utilized for modeling TB disease and have characteristics that may be important for gaining insight into PTLD. The rabbit model, which can develop cavitory lung lesions, has yielded several essential insights regarding how cavities and their scar influence lung function (14, 104). The non-human primate (NHP) model, using either cynomolgus or rhesus macaques, is the most human-like animal model for TB infection and disease. Upon intrabronchial instillation of *Mtb*, approximately 50% of cynomolgus macaques develop progressive lung disease with various features including lymphadenopathy, granulomas, cavitation, and tuberculous pneumonia (105, 106). However, even with the ability to control for *Mtb* strain during inoculation,

there remains substantial heterogeneity in the spectrum of pulmonary involvement and eliciting consistent disease outcomes remains challenging.

To date, animal model studies of TB have not traditionally incorporated measures and outcomes specifically focused on PTLD. However, a recent report describes the development of a mouse model to quantify fibrosis during and after TB treatment, opening the door to much-needed mechanistic studies (107). Ideally, such studies would incorporate multiple, complementary measures, including pathogen factors, histopathology, radiographic assessments, and dynamic measures of lung function, which are increasingly available, even for small animal models (108). Further, these outcomes could be assessed at multiple time points during and after TB treatment, to provide longitudinal insights into the trajectory of lung damage, remodeling, and recovery which could be paired with multi-omics measures of host responses (Table 2).

Given the limited availability of applicable *in vivo* models, there is also a need to develop *in vitro* models for studying PTLD (109). An ideal *in vitro* model would utilize primary human cells and replicate the complex three-dimensional, multi-cellular environment of the lung while also reflecting the physiologic and cellular characteristics of TB-affected human lungs (110). Three-dimensional lung matrix models and airway organoids have been used to study immune responses in TB, but do not yet model long term outcomes (111, 112). These pre-clinical models, however, have the potential to be utilized for studying specific pathogen factors and toxicity of novel therapeutics, thus speeding up the discovery and evaluation of new host-directed therapies. Incorporating fibrotic elements or matrix-producing cell types would make it possible to experimentally mimic the pathological changes observed in PTLD and investigate host-pathogen interactions. The integration of high-throughput methodologies would also facilitate the rapid screening of potential therapeutic agents to prevent or mitigate the development of PTLD.

### **Interactions between clinical risk factors and pathogenesis of PTLD**

There are substantial gaps in our understanding of how the five key risk factors for TB disease—undernourishment, HIV infection, alcohol use disorders, smoking, and diabetes mellitus—impact the pathogenesis and molecular pathways associated with the development of PTLD (1). Rather, many studies have examined the association between clinical risk factors and the extent of radiographic involvement at TB diagnosis, which has, in turn, been associated with the risk of PTLD (44, 113). There is a similar paucity of information about the impact of other key clinical risk factors for TB, including indoor and outdoor air pollution and occupational exposures like silica dust on the long-term risk for PTLD (114-116). It is also important to recognize that these clinical risk factors are often associated with social determinants of health, which could result in delayed care seeking behavior and delays in TB diagnosis, which would likely increase the extent of lung damage prior to treatment initiation.

#### *HIV*

Although HIV is the greatest individual risk factor associated with TB, it remains uncertain how HIV may impact the risk of PTLD. A cross-sectional study conducted at community health clinics in Nigeria found that people with HIV had an increased risk of chronic lung disease (defined by either self-report or abnormal spirometry) in models adjusted for a history of TB (adjusted odds ratio [aOR] 2.35, 95% CI 1.33-4.17), reflecting a greater risk for PTLD in people with HIV (117). However, in a similar analysis of people with and without HIV in western Kenya, HIV infection was not associated with impaired spirometry after adjustment for a history of TB (aOR 1.1, 95% CI 0.60-1.9), nor was HIV coinfection associated with symptomatic PTLD (aOR 1.1, 95% CI 0.4-2.9) in a cohort study from Tanzania (118, 119). Further, in a longitudinal study of TB survivors, HIV coinfection was associated with higher lung function 3 years after treatment completion (120). However, this association was no longer significant after adjustment for radiologic extent of disease at TB treatment completion, suggesting that people with and without HIV with similar extent of lung involvement may have a

similar risk of PTLD. No studies have explicitly examined the association between CD4 count and PTLD, but CD4 count has been linked to the extent of radiographic disease at diagnosis (41, 121), indicating that individuals with a higher CD4 count may be at greater risk for PTLD. Of note, for the studies in Nigeria, Kenya, and Malawi, more than 80% of the people with HIV were on antiretroviral therapy and/or had an undetectable viral load. While few studies have explicitly examined the pathogenesis of PTLD according to HIV status, higher plasma levels of MMP-8, but not MMP-9, were seen in patients with TB/HIV co-infection than those with TB alone in a study in Indonesia (122). Further, in two studies of people with and without HIV with pulmonary TB in South Africa, baseline peripheral blood neutrophil count, and circulating IL-6 and IL-1RA expression were associated with the extent of chest radiograph involvement and cavitation respectively, but these associations were independent of HIV status (41, 123).

### *Diabetes mellitus*

The epidemiologic association between TB and diabetes mellitus is well established, including the greater risk of poor treatment outcomes and greater mortality both during and after TB treatment completion among those with TB and diabetes (124-126). In a study from Peru, people with TB who had dysglycemia had significantly more cavities, alveolar infiltrates, and fibrous tracts at the time of TB diagnosis, and the odds of abnormal radiographic findings increased per unit increase in HbA1c (127). A recent review of the converging epidemics of TB and diabetes by Ronacher et al. focused primarily on susceptibility to TB and impaired antimycobacterial immunity for people with diabetes, but they also discussed the altered inflammatory milieu in diabetes (128). Such alterations range from lower frequencies of *Mtb*-specific T cells and higher Th1 and Th17 cytokines to the accumulation of advanced glycation end products and reactive oxygen species, all of which have implications for the risk of lung damage, delayed healing, and PTLD. Likewise, a series of studies by Kumar et al. conducted in India identified a number of features of TB disease among those with diabetes that might increase the risk for PTLD, including: (1) delayed resolution of systemic

inflammation at the end of TB treatment (129), (2) upregulation of inflammatory lipid eicosanoids during and after TB treatment (130), (3) higher levels of multiple MMPs at the time of TB diagnosis for people with diabetes, which were also significantly correlated with HbA1c (131), and (4) higher levels of the inflammatory mediators advanced glycation end products, sRAGE (soluble receptor for advanced glycation end products), and the neutrophil-derived protein S100A12 in people with diabetes and TB that persisted throughout TB treatment (132). Another study from India found both greater correlations between pro-inflammatory lipid profiles and greater network connectivity between pro-inflammatory and pro-resolving lipid mediators in those with TB and diabetes as compared to those with TB alone (133). In the context of PTLD, such greater connectivity may have implications for the trajectory of lung damage and remodeling over the course of TB treatment.

#### *Undernutrition*

While no studies have investigated the relationship between undernutrition and PTLD, several studies have found an association between undernutrition, typically defined as a low body-mass index (BMI), and the extent of chest radiograph involvement or the presence of cavitation at the time of TB diagnosis (134-137). Low BMI has also been associated with poor treatment outcomes of treatment failure, relapse, and death (138). A recent randomized trial in India found that nutritional supplementation led to reduced mortality for TB patients and a reduced incidence of TB among their household contacts, but outcomes relevant to PTLD were not reported and so it is unknown whether nutritional supplementation would be associated with less chest radiograph involvement at the time of TB diagnosis or better lung function after TB treatment completion (139, 140). Finally, in a cross-sectional study of people with PTLD in Nigeria, weight loss was associated with poor health-related quality of life (141).

#### *Smoking and alcohol use disorders*

A recent systematic review and meta-analysis found that tobacco use increased the risk of poor TB treatment outcomes, including recurrence, relapse, and death during treatment (142). With regards to the risk of PTLD and smoking, prior TB was associated with airflow limitation even after adjustment for smoking in a nationally representative survey from Korea (143). There was also evidence of statistical interaction between smoking and previous TB on airflow limitation, with a greater risk of airflow limitation according to the number of cigarette pack-years smoked (143). In addition, in a prospective cohort study in Mozambique, a greater than 10 pack-year smoking history was associated with a greater risk of PTLD (RR 7.5, p 0.06) (144). Finally, in a study from Cameroon, inflammatory cytokine levels in sputum were lower among those with PTLD as compared to smoking-related COPD (145), suggesting that people with airflow obstruction after successful TB treatment may have less persistent inflammation in the lungs than those with ongoing smoke exposure.

In contrast to the growing literature supporting associations between smoking at PTLD, there are no studies examining the impact of alcohol use disorders on PTLD. However, as with other clinical risk factors, multiple studies have reported an association between alcohol use disorders and both cavitation and radiographic involvement at the time of TB diagnosis and poor TB treatment outcomes (146-148), all of which would point to a higher risk for PTLD.

### **Research priorities**

Despite the progress made in understanding matrix destruction in TB, many knowledge gaps with respect to the pathogenesis of PTLD still exist (Table 1). There is a need for small animal models that better represent and recapitulate key features of human pulmonary TB and PTLD, including matrix destruction, cavitation, and fibrosis, and the extent of residual disease following treatment (Table 2). With such models, the contributions of individual molecules and cellular subsets in either matrix

destruction and/or pathologic repair can be established. Determining which cell death pathways in which cells lead to which pathology, how bacterial factors and different *Mtb* strains regulate these pathways, and whether different timing in skewing towards certain cell death pathways leads to different outcomes will also be critical to understanding the contributors to necrotic tissue destruction in TB. Likewise, models that allow for the evaluation of key comorbidities will help elucidate how various molecular mechanisms may be impacted by clinical risk factors known to exacerbate disease severity (e.g., HIV coinfection, diabetes mellitus, undernutrition, alcohol use, and cigarette smoking). In the case of the NHP model, given that cynomolgus macaques are known to develop many features of human pulmonary TB, repeated lung function measures should be included in NHP studies of TB pathogenesis and treatment. The development and validation of these small and large animal models will ultimately enable the development and preclinical testing of novel therapeutics designed to prevent or reduce PTLD.

Ultimately, any findings from animal models will need to be validated in humans. In-depth analysis of human lung tissue samples from people with TB disease, whether from surgical resections or autopsy samples, will help to confirm the relevance of any pre-clinical findings in human disease. The addition of endpoints relevant to PTLD in both observational cohorts and clinical trials will also enhance our understanding of the clinical risk factors and biomarkers associated with PTLD and allow for the definition of both clinical phenotypes and biological endotypes of PTLD. Such studies would require time points extending beyond TB treatment completion to shed light on how lung remodeling may continue to evolve even after microbiologic cure. It will be equally important to include pediatric cohorts in these studies, as recent data indicate that children who complete TB treatment also suffer from post-TB morbidity and reduced lung function (149). The incorporation of advanced imaging modalities has the potential to provide further insight into both cellular metabolism and activity, with PET imaging using fluorodeoxyglucose (FDG) or novel tracers targeting type-1 collagen (150-152), and gas exchange in the lung, with hyperpolarized xenon MRI (153).

Funders of TB treatment trials need to acknowledge that extended follow up is essential and allocate funds accordingly. Efforts to establish consensus around standardized measures and definitions for PTLD in order to facilitate comparisons between studies are underway (154). Validation of these definitions is an important next step in this process.

## **Conclusion**

Given increasing recognition of PTLD as a major contributor to global pulmonary morbidity, a greater understanding of the underlying pathogenesis is necessary to identify therapies to improve long-term outcomes for TB survivors. Yet, large gaps exist in our understanding of the biological mechanisms underlying PTLD and how key clinical risk factors influence those mechanisms and the accompanying long-term risk of PTLD. Addressing these gaps will require concerted efforts from researchers, clinicians, policymakers, and funders in order to identify synergies that can be gained by extending existing studies of TB pathogenesis with the addition of clinical endpoints relevant to PTLD. Such studies, in both animal models and humans, will be critical to understand how best to prevent excessive matrix damage, aberrant remodeling, and fibrosis in people with pulmonary TB. Over the last decade, we have witnessed significant progress in the prevention, diagnosis, and treatment of TB (155); it is now time to meaningfully advance our understanding of PTLD.

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**Box 1.** Consensus definition and proposed clinical patterns of post-TB lung disease (PTLD) from the First International Post-Tuberculosis Symposium(156)

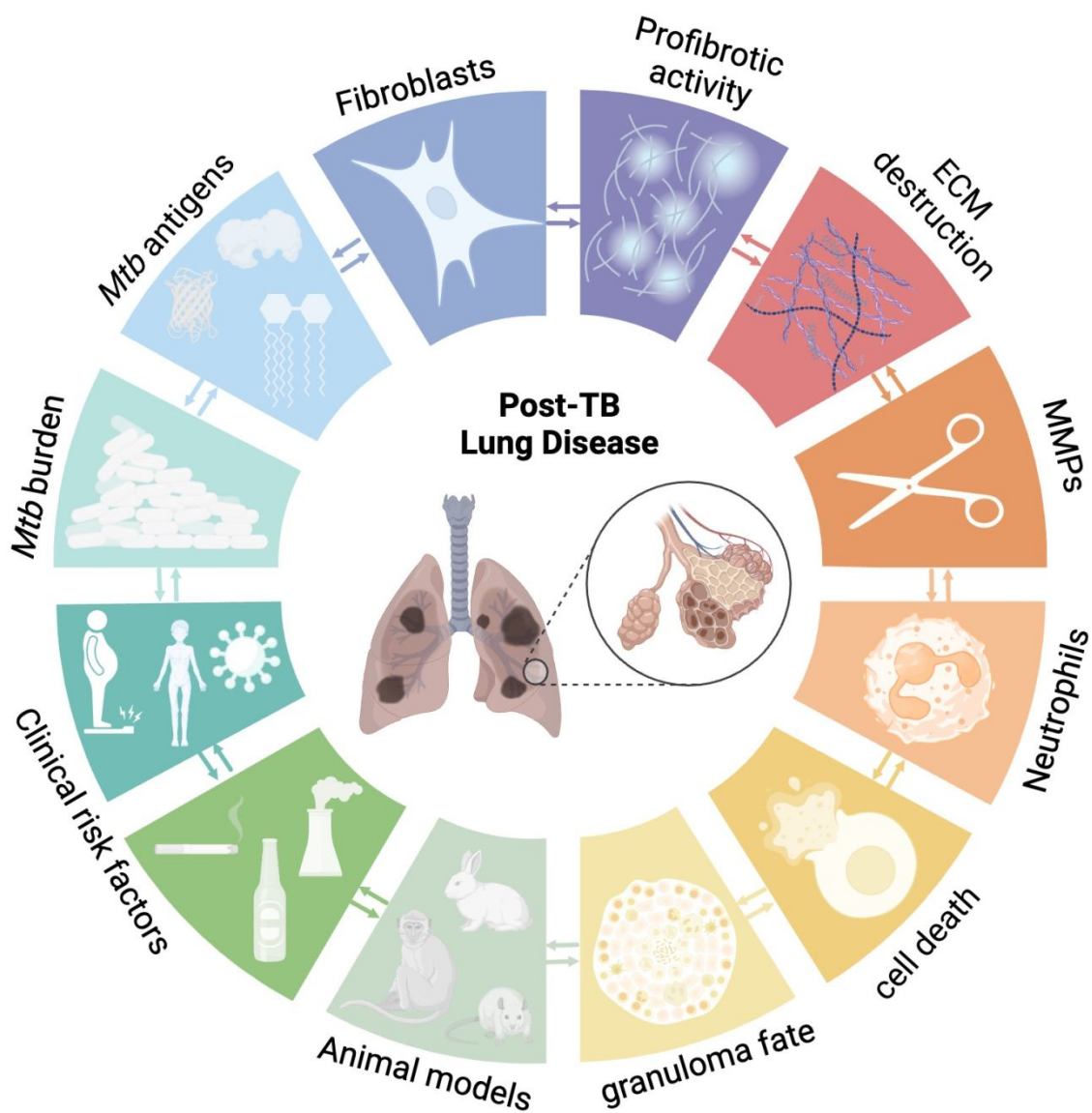
*Minimum case definition of PTLD:* Evidence of chronic respiratory abnormality, with or without symptoms, attributable at least in part to previous tuberculosis.

*Proposed clinical patterns of PTLD:*

- (1) Airways: TB-associated **obstructive lung disease** with airway obstruction on spirometry OR **bronchiectasis** on chest radiography
- (2) Parenchyma: **Cavitation, parenchymal destruction**, or fibrotic change on chest radiography OR **aspergillus-related lung disease** on imaging and/or blood testing
- (3) Pleural: **Pleural thickening** on chest imaging
- (4) Pulmonary vascular: **Pulmonary hypertension** with elevated pulmonary artery pressures on echocardiography and/or right heart catheterization
- (5) Other: Other pathology not meeting the above criteria.

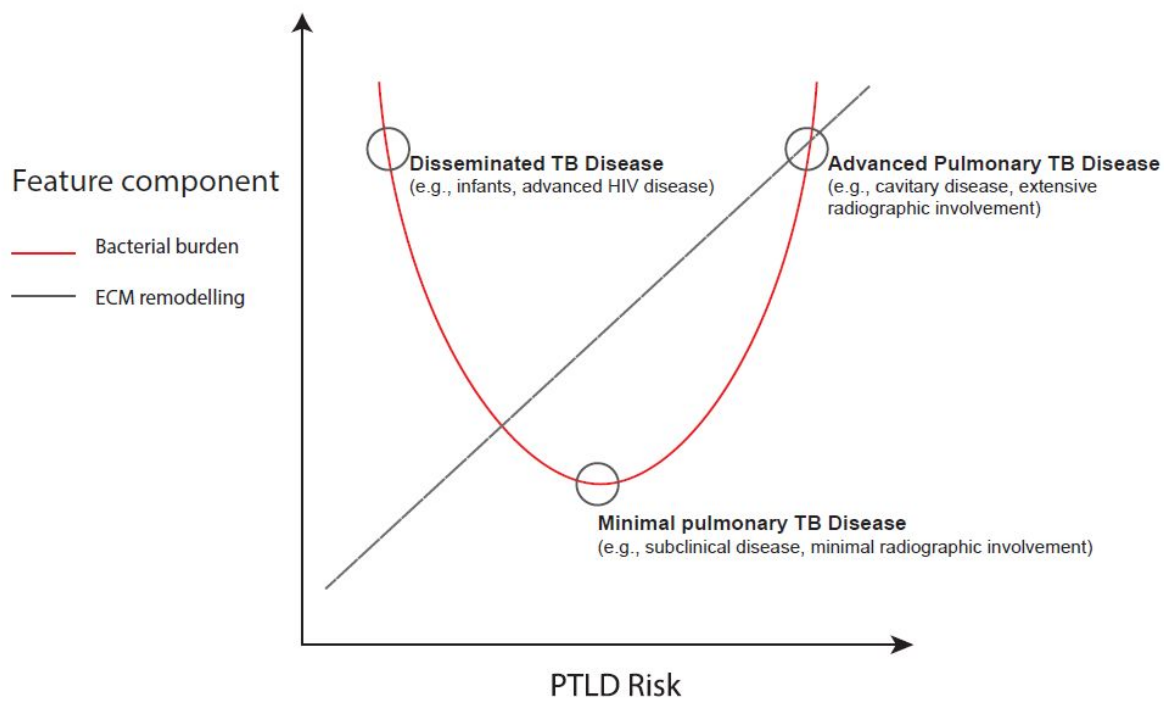
**Figure 1.** Consensus key areas of importance in Post-Tuberculosis Lung Disease (PTLD) pathogenesis.

Created with BioRender.com. *ECM* = extracellular matrix; *MMPs* = matrix metalloproteinases; *Mtb* = *Mycobacterium tuberculosis*.



**Figure 2. Proposed relationship between *Mtb* bacterial burden, extracellular matrix (ECM)**

**remodeling, and risk of PTLD.** Disseminated TB disease often occurs in the setting of immunodeficiency (e.g., advanced HIV) or a less developed immune system (e.g., infants) and is characterized by less clinically evident ECM damage and remodeling. In disseminated disease, sputum *Mtb* burden may be low due to lack of cavitation but there is a high *Mtb* burden in tissues on histopathological analysis and/or post-mortem findings and mortality is typically high. In patients with minimal pulmonary disease, there is typically a low *Mtb* burden with moderate pulmonary immune responses and accompanying ECM damage remodeling, in addition to a relatively lower risk of PTLD. In advanced pulmonary disease, including classical post-primary TB with upper lobe cavitation, there is a high *Mtb* burden, often with accompanying sputum smear acid-fast bacilli (AFB) positivity, and more extensive ECM damage and remodeling, with a greatly increased risk of PTLD.



**Table 1. Major findings and research priorities for pathogenesis of post-TB lung disease.**

Topic area	Major findings to date		
	Findings from animal models of TB	Findings from human studies of TB	Findings specific to post-TB lung disease
<b>Matrix destruction, MMPs, and neutrophils</b>	<ul style="list-style-type: none"> <li>• Cipemastat (MMP-1 inhibitor) worsened TB pathology in the absence of anti-tuberculosis treatment in a mouse model</li> <li>• Combining MMP inhibition with TB treatment reduced tissue damage</li> <li>• Marimastat (broad MMP inhibitor) improved delivery and retention of anti-TB drugs in the lungs of <i>Mtb</i>-infected mice via effects on vasculature</li> <li>• IL-17 implicated in neutrophil accumulation through S100A8/9 and proinflammatory cytokine networks, regulated by IFN-<math>\gamma</math></li> </ul>	<ul style="list-style-type: none"> <li>• Elevated MMP-1, -2, -3 and -8 in sputum, associated with cavitation</li> <li>• Neutrophil abundance, phenotype, and activity associated with extracellular matrix destruction and immunopathology</li> <li>• MMPs, advanced glycation end products, sRAGE, and neutrophil-derived S100A12 levels higher at the time of TB diagnosis in people with diabetes mellitus; MMP levels correlated with HbA1c</li> <li>• Adjunctive doxycycline (MMP inhibitor) with TB treatment reduced sputum MMPs, suppressed collagenase activity, and decreased cavity volume</li> </ul>	<ul style="list-style-type: none"> <li>• MMP-1 polymorphisms associated with more extensive pulmonary fibrosis and increased risk of tracheobronchial stenosis after TB treatment completion</li> </ul>
<b>Fibroblasts and profibrotic activity</b>	<ul style="list-style-type: none"> <li>• Exposure of human fibroblasts to <i>Mtb</i> results in pro-fibrotic connective tissue growth factor secretion, in a TLR2-dependent fashion</li> <li>• In non-human primates, TGF<math>\beta</math> and <math>\alpha</math>SMA were associated with fibrotic granuloma formation following <i>Mtb</i> infection; macrophage-to-fibroblast transformation and local cytokine milieu implicated by computational modelling</li> </ul>		<ul style="list-style-type: none"> <li>• Elevated levels of TGF<math>\beta</math> and IL-6 associated with lower lung function and more symptoms after TB treatment completion</li> </ul>
<b>Granuloma fate and cell death pathways</b>	<ul style="list-style-type: none"> <li>• Interplay of pathogen and host factors within granuloma, including cell death pathways, determines <i>Mtb</i> elimination, control, or disease</li> <li>• In NHP models, timing of granuloma formation after <i>Mtb</i> infection corresponds to <i>Mtb</i> burden and cytokine profile</li> </ul>	<ul style="list-style-type: none"> <li>• Radiographic severity of TB linked to differential regulation of inflammasome pathway and pyroptosis</li> </ul>	

<b>Mycobacterial factors and pathogen burden</b>	<ul style="list-style-type: none"> <li>• Multiple <i>Mtb</i>-derived molecules can trigger host inflammatory responses, including trehalose-6,6' dimycolate, lipomannan and bacterial nucleic acids</li> <li>• Early Secreted Antigenic Target 6, Culture Filtrate Protein 10) and phthiocerol dimycocerosates damage host cell walls and promote cellular necrosis</li> </ul>	<ul style="list-style-type: none"> <li>• High bacterial burden inside a TB cavity is reflected in higher <i>Mtb</i> loads in sputum in cavitory disease</li> <li>• Conflicting data on association between <i>Mtb</i> strain and risk of cavitory disease</li> </ul>	<ul style="list-style-type: none"> <li>• MDR-TB survivors in Peru more likely to have a restrictive pattern on spirometry than survivors of drug-susceptible TB</li> </ul>
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**Table 2. Research priorities and proposed strategies.**

<b>Overarching priorities</b>	<ul style="list-style-type: none"> <li>• Consensus and standardization of endpoints to quantify and classify the extent, course, and nature of matrix damage in both animal models and humans</li> <li>• Longitudinal studies with extended follow-up periods beyond completion of TB treatment, with sampling at multiple timepoints to investigate early and late events associated with post-TB lung disease in both preclinical and clinical studies</li> </ul>
<b>Preclinical model systems</b>	<ul style="list-style-type: none"> <li>• Development of new cellular and animal models to reflect the full spectrum of human disease, including cavitation and fibrosis, and with inclusion of post-treatment outcomes</li> <li>• Adoption of cellular and animal models developed for studying other pulmonary diseases (e.g., IPF, pulmonary hypertension) and assessing their utility for PTLTD</li> <li>• Inclusion of physiologic measures of lung function in animal model studies</li> <li>• Evaluation of mechanisms of bacterial invasion and dissemination that lead to prolonged host inflammatory responses</li> <li>• Investigation of mechanisms implicated in granuloma trajectory towards <i>Mtb</i> clearance or immunopathology</li> <li>• Identification of <i>Mtb</i> mutants that increase lung pathology</li> <li>• Development of advanced <i>in vitro</i> models utilizing primary human cells to replicate the complex 3D environment of the lung</li> <li>• Use of high-throughput multiparameter readout platforms for breadth and efficiency</li> <li>• Inhibition studies to identify key pathways amenable to intervention and host-directed therapies</li> <li>• Simulation of risk factors in model systems, e.g., dysglycemia and HIV coinfection</li> </ul>
<b>Human cohort studies and interventional trials</b>	<ul style="list-style-type: none"> <li>• Incorporation of translational and mechanistic sub-studies within ongoing clinical cohorts</li> <li>• Use of advanced imaging modalities (e.g., PET, hyperpolarized gas MRI) to gain insight into cellular metabolism and processes in clinical cohorts</li> <li>• Collection of lung tissue samples from sites of TB disease and from people with post-TB lung disease</li> <li>• Collection of <i>Mtb</i> isolates to enable analysis of the relationship between pathogen burden, strain, and resistance profiles with post-TB sequelae</li> </ul>

	<ul style="list-style-type: none"><li>• Cellular and tissue-based studies with samples from disease sites, including advanced high-throughput sequencing and multi-omics analyses</li><li>• Larger randomized trials of MMP inhibition (and other host-directed strategies) to prevent or reduce post-TB lung disease</li></ul>
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## Pathogenesis of Post-Tuberculosis Lung Disease: Defining Knowledge Gaps and Research Priorities at the 2<sup>nd</sup> International Post-Tuberculosis Symposium

**Supplemental Table 1.** Research areas considered for in-depth review, listed according to ranked choice voting results.

<b>Rank</b>	<b>Research area</b>
1	Extracellular matrix destruction
2	Cell death pathways
3	MMP dysregulation
4	Granuloma fate (e.g., loss of control & progression to active disease)
5	Pro-fibrotic pathways
6	Neutrophil recruitment
7	Pro-inflammatory cytokines
8	Mycobacterial load/burden
9	Myeloid cells (incl. MDSCs) and dysfunction
10	Mycobacterial virulence factors
11	Fibroblasts
12	NETs
13	Immunometabolism
14	Arachidonic acid pathway
15	Autophagy
16	PD-1/PD-1L axis
17	Loss of regulatory control
18	Epigenetic modifications