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## Tuberculous meningitis: a roadmap for advancing basic and translational research

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### Abstract

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Competing interests

The authors declare no competing interests.

Tuberculous meningitis is a serious, life-threatening disease affecting vulnerable populations, including HIV-infected individuals and young children. The US National Institutes of Health convened a workshop to identify knowledge gaps in the molecular and immunopathogenic mechanisms of tuberculous meningitis and to develop a roadmap for basic and translational research that could guide clinical studies.

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The World Health Organization (WHO) estimates that 10.4 million new cases and 1.7 million deaths occurred from tuberculosis (TB) in 2016 alone. Tuberculous meningitis (TBM) is a devastating disease, especially among HIV-infected individuals and young children, and accounts for 1–2% of all TB cases. Early diagnosis is challenging; outcomes are poor even with treatment<sup>1</sup>, and prolonged (12 months) antimicrobial treatments are recommended, but not on the basis of clinical trial evidence. Bacillus Calmette-Guérin vaccine, developed almost 100 years ago, provides limited protection, but only during early childhood. Although immunoinflammatory damage is the critical pathological process in TBM and is amenable to host-directed treatments (HDTs)<sup>2</sup>, pulmonary TB is still used as the treatment paradigm and limited efforts have been made to utilize preclinical models of TBM to guide clinical studies<sup>3</sup>. Increasing numbers of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* and limited access to the central nervous system (CNS) by key antimicrobials are additional challenges in combating this disease. Therefore, in May 2017, the US National Institutes of Health convened a two-day workshop to address these knowledge gaps (Table 1) and develop a roadmap for advancing TBM research.

## Pathogenesis and molecular mechanisms

Studies performed by Arnold Rich in the 1930s remain the current basis for TBM pathogenesis<sup>4</sup>. After initial infection of the lung, bacteria replicate and disseminate via the bloodstream. ‘Rich foci’ develop around bacteria deposited in the meninges and brain parenchyma during this initial bacteremia (Fig. 1). Months to years later, these foci rupture, with bacterial dissemination into the subarachnoid space, causing inflammatory meningitis. Although late-onset TBM occurs in adults and older children, meningitis in young children is not due to reactivation and often is concurrent with disseminated TB. ‘Paradoxical reactions’, that is, delayed development or worsening of brain lesions, can occur several months after the initiation of TB treatments, even in HIV-negative individuals<sup>5</sup>. Although the reasons for these delayed immunological responses in TBM are poorly understood, intracranially injected mycobacteria in rats escape immune recognition for months and delayed CNS lesions develop only after peripheral sensitization in these animals<sup>6</sup>. HIV-infected patients with TB can also develop immune reconstitution inflammatory syndrome (IRIS) after initiating antiretroviral treatments, and upregulation of certain innate signaling pathways that can predict IRIS has been identified recently. Overall, the dynamics of *M. tuberculosis*-induced immune responses and the development of granuloma-like lesions remain poorly characterized.

The CNS is protected from systemic circulation by tightly apposed brain microvascular endothelial and epithelial cells forming the blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier (BCSFB), respectively. Although these barriers are impermeable

to many large, hydrophilic molecules and circulating pathogens, many neuroinvasive bacteria, including *M. tuberculosis*, can invade the CNS, aided by specific microbial virulence determinants<sup>7</sup>. Although the mechanism(s) of *M. tuberculosis* traversal are poorly understood, endothelial cell invasion observed in vitro<sup>8</sup> and in animal models<sup>3</sup> and the extensive endovascularitis associated in humans with TBM<sup>4</sup> suggest that ‘extracellular’ bacteria can invade the CNS. Infected dendritic cells can also disseminate bacteria, and entry of intracellular *M. tuberculosis* through the choroid plexus (BCSFB) via infected monocytes (‘Trojan horses’) was also presented in mouse models. Understanding the molecular mechanism(s) of CNS bacterial invasion and identification of key microbial determinants would be helpful in developing novel therapeutic strategies for TBM<sup>9</sup>.

## Immune mechanisms and host-directed treatment strategies

Inflammation in response to bacterial infection in TBM causes endovascularitis, exudative meningitis, infarction, hydrocephalus and neuronal damage (Fig. 1). Experimentally induced intracisternal or intracerebral mycobacterial infections in mice and rabbits have demonstrated interferon- $\gamma$  (IFN- $\gamma$ )-dominated immune responses, microglial activation and elevated concentrations of proinflammatory mediators, including damage-associated molecular patterns (DAMPs), in the CSF<sup>3</sup>. Microglia are also critical for neurodevelopment, making the immature brain of young children uniquely vulnerable to injury. Host genetics may also have a key role. There is evidence that a variant of *LTA4H*, which encodes leukotriene A4 hydrolase, affects TBM-related mortality in multiple Vietnamese cohorts<sup>10</sup>, although a study in Indonesia did not find this association<sup>11</sup>. Analysis of CSF and serum metabolites in TBM has also identified an association between tryptophan metabolism and survival, likely mediated by host responses<sup>12</sup>.

Adjunctive corticosteroids improve survival by 25% in patients with TBM in the short term<sup>2</sup> but are ineffective against neurological deficits, at least in adults. Therefore, development of improved HDTs specifically for TBM is a critical need. Glutathione is an important antioxidant with potential anti-mycobacterial and immune-enhancing properties. Augmenting glutathione abundance in macrophages derived from HIV-infected individuals resulted in improved control of infection<sup>13</sup>. Thalidomide and its analogs effect several immune functions, and adjunctive thalidomide improved survival in a rabbit model of TBM<sup>14</sup>. However, a trial evaluating thalidomide (24 mg/kg) in children with TBM was discontinued prematurely because of serious adverse events and deaths, although subsequently, lower doses (4 mg/kg) of thalidomide have benefited individuals with refractory CNS masses, optic neuritis and steroid-resistant paradoxical reactions associated with TBM. Recently, a phase 2 trial in HIV-negative adults suggested that addition of aspirin to dexamethasone could reduce new infarcts or deaths in microbiologically confirmed TBM<sup>15</sup>.

Pathogen-associated molecular patterns (PAMPs) are microbial products recognized by host cell receptors that activate innate immune responses. DAMPs such as high-mobility-group box 1 (HMGB1) and S100B are released by damaged host cells and react with PAMPs, causing a vicious cycle of inflammation and cell death. Host poly(ADP-ribose) polymerase 1 (PARP1; also known as ARTD1) activates microglial cells to release HMGB1 and

potentiates the effects of S100B, resulting in brain injury. Inactivation of PARP1 in leukocytes diminishes inflammation and the migration of these cells across the BBB. Current US Food and Drug Administration (FDA)-approved inhibitors of DAMP, PARP1<sup>16</sup> and other innate immune pathways, for example, microbial phosphodiesterase subverting the stimulator of interferon genes (STING), are therefore promising HDT candidates for TBM. Paradoxical reactions and TB-IRIS would especially benefit from HDTs.

## Antimicrobial treatment

TBM is complicated by the limited CNS penetration of several key antimicrobials, particularly rifamycins. Compartmental modeling for solute exchange and drug delivery to the brain has been attempted, but lack of data on intralésional drug concentrations in infected tissues where *M. tuberculosis* reside<sup>17</sup> has limited these efforts. In a phase 2 trial, reduction in mortality was noted in patients with TBM treated with an intensified regimen using 'high-dose' intravenous rifampin (13 mg/kg) during the first two weeks<sup>18</sup>, although a subsequent larger randomized trial using oral rifampin (15 mg/kg) did not show this benefit<sup>19</sup>. These differences in clinical outcome may be due to suboptimal CNS exposures with the oral regimen; alternatively, outcomes could be more strongly associated with changes in intracerebral inflammation than with bacterial killing. Linezolid, an oxazolidinone, is a potent sterilizing antimicrobial with excellent CSF penetration, and retrospective studies from China suggest that addition of linezolid to the background TB regimen benefits patients with life-threatening TBM<sup>20</sup>. Prolonged antimicrobial treatments are recommended for TBM, but these recommendations are not based on clinical trial data and a shorter regimen (6 months) may be as effective, at least in HIV-negative individuals<sup>21</sup>. The CNS penetration and biodistribution of new or repurposed drugs (for example, bedaquiline, clofazimine and pretomanid), active against MDR-TB, are not well established. Developing TBM-specific antimicrobial regimens, for example, ones using high-dose rifampin and/or new drugs, is a priority and needs to be addressed in preclinical models, and ultimately in clinical studies. Finally, supportive care and neurosurgical interventions also have crucial roles in the outcomes for TBM and need to be optimized.

## Modeling

The interaction of neuroinvasive bacteria, including *M. tuberculosis*, with in vitro cellular barriers has been investigated previously<sup>7,8</sup>, and efforts to develop an in vitro granuloma model using primary murine cells were also presented. Animal models of TBM have been summarized<sup>3</sup>, and several models using intracerebral or intracisternal infections have been described<sup>3,14</sup>. Recently, *M. tuberculosis* infections in young rabbits were performed to mimic TBM in children, highlighting the role of microglial activation<sup>22</sup>. Although intracisternal and intracerebral infections are useful for studying pathogenesis and evaluating novel therapeutics, they do not simulate natural disease, caused by hematogenous dissemination of *M. tuberculosis*. Murine and guinea pig models using intravenous or aerosol infections have been used to identify microbial factors associated with TBM<sup>3</sup> and to develop novel vaccine strategies<sup>9</sup>. Zebrafish afford optical transparency for live in vivo imaging using *Mycobacterium marinum*<sup>3</sup> and have been used to study the dynamics of granuloma formation and bacterial dissemination and to identify a role for *LTA4H*-

dependent inflammation<sup>23</sup>. Live imaging using novel positron emission tomography (PET) tracers has also been applied in mammalian species to noninvasively measure inflammation<sup>22</sup> and rifampin biodistribution in infected tissues in animals<sup>17</sup> and patients with TB and for specific detection of bacteria<sup>24</sup>. These data suggested heterogeneous intralésional physiology and antimicrobial concentrations in infected tissues, which are different from those measured in clinical samples, for example, serum or CSF. Although PET may not be widely available in the developing world, it could enable research studies (for example, phase 0 studies with 10–20 patients) or be of clinical use at major referral centers. Finally, although not able to productively model all aspects of human disease, animal and other preclinical models can provide cellular and molecular insights into the pathogenesis of TBM in ways not possible in humans, as well as allow for screening and initial evaluation of therapeutics (Fig. 2).

## Diagnosics and imaging

Poor outcomes in TBM are related to diagnostic delay, and bacteriological testing is not sensitive. Assays based on nucleic acid amplification, such as Xpert MTB/RIF Ultra (Xpert Ultra), were found to be promising for diagnosing TBM in a small cohort of HIV-infected adults<sup>25</sup>. Xpert Ultra is now recommended by the WHO, especially for specimens with a low pathogen burden, such as CSF. Development of sensitive and specific lateral flow assays for CSF, which can occur at point of care and are inexpensive (for example, urine lipoarabinomannan), could also improve diagnosis, but current tests fall substantially short of this goal, possibly because of the low pathogen burden in CSF. An ELISA to measure CSF HMGB1 was also found to be promising for diagnosing TBM. It should be noted that, in comparison with a comprehensive ‘shotgun’ approach, a stepwise decision analysis has demonstrated better diagnostic yield and cost-effectiveness. In children with TBM, markers of neuronal and astroglial injury, neuron-specific enolase (NSE), S100B and glial fibrillary acidic protein (GFAP) have been found to be elevated in ventricular but not lumbar CSF or serum. Lastly, the field of TBM diagnostics suffers from the lack of a gold standard, and post-mortem studies could help determine the true sensitivity of diagnostics.

Imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are increasingly available in developing countries and, when available, are used in the evaluation and management of TBM (Fig. 3). In children with TBM in South Africa, angiographic abnormalities and infarcts were commonly noted on MRI<sup>5</sup>. MR spectroscopy, PET and pathogen-directed imaging could provide novel insights into pathophysiology and help in the management of TBM.

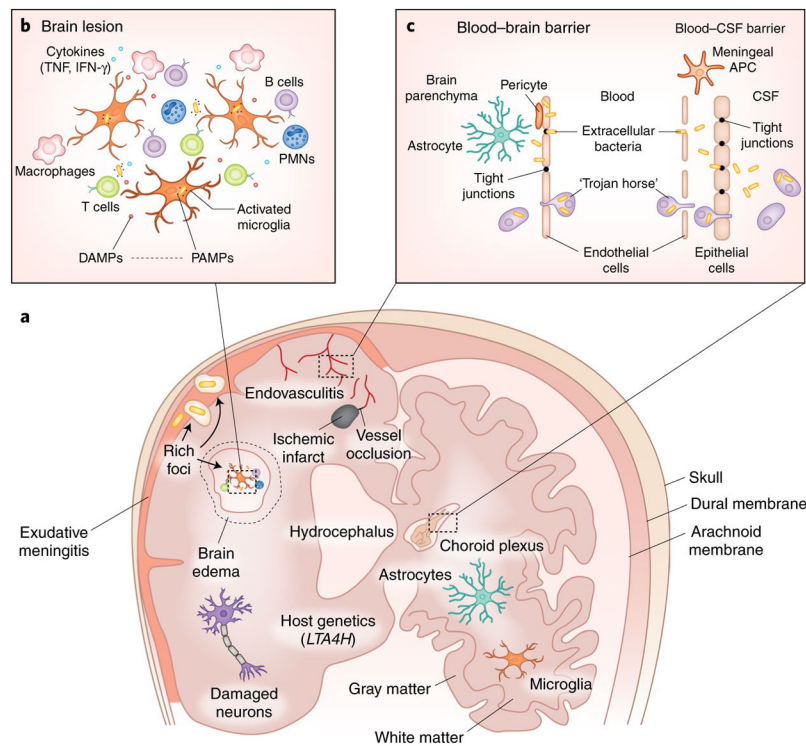
## Conclusions

TBM continues to be a serious, life-threatening disease affecting vulnerable populations including HIV-infected individuals and young children. Key gaps exist in understanding the molecular basis and immunopathogenesis of TBM as well as the mechanisms of bacterial invasion into the CNS. New research in these areas is required to develop novel therapeutic strategies for TBM. Evidence that TBM-specific HDTs can improve survival is promising and should lead to proof-of-concept studies. Antimicrobial treatments, currently based on

pulmonary TB regimens, need to be optimized for TBM, although a critical unanswered question is whether new regimens should be directed at decreasing inflammation, enhancing bacterial killing or both. To date, preclinical models have not been fully used to guide TBM clinical studies. Preclinical models could provide important mechanistic insights as well as a platform for the screening and initial evaluation of therapeutics. Live in vivo imaging techniques could provide vital information on the spatial and temporal kinetics of inflammation and intralesional antimicrobial exposures and the effects of bacterial killing at sites of infection, and some technologies such as MR spectroscopy and PET have the potential for clinical translation. Lastly, the field of TBM diagnostics suffers from the lack of a gold standard, and post-mortem studies could help determine the true sensitivity of current diagnostics. Substantially increased support for sustained basic and translational research to guide clinical studies is needed to advance the management of TBM and is an urgent priority.

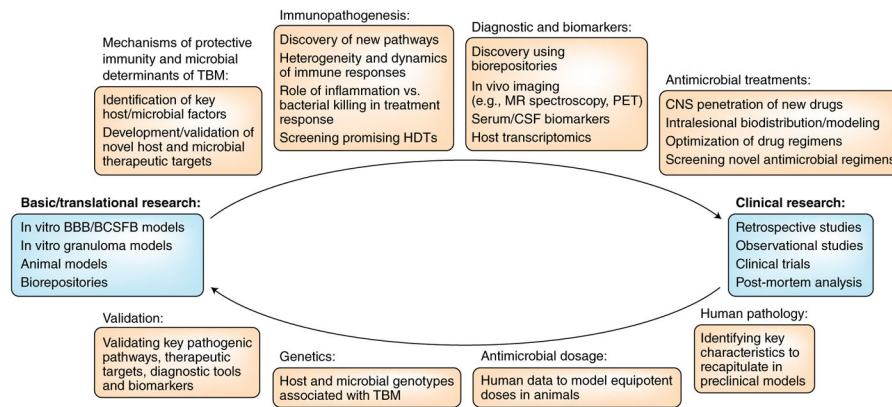
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**Fig. 1. Knowledge gaps in molecular mechanisms and immunopathogenesis**

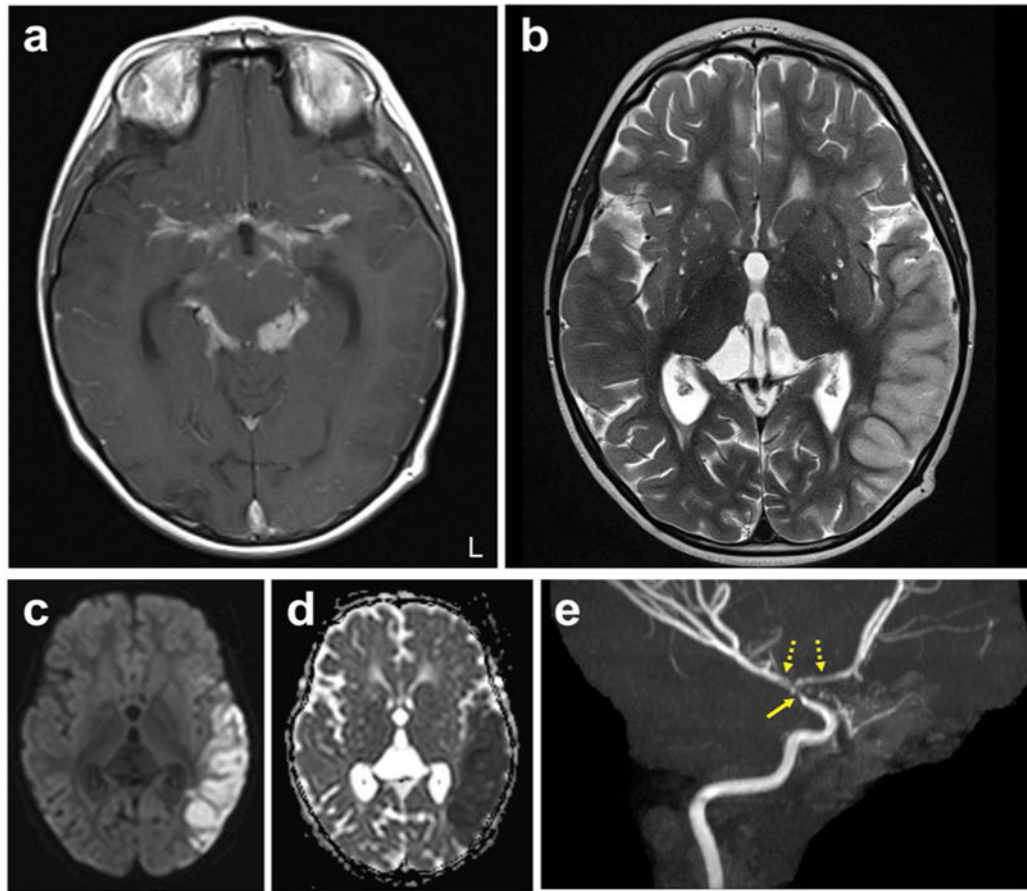
TBM continues to be a serious, life-threatening disease affecting vulnerable populations, including HIV-infected individuals and young children. Key gaps exist in understanding the molecular basis, immunopathogenesis and mechanisms of bacterial invasion. New basic and translational research is required to develop novel therapeutic strategies for TBM. **a**, Cartoon of the brain highlighting multiple immunopathological processes. *M. tuberculosis* are shown as small yellow bacilli, and exudative meningitis is shown in pink. **b**, Enlarged view of a TBM lesion in the brain parenchyma demonstrating interaction between immune cells and bacteria. **c**, Details of the specialized barriers that separate the CNS from the systemic circulation are shown. CSF, cerebrospinal fluid; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; APC, antigen-presenting cell; PMNs, polymorphonuclear leukocytes.



### Fig. 2. Advancing preclinical and translational research

Preclinical models have not been fully used to guide TBM clinical studies and could provide important mechanistic insights as well as a platform for screening and initial evaluation of therapeutics. Substantially increased support for sustained basic and translational research to guide clinical studies is needed to advance the management of TBM. HDTs, host-directed treatments; MR, magnetic resonance; PET, positron emission tomography; CSF, cerebrospinal fluid; CNS, central nervous system; BBB, blood–brain barrier; BCSFB, blood–cerebrospinal fluid barrier.





**Fig. 3. Paradoxical reaction in a young child with TBM**

Brain MRI of a four-year-old, HIV-negative child with microbiologically confirmed TBM presenting with paradoxical reaction ten weeks after initiation of TB treatment. **a–e**, Representative enhanced T1-weighted (**a**) and T2-weighted (**b**) images, diffuse weighted imaging (DWI) (**c**), ADC (apparent diffusion coefficient) map (**d**) and MR angiography (maximum-intensity projection) (**e**) are shown. T2 hyperintensity involving the left temporal cortex is seen, with corresponding increased signal on DWI and decreased signal on ADC, consistent with left middle cerebral artery distribution acute stroke. The enhanced T1-weighted image shows extensive thick basilar meningeal enhancement. Narrowing of the distal left internal carotid artery (solid arrow) with irregularity and narrowing of the M1 and A1 segments (dashed arrows) are noted on the angiogram. Corticosteroids were reinitiated at this time, and the child improved considerably, with sustained improvement noted at follow-up many months later. L, left. (Credit: unpublished data, S.K.J. The author is in compliance with Johns Hopkins School of Medicine institutional policy regarding single-case deidentified patient data.)

**Table 1**

Key gaps in understanding the molecular basis and immunopathogenesis of TBM

	<b>Knowledge gaps</b>	<b>Directions</b>
<b>Vaccines</b>		
BCG ineffective in adults/variable efficacy in children Live vaccine avoided in HIV-infected individuals	Mechanisms of protective immunity and microbial targets poorly characterized	Studies to understand Host-microbial interactions Mechanism(s) of infection Microbial determinants of disease Developing subunit- and TBM-specific vaccines
<b>Host-directed treatments</b>		
HDTs improve survival in the short term but are ineffective against neurological deficits	Poor understanding of immunopathogenesis Dynamics of immune responses and lesion formation remain poorly characterized	Discovery of new pathways Thalidomide and its analogs PARP1/DAMP inhibitors Role of inflammation vs. bacterial killing in treatment response
<b>Antimicrobial treatments</b>		
Limited CNS penetration of key antimicrobials CNS tissues difficult to sample	Data on intralésional drug concentrations in infected tissues lacking Spatial and temporal changes in drug penetration over time unknown	Intralésional biodistribution Post-mortem studies in animals/fresh tissue analysis Live in vivo imaging (for example, PET) Developing TBM-specific antimicrobial regimens
<b>Diagnostic/biomarkers</b>		
Paucity of bacteria in CSF leading to poor diagnostic sensitivity	Lack of a gold standard	Post-mortem studies in humans Host transcriptomics Serum/CSF biomarkers Diagnostic imaging (for example, MR spectroscopy, PET)

BCG, bacillus Calmette-Guérin; PARP1, poly(ADP-ribose) polymerase 1 (also known as ARTD1); DAMPs, damage-associated molecular pattern; CNS, central nervous system; PET, positron emission tomography; CSF, cerebrospinal fluid; MR, magnetic resonance.