Progress in tuberculosis vaccine development and host-directed therapies—a state of the art review

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Tuberculosis continues to kill 1·4 million people annually. During the past 5 years, an alarming increase in the number of patients with multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis has been noted, particularly in eastern Europe, Asia, and southern Africa. Treatment outcomes with available treatment regimens for drug-resistant tuberculosis are poor. Although substantial progress in drug development for tuberculosis has been made, scientific progress towards development of interventions for prevention and improvement of drug treatment outcomes have lagged behind. Innovative interventions are therefore needed to combat the growing pandemic of multidrug-resistant and extensively drug-resistant tuberculosis. Novel adjunct treatments are needed to accomplish improved cure rates for multidrug-resistant and extensively drug-resistant tuberculosis. A novel, safe, widely applicable, and more effective vaccine against tuberculosis is also desperately sought to achieve disease control. The quest to develop a universally protective vaccine for tuberculosis continues. So far, research and development of tuberculosis vaccines has resulted in almost 20 candidates at different stages of the clinical trial pipeline. Host-directed therapies are now being developed to refocus the anti-*Mycobacterium tuberculosis*-directed immune responses towards the host; a strategy that could be especially beneficial for patients with multidrug-resistant tuberculosis or extensively drug-resistant tuberculosis. As we are running short of canonical tuberculosis drugs, more attention should be given to host-directed preventive and therapeutic intervention measures.

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Introduction

At present, the development of new drugs for the treatment of tuberculosis does not keep pace with the development of *Mycobacterium tuberculosis* drug resistance. Evidently, innovative interventions are needed to combat the emerging pandemic of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. At present, research and development of tuberculosis vaccines is financed via global financial investments in the order of US$100 million per year.[1] Investment in preclinical research and development has yielded almost 20 vaccine candidates for tuberculosis—most of which still remain at different stages of the clinical trial pipeline with few dropouts. Moreover, several new candidates are ready to enter the pipeline soon. The development of most vaccine candidates is jointly sponsored by the public sector with the aim to advance the research and development of tuberculosis vaccines, with substantial contributions from private companies.

The BCG vaccine, which is extensively used as part of the Expanded Program on Immunisation, prevents against only severe forms of childhood tuberculosis, and does not protect against the most prevalent form of this disease, pulmonary tuberculosis, in all age groups.[2] Thus, an improved vaccine against tuberculosis is desperately needed. Preclinical research and development can benefit from research in related specialties—notably, with regards to the development of novel adjuvants and vectors. New vaccine candidates are being created in other specialties with new vectors such as simian adenovirus, cytomegalovirus, and lymphocytic choriomeningitis virus.[3] Although research and development for a universally protective tuberculosis vaccine continues, novel host-directed therapies might be helpful to augment biologically relevant host immune responses; a strategy that could be particularly beneficial for patients with multidrug-resistant and extensively drug-resistant tuberculosis. This Review discusses advances and progress being made in host-directed interventions,

Key messages

- Tuberculosis continues to kill 1·4 million people annually, and numbers of patients with drug-resistant tuberculosis have increased alarmingly
- A novel, safe, widely applicable and more effective vaccine against tuberculosis is needed for disease control
- Vaccine research and development for tuberculosis has brought forward almost 20 vaccine candidates, many of which are at different stages of the clinical trial pipeline
- Vaccines for tuberculosis can be classified according to their target population; therapeutic or preventive vaccines; composition (ie, killed mycobacteria, viable recombinant mycobacteria, viral-vectored and adjuvanted subunit vaccines); time of administration (pre-exposure and postexposure vaccines), and according to BCG (ie, replacement and heterologous prime-boost vaccines)
- Host-directed therapies aim to eliminate *Mycobacterium tuberculosis* in the host—eg, by augmenting focused, clinically effective anti-*M tuberculosis*-directed immune responses, or by limiting non-productive, tissue-damaging inflammation in tuberculosis, a strategy that could be particularly beneficial for patients with drug-resistant tuberculosis
- Host-directed therapies contain different groups of compounds, including cytokines and so-called repurposed drugs, that target biologically and clinically relevant checkpoints in anti-*M tuberculosis*-directed host response pathways

including the clinical trial pipeline for new tuberculosis vaccines and therapeutic concepts targeting host defense mechanisms to improve treatment outcomes.

Immune orchestration against *M tuberculosis*

Tuberculosis is primarily a pulmonary disease; the respiratory tract serves as port of entry and the lung as the prime organ of disease manifestation.[4,5] At the site of *M tuberculosis* infection, granulomas are formed. Protection against and pathogenesis of tuberculosis are cell mediated,[6,7] primarily comprising T lymphocytes and mononuclear phagocytes focused on granulomas (figure 1). Granulomas are composed of different T-lymphocyte subsets and different myeloid cell types, which, within the granuloma, stay in close and dynamic contact. As long as these granulomas are confined and well structured, they successfully contain *M tuberculosis* without major harm to the affected organ.[8] Once the lesion liquefies and becomes caseous, tissue damage prevails and *M tuberculosis* can no longer be restrained.[8] CD4+ T cells, which produce T helper 1 (Th1) cytokines—notably, interferon γ and tumour necrosis factor α

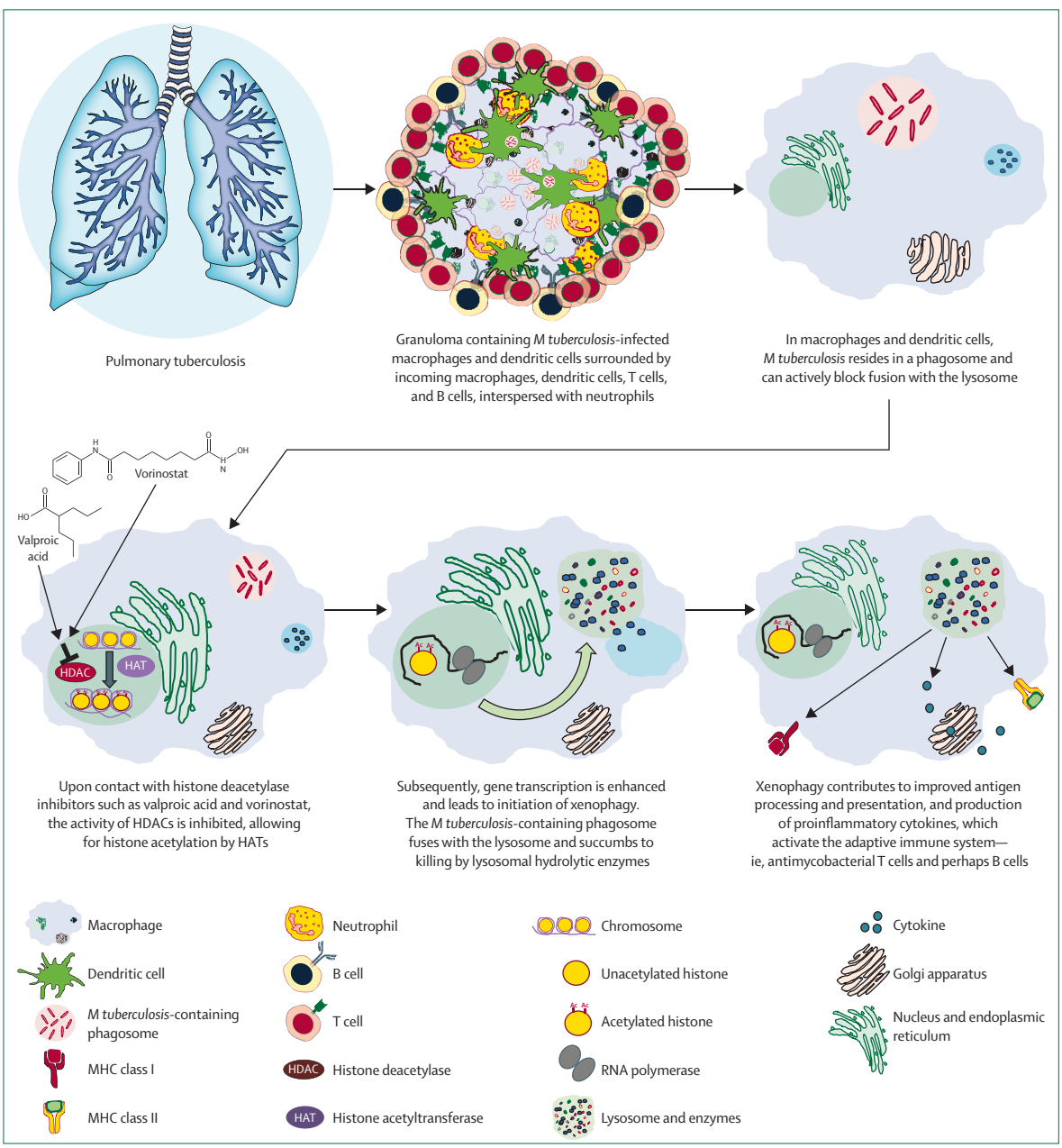

Figure 1: **Physiology of *Mycobacterium tuberculosis* antigen processing and presention**
Histone deacetylase inhibition (by valproic acid and vorinostat) might contribute to immunological control of *M tuberculosis* infection.

(TNFα) are considered major mediators of protective immunity.[6,7] During the early stage of the immune response against tuberculosis, Th17 cells, which among other functions regulate neutrophils, participate in protection.[9] However, because of the detrimental role of neutrophils in chronic inflammation, including in tuberculosis, the Th17 response needs to be tightly regulated to avoid pathological sequelae.[10]

It is likely that additional cells and mediators contribute to protection, including CD8+ T cells.[11,12] CD8+ T cells not only produce similar cytokines as CD4+ T cells, but also have cytolytic activities[13] that can directly harm *M tuberculosis*.[14] Unconventional T cells (γδ T cells, CD1-restricted T cells, mucosal-associated invariant T cells)[15–17] and a broad array of soluble mediators[18] contribute to orchestration of anti-*M tuberculosis*-directed immune responses. After antigen-specific priming, the conventional CD4+ and CD8+ T cells first become T effector cells that orchestrate early host defence, and then mature into T-memory cells that coordinate coexpression of multiple cytokines. These T-memory cells orchestrate protection in solid granulomas. Antimycobacterial properties are activated in mononuclear phagocytes, which thus assume the capacity to control *M tuberculosis*.[6,7] However, the pathogen is not eliminated and persists in these phagocytes in an altered metabolic activity state over long periods.[19] This scenario occurs in solid granulomas during latent *M tuberculosis* infection.

The fine regulatory mechanisms between various components of the immune system needed for protection against *M tuberculosis* are mainly understood on the surface, but remain undefined. Importantly, dysregulation of this fine balance results in loss of an organised structure of the lesion. As a result, *M tuberculosis* reverts into a highly replicative stage, leading to caseous lesions that expand and cause major lung damage, which is characteristic of active tuberculosis.[8,19,20] Tight regulation of different immune mechanisms needs to be viewed in a spatio-temporal framework: some elements such as Th17 cells are protective in early, but pathogenic in late, stages of tuberculosis.[9,10] Similarly, regulated activation of mononuclear phagocytes within solid granulomas promotes *M tuberculosis* containment, whereas their uncontrolled activation and lysis of phagocytes results in necrosis and later in caseation of granulomas, which severely damage the affected organ.[8,19,20]

Primarily, current vaccine development assumes that viable *M tuberculosis* are present in granulomas, and thus is aimed towards preventing progression to tuberculosis disease after primary infection. Although epidemiological evidence suggests that *M tuberculosis* can occasionally be eradicated in infected individuals,[21] the underlying mechanisms for this eradication remain elusive. Strategies for vaccine research and development are focused on the mimicking and modification of immune mechanisms operative during latent *M tuberculosis* infection, which contains *M tuberculosis* and prevents active tuberculosis disease, but fails to achieve sterile eradication of the bacteria.[22,23] Next generation vaccines, intended to prevent or eradicate *M tuberculosis* infection, would not only require improved knowledge about immune mechanisms induced during natural infection, but also about alternative immune mechanisms that perform better than does naturally induced immunity.[24]

Host-directed therapies

At first glance, vaccination and therapy seem to be two unrelated topics. About 120 years ago, Robert Koch[25] attempted to combine both themes by vaccinating patients with tuberculosis with tuberculin; his efforts, however, were met with failure. Tuberculosis vaccines aim to induce long-lasting immune responses that would eliminate or effectively contain *M tuberculosis* upon encounter. These (adaptive) immune responses are thought to be proinflammatory and Th1 T cell oriented. *M tuberculosis* resides intracellularly within phagosomal compartments in professional antigen-presenting cells—notably, macrophages and dendritic cells—and in non-professional antigen-presenting cells—eg, epithelial and fat cells.[19,26–29] Host-directed therapies target host pathways and aim to shorten the duration of standard drug treatments against tuberculosis, restrict damage of overt (pulmonary) inflammation, and possibly reduce the risk for reinfection with *M tuberculosis*.[30]

Although host-directed therapies have been hailed as a breakthrough for cancer, new concepts and clinically relevant trials are needed to achieve similar life-changing progress in infectious diseases. Immunotherapeutic approaches in tuberculosis have been discussed and reviewed in the past,[30] both in the advent of drug-resistant tuberculosis and with the need to offer alternative strategies to induce or expand clinically relevant anti-*M tuberculosis* immune responses. Because of space restrictions, we are only able to review a restricted range of approaches to host-directed therapies for anti-*M tuberculosis* with a particular focus on drugs or compounds that have already been tested in clinical phase 1 trials (for adjunct treatment of tuberculosis) or so-called repurposed compounds—drugs that have been used for other (non-tuberculosis) indications, yet are a rational choice based on their method of action and tuberculosis immunopathology. The appendix provides some additional sections and reading about *M tuberculosis* and HIV co-infection, cytokines in *M tuberculosis* immunotherapy, preclinical models of host-directed therapies, and biological intervention with vitamin D.

See Online for appendix

Immunotherapeutic approaches, such as host-directed therapies, require carefully designed clinical protocols with biologically relevant biomarkers to gauge the best timepoint for immune intervention and to monitor response to therapy. Preclinical models can facilitate immunotherapeutic efforts and might help to dissect immunological pathways that could be safely and

successfully exploited in host-directed therapies.[31] Immunotherapeutic approaches often focus on cellular immune (adaptive or innate) responses, but other viable options include antimicrobial peptides[32] or antibody-based therapies.[33,34]

Not only do genetic differences in the host or pathogen shape the quality of immune responses to *M tuberculosis*,[35] but also genetic variants in immune response elements help to orchestrate the quality and quantity of anti-*M tuberculosis*-directed immune responses.[36,37] Host-directed therapies target biologically relevant cellular checkpoints with the aim to increase clearance or containment of *M tuberculosis* and—not mutually exclusive—to restrict so-called collateral organ damage associated with *M tuberculosis*-induced immune responses.

Identification of host mediators that influence mycobacteria–host crosstalk include host signalling pathways and their epigenetic regulation. Studies show that microRNAs (miRNA) contribute to regulation of host signalling pathways and might therefore help to shape the mycobacteria–host crosstalk. Although attempts to identify the molecular biomarkers of tuberculosis in terms of responsive miRNAs have been made,[38] the exact mechanisms and regulatory circuits that modulate miRNA expression and their functions have not been sufficiently characterised up to now. For the sake of clarity, we segregate host-directed therapies into drugs that induce (productive) anti-*M tuberculosis*-directed inflammation and drugs that decrease non-productive (damaging) inflammation favouring targeted anti-*M tuberculosis* immune responses (figure 2, table 1). Some of the compounds or biologicals show either effect, dependent on the time of administration, dose, or the local milieu.

Corticosteroid treatment[48] and antiretroviral therapy (ART)[99] for patients with HIV and tuberculosis co-infection have long and successful track records in the management of tuberculosis. Improved AIDS-free survival in patients with tuberculosis can be achieved with early ART initiation,[100] except for patients with tuberculosis meningitis. However, the survival benefit was associated with increased immune reconstitution inflammatory syndrome events.[101] This association emphasises an important point in the management of clinical tuberculosis and particularly HIV and *M tuberculosis* co-infection, a topic not covered by this Review. ART affects the quality and quantity of *M tuberculosis*-directed immune responses and is therefore also a form of host-directed therapy for

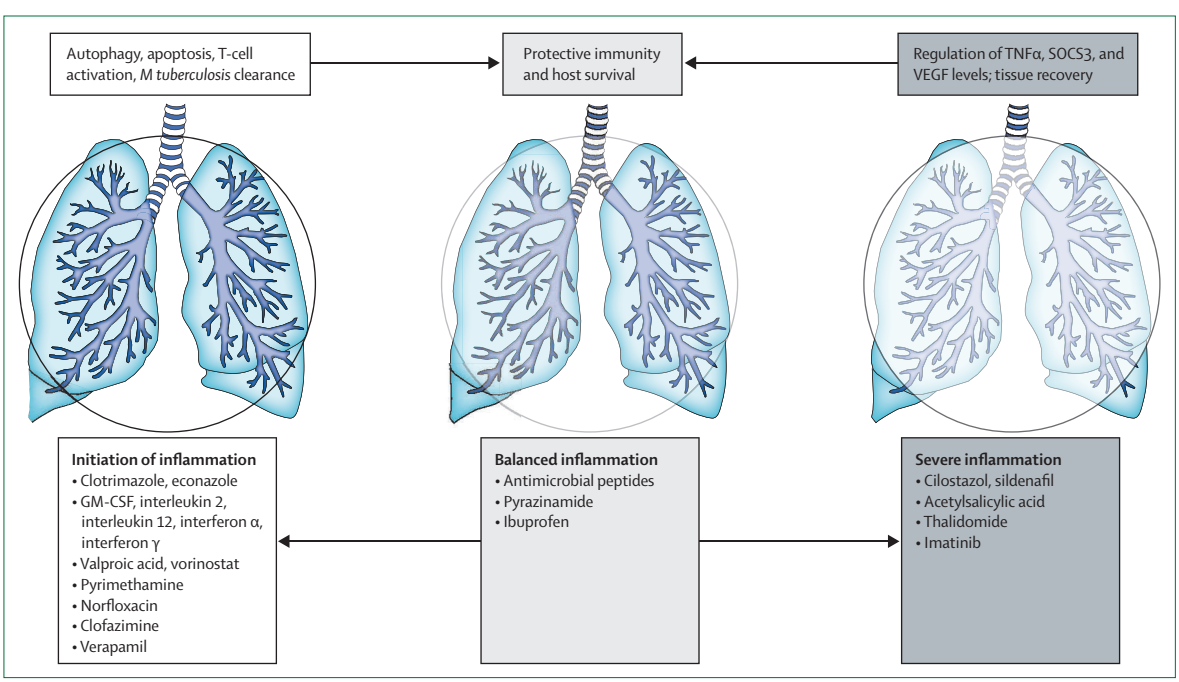

Figure 2*: Overview of selected host-directed therapies for *Mycobacterium tuberculosis
Left: Examples of compounds or recombinant cytokines that induce proinflammatory reactions aiming to kill or contain *M tuberculosis*. Different host pathways are targeted. Centre: Balanced inflammation without overt tissue damage helps to contain viable *M tuberculosis* bacilli, and warrants host survival. Right: Unproductive and excessive inflammation might deteriorate focused anti-*M tuberculosis* immune responses, resulting in harmful tissue damage and T-cell dysregulation. Uncontrolled inflammation also induces factors which increase *M tuberculosis* mutation rates owing to error-prone DNA polymerase activity triggered by mycobacterial stress response to low pH.[39] Excessive host inflammation drives the selection of *M tuberculosis* genetic variants. Anti-inflammatory strategies can help to remove the so-called editing function of inflammation that drives *M tuberculosis* mutations. Bottom row, centre box: compounds or drugs listed can act either at initiation of tuberculosis inflammation or at a later stage, hence the bidirectionality. Top row: compounds or cytokines that act either early or late in tuberculosis inflammation with the aim to establish protective immune responses in the host via controlled, balanced inflammatory responses, hence arrows point to the centre from both left and right. VEGF=vascular endothelial growth factor. TNFα=tumour necrosis factor α. GM-CSF=granulocyte-macrophage colony-stimulating factor.

	Classification	Host biological process or pathway targeted and effects	Comment
In use or under clinical evaluation			
Thalidomide[40–42]	Immuno-modulatory drug	Inhibition of VEGF activity and angiogenesis; downregulation of TNFα production	Validated adjunctively in paediatric CNS tuberculosis
Clofazimine[43–47]	DNA-binding lipophilic riminophenazine	Caspase 3 activation and subsequent induction of PARP-mediated apoptosis; release of vesicles containing immunomodulatory molecules (cytokines, nucleic acids) and antigens	In clinical trials for patients with multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis
Prednisolone, dexamethasone[48]	Corticosteroid	Blockade of glucocorticoid receptors, exerting immunosuppressive effects—ie, reduces MMP-9 and VEGF concentrations in CSF of patients with tuberculosis meningitis; exact mechanism of action unknown	Validated adjunctively to standard antituberculosis regimens
Vitamin D3[18,49–52]	Secosteroid	Activation of vitamin D receptor and downstream production of cathelicidin; mediates initiation of interferon γ-mediated autophagy	Successful clinical trials involving patients with pulmonary tuberculosis
Pyrimethamine plus sulfadoxine[53–56]	Antiparasitic/ antiprotozoal	Activation of caspase-dependent and cathepsin B-dependent apoptotic pathways	Trialled in patients with multidrug-resistant tuberculosis
Pyrazinamide[57]	Antimycobacterial	Downregulation of proinflammatory cytokines—ie, TNFα, interleukin 6; CCL2 release	In use as antituberculosis drugs; immunomodulatory activity observed in mice
Interferon γ[58–62]	Recombinant human cytokine	Activation of lung macrophages and dendritic cells and expression of many interferon γ-induced genes; nitric oxide for enhanced mycobacterial killing and robust T-cell responses	Successful clinical trials involving patients with pulmonary tuberculosis
Interleukin 2[63–66]	Recombinant human cytokine	T-cell proliferation and activation; production of proinflammatory cytokines—ie, interferon γ and TNFα—for activation of *Mycobacterium tuberculosis*-infected macrophages and dendritic cells	More pronounced effect in patients with multidrug-resistant tuberculosis
Granulocyte-macrophage colony-stimulating factor[67,68]	Recombinant human cytokine	Differentiation, proliferation, and activation of macrophages and dendritic cells; promotes T-cell activation and subsequent antimycobacterial immune responses in the lung	Clinical trials in patients with pansusceptible tuberculosis
Interferon α[69,70]	Recombinant human cytokine	Involved in innate immune responses to viral infections	Clinical trials involving patients with multidrug-resistant tuberculosis
Interleukin 12[71]	Recombinant human cytokine	Triggered by *M tuberculosis* infection to activate innate immune responses; augments potent antimycobacterial Th1 effector responses	Tried in a patient who failed conventional treatment
Under preclinical investigation			
Acetylsalicylic acid, ibuprofen[72–74]	Non-steroidal anti-inflammatory drug	Blockade of arachidonic acid metabolism by inhibition of COX-1 and COX-2; eicosanoid level balance and regulation of TNFα production; promotes contol of tuberculosis; immunopathology and patient survival	In-vivo validation in mice
Imatinib[75–80]	Tyrosine-kinase inhibitor	Failed tyrosine residue phosphorylation on ABL family tyrosine kinases leading to intracellular killing of *M tuberculosis* and *Mycobacterium marinum* in macrophages	In-vivo validation in mice
Cilostazol, sildenafil[81,82]	Phosphodiesterase inhibitor	Hydrolysis of cAMP and cGMP; modulation of NF-κB activity; regulation of TNFα levels and reduced lung immunopathology to promote host survival	In-vivo validation in mice
Alisporivir, desipramine[83]	Cyclophilin inhibitor	Inhibitory effect on cyclophilin D and acid sphingomyelinase, respectively; induces mitochondria-mediated oxidative stress in macrophages; killing of intracellular *M marinum*	In-vivo validation in zebrafish
Promising avenues			
Valproic acid, vorinostat[84–94]	Histone deacetylase inhibitor	Inhibition of histone deacetylase leading to histone acetylase activity and DNA unwinding; enhances gene expression, promoting autophagy and improved antigen presentation	In clinical trials for patients with HIV; yet to be evaluated for tuberculosis
Clotrimazole, econazole[95–98]	Cytochrome P450 blockers	Modulation of Ca^{2+} flux-driven K^+ channel, subsequently increasing K^+ efflux from cell cytosol; proautophagic, anti-inflammatory and antiapoptotic attributes in cardioprotection and neuroprotection	In-vitro antituberculosis and proapoptotic activity

VEGF=vascular endothelial growth factor. TNFα=tumour necrosis factor α. CNS=central nervous system. PARP=poly (ADP-ribose) polymerase. CSF=cerebrospinal fluid.

Table 1: **Repurposed clinically approved drugs for treatment of tuberculosis that target metabolic pathways involved in the host defence against *M tuberculosis* or target *M tuberculosis* directly**

tuberculosis. The appendix provides information about new insights concerning the immune reconstitution inflammatory syndrome and *M tuberculosis* co-infection.

Autophagy and host-directed therapies

Autophagy is the physiological response to stimuli that activate autophagy and related genes (*ATG*), and is a catabolic pathway that leads to destruction of cellular components via lysosomal compartments.[102] Autophagy is relevant to treatment because conventional anti-tuberculosis drugs work, partly, via regulation of autophagy; autophagy plays a part in BCG vaccination; and candidate repurposed drugs for antituberculosis treatment might work via modulation of host autophagy.

The physiological role of autophagy is multifaceted and aims to ensure cellular survival. Infection with intracellular pathogens, including *M tuberculosis*, is a stressful event for cells.[103] Intracellular survival of the bacteria is affected by autophagy in several ways; *M tuberculosis* is delivered to lysosomal compartments and antimicrobial peptides are produced, including a particular set of so-called cryptides—antimicrobial peptides generated via proteolysis of endogenous cellular proteins.[104] *M tuberculosis*-associated proteins—eg, ESAT-6—interfere with maturation of the phagosome and inhibit autophagy.[105] The *IRGM* locus, which participates in autophagy, is associated with tuberculosis risk.[106–108] *LRG47* gene-deleted mice (the murine homologue of *IRGM*) fail to control *M tuberculosis* infection.[109] Genes involved in autophagy[109–111] shape the course of mycobacterial disease. These autophagy-related genes are shared with the genetic predisposition to inflammatory bowel disease.[112] A similar association has been noted between inflammatory bowel disease[49] and variants of the vitamin D receptor-encoding genes contributing to *M tuberculosis* clearance and autophagy,[50] suggesting that the nature of the autophagy-driven inflammatory host response is an important factor in the clinical presentation of tuberculosis.

Autophagy contributes to the downregulation of inflammatory responses including production of interleukin 1β and interleukin 18.[104,113] Starvation and a Th1-geared cytokine milieu promote *M tuberculosis* killing by autophagy,[114,115] suggesting that therapeutic, targeted activation of the autophagic pathway helps to kill *M tuberculosis* bacilli in the host. Autophagy has been shown to be instrumental in the mediation of antitubercular activity in standard antituberculosis treatment with conventional antibiotics.[116]

Treatments to target overt inflammatory responses have shown clinical benefits, particularly for tuberculosis meningitis.[48,117] Phosphodiesterase inhibitors[72,73,118] and thalidomide (anti-TNFα effects) have been tested in safety trials; however, the thalidomide study was terminated because of adverse events.[40] Although TNFα antagonists are contraindicated for individuals who are latently infected with tuberculosis, drugs such as etanercept or infliximab are effective as adjunctive therapy in animal models.[119,120] In an open-label, non-randomised study with autologous mesenchymal stromal cells, Skrahin and colleagues[121] showed that cellular treatment to reduce non-productive inflammation in patients with multidrug-resistant tuberculosis or extensively drug-resistant tuberculosis is safe and might help to restore focused, anti-*M tuberculosis* reactivity.

Autophagy is a crucial component of innate and adaptive immune responses, antigen processing and presentation—particularly within the MHC class II antigen compartments[122]—and the subsequent shaping of antigen-specific adaptive cellular immune responses.[123–125] Deletion of *ATG5* in murine studies confirmed the role of autophagy in *M tuberculosis* infection: *ATG5*$^{-}$ mice show increased susceptibility to tuberculosis.[104] However, experimental evidence suggests the presence of autophagy evasive strategies during mycobacterial infection.[126] *M tuberculosis* stimulates the class I phosphatidylinositol 3-kinase mammalian target of rapamycin (mTOR) pathway that negatively regulates several autophagy-related genes.[127]

Recent investigations identified host factors used by *M tuberculosis* to restrict autophagy. Host signalling cascades responsive to *M tuberculosis* such as Wnt and sonic hedgehog pathways induce eicosanoids, lipoxygenases and lipoxins, that downregulate interferon γ-mediated autophagy.[128] Epigenetic regulation of these signalling pathways, by miR-155 and miR-31, facilitates evasion of autophagy by mycobacteria. Several other studies show the crucial roles of miRNAs during *M tuberculosis* infection. miR-29s, for example, suppresses interferon γ production by targeting interferon γ mRNA.[129] miR-125b blocks TNFα biosynthesis,[130] whereas miR-99b negatively regulates production of proinflammatory cytokines.[131] miR-223 is differentially upregulated in patients with tuberculosis versus healthy controls.[132,133] Studies in mice showed that miR-223 directly regulates the chemokines, CXCL2 and CCL3, and the proinflammatory cytokine interleukin 6.[132] In this way, miR-223 controls neutrophil development[134] and their influx into the *M tuberculosis*-infected lung.[132] Exacerbated neutrophil activation and influx causes severe damage in mice infected with *M tuberculosis* with deleted miR-223. Thus, miR-223 is a promising target for host-directed RNA-based therapy. *M tuberculosis*-induced miRNAs and host signalling pathways are promising candidates to design new targets for host-directed therapies.

Host-directed pathways: controlling immunopathology and favouring *M tuberculosis* clearance

Establishment of fine-tuned immune responses plays a key part in improvement of clinical outcomes of tuberculosis. Additionally, the increasing need for new chemotherapeutic interventions has encouraged investiture of much effort into repurposing clinically

approved drugs targeting biological pathways involved in the host defence against *M tuberculosis*. This section discusses pharmacological and biological interventions of immunologically relevant indications that might contribute to effective management for tuberculosis in the form of adjunct therapies.

Pharmacological interventions

cAMP regulation

Many intracellular pathways in both prokaryotes and eukaryotes require participation of the second messenger cAMP. Immunologically, cAMP can suppress innate immune mechanisms via downregulation of phagocytic function, cytokine release (ie, TNFα and interleukin 12), and generation of reactive oxygen and nitrogen intermediates, among others.[135] A virulence characteristic of *M tuberculosis* is to increase cytosolic concentrations of cAMP in macrophages to promote intracellular survival.[136] Synthesis of biochemically active cAMP requires two enzymatic processes; the first implemented by adenylate cyclases, and the second by phosphodiesterases. Phosphodiesterase inhibitors such as cilostazol and sildenafil are currently used for treatment of vascular diseases and erectile dysfunction, respectively. Coadministration of cilostazol (phosphodiesterase inhibitor type 3) and sildenafil (phosphodiesterase inhibitor type 5) with isoniazid, rifampicin, and pyrazinamide for treatment of *M tuberculosis*-infected mice shortens the course of antituberculosis therapy by 1 month, augmenting reduced lung immunopathology and quicker bacterial clearance.[81,82] The absence of negative drug–drug interactions between the standard antituberculosis regimen and phosphodiesterase I might warrant clinical trials in the near future.

Eicosanoid pathway

Arachidonic acid metabolism propels the release of biochemical lipid mediators called eicosanoids—ie, lipoxin A4, leukotriene B4, and prostaglandin E2—in the onset of inflammation-induced fever. Acetylsalicylic acid, the active ingredient in aspirin, is an effective antagonist of cyclooxygenase 1 and cyclooxygenase 2. Through its inhibition of cyclooxygenase activity, the breakdown of arachidonic acid is perturbed and the downstream release of eicosanoids is blocked. As a result, TNFα production is halted, which directly dampens inflammation. However, should this event be totally abrogated, the host is rendered hypersusceptible to infection with intracellular pathogens such as *M tuberculosis*. Contrastingly, too much TNFα-driven inflammation in response to infection can lead to host cell necrosis, causing severe tissue damage and death.[83,137] Fine tuning the lipoxin A4–leukotriene B4 balance regulates TNFα concentrations in tissue and prolongs patient survival, a feat that might be achieved with acetylsalicylic acid administration alongside antituberculosis drugs.[72]

Tyrosine kinase pathways

ABL family kinases are involved in several important biological processes ranging from physiological cell maintenance to T-cell receptor-mediated T-cell activation. In the case of chronic myelogenous leukaemia and gastrointestinal stromal tumours, chromosomal fusion between the *ABL* and the *BCR* genes eventually leads to expression of an aberrant protein, BCR–ABL, that causes cancer.[138] Imatinib is an efficacious BCR–ABL tyrosine kinase inhibitor that has been clinically used since 2001 for the treatment of chronic myelogenous leukaemia and gastrointestinal stromal tumours.[75,139] With the chronic myelogenous leukaemia cell line K562, imatinib was shown to exert its effects by inducing caspase 3-mediated and caspase 9-mediated apoptosis in cells expressing the BCR–ABL fusion protein.[76]

A few years after the drug was licensed for use, imatinib was reported to abrogate T-cell function by disrupting T-cell receptor-mediated activation of T cells and subsequent interleukin 2 production, a process requiring ABL kinase activity.[140] Imatinib ameliorated rheumatoid arthritis-associated pathology in four patients via unknown mechanisms, although inflammation-induced symptoms were reduced.[77,141] The anti-inflammatory properties of imatinib were substantiated by studies showing its ability to downregulate TNFα production by hepatocytes and in a mouse model of arthritis.[78,79] A more recent study suggested a protective role for imatinib in tuberculosis. Treatment of *M tuberculosis*-infected mice with imatinib led to reduced pulmonary bacterial burden.[80] This effect was further boosted when imatinib was coadministered with rifampicin, suggesting positive synergism between the two drugs in tuberculosis treatment.[80] This accentuates the immunomodulatory role of imatinib and could be useful to dampen hyperinflammation in tuberculosis.

Histone modification and gene transcription

Histone modification is a pivotal epigenetic strategy to control gene expression. As such, acetylation of lysine residues on histone ε amino tails by histone acetylases promotes chromatin unwinding and enhanced gene transcription.[84] This process can be reversed by histone deacetylases, and a balance between activities of histone acetylases and histone deacetylases secures genetic stability. Herein, histone deacetylase inhibitors qualify as potent immunomodulators owing to their ability to orchestrate a range of cellular processes including autophagy and apoptosis.[84–86] FDA-approved histone deacetylase inhibitors comprising, but not restricted to, valproic acid, romidopsin, and vorinostat are used for the treatment of psychiatric and neurological disorders, such as depression and epilepsy, and more recently, cutaneous T-cell lymphoma.[87] Additional histone deacetylase inhibitors are undergoing clinical investigation for various

cancers including melanomas, glioblastomas, and other solid tumours associated with increased MHC expression and presentation of nominal target epitopes to antigen-specific T cells.[88] Valproic acid and vorinostat were shown to trigger the expression of HIV genes in latently-infected human T cells, leading to viral replication, improved efficacy of ART, and immune attack by autologous CD8+ T cells due to high antigen turnover.[89–92] Collectively, these findings prompted clinical trials investigating combination therapy of valproic acid or vorinostat with ART in individuals positive for HIV.[93,94]

The pleiotropic, immunomodulatory effects of histone deacetylase inhibitors might help to enhance intracellular killing of *M tuberculosis* and antigen processing and presentation. Collectively, more efficient T cell priming and a different quality of the ensuing antituberculosis immune response might take place (figure 1).

Apoptotic cell death

Studies have shown the immunomodulatory facets of the antileprotic drug clofazimine. Clofazimine induces caspase 3-dependent apoptosis in THP-1-derived macrophages, and has been assessed in the context of adjunctive therapy for patients with drug-sensitive or multidrug-resistant tuberculosis with much success.[43,44]

In a clinical study in the Ivory Coast, prophylactic coadministration of the combination antimalarial drug sulfadoxine-pyrimethamine and isoniazid to HIV-seropositive individuals who had recently recovered from pulmonary tuberculosis improved their health status and aided better clinical management of an eventual episode of active tuberculosis than did isoniazid monotherapy.[53] This improvement could be attributed to the proinflammatory properties of pyrimethamine. Human melanoma and rodent pituitary adenoma cells treated with pyrimethamine succumb to caspase-mediated apoptotic activity and subsequent cell death.[54,55] Pyrimethamine also effectively blocks STAT3,[56] which has been associated with MMP1-driven immunopathology and tissue destruction in patients with tuberculosis.[142] Timely blockage of STAT-3 might therefore benefit clinical outcomes in tuberculosis treatment.

Anti-inflammatory regulation

New light has been shed on the first-line antituberculosis drug pyrazinamide, which might act as a double-edged sword by simultaneously exerting antimycobacterial effects, while downregulating inflammatory responses mediated by TNFα, interleukin 1β, interleukin 6, and CCL2.[57] The anti-inflammatory effect of pyrazinamide might be instrumental in abatement of excessive immunopathology in the host during severe disease.

Thalidomide, another antileprotic drug, possesses anti-inflammatory properties, as opposed to clofazimine, by reducing TNFα concentrations albeit with an increase in interferon γ, interleukin 2, and interleukin 12 concentrations, which share protective attributes in tuberculosis.[41] An analogue of thalidomide, lenalidomide, produced similar finding in rabbits with CNS tuberculosis; cerebrospinal fluid from these animals contained reduced amounts of TNFα compared with controls.[42] Thalidomide has been used to treat childhood CNS tuberculosis, which is refractory to standard antituberculosis regimens with unlicensed use.[143]

Anti-inflammatory drugs might also exhibit direct effects on *M tuberculosis*. The non-steroidal anti-inflammatory drug compound ibuprofen, among other propanoate-based compounds, displayed potent bactericidal activity against replicating and non-replicating *M tuberculosis* and multidrug-resistant isolates in a high-throughput in-vitro drug screen.[74] This observation was substantiated in mice with severe tuberculosis disease that showed improved survival and decreased immunopathology after receiving ibuprofen without previous exposure to antituberculosis drugs,[73] thus endowing the drug with a dual role in tuberculosis.

The glucocorticoid receptor agonists prednisolone and dexamethasone are synthetic corticosteroids used for the treatment of a variety of immunological disorders, including arthritis, asthma, and leukaemia. Additionally, both drugs have been trialled in the treatment of tuberculosis meningitis, wherein decreased matrix metalloproteinase 9 and vascular endothelial factor concentrations were observed in the cerebrospinal fluid of treated patients.[144] The overall efficacy of prednisolone and dexamethasone was manifested in improved survival in treated patients with tuberculous meningitis; up to 17% reduction in mortality across 41 clinical trials between 1960 and 2012.[48]

SOCS3, a suppressor of cytokine signalling, has been reported to maintain an antiapoptotic state in human psoriatic keratinocytes by inhibiting deactivation of the PI3K/AKT pathway, and its depletion led to interferon γ/TNFα-induced cell death.[145] Pertinent to tuberculosis, SOCS3 expression in human T cells elevates interleukin 17 production, whilst reducing T-cell proliferation, thus increasing host susceptibility to severe tuberculosis.[146] By contrast, recent studies in mice showed that SOCS3 helps to fine-tune interferon γ-mediated control of *M tuberculosis* infection contributed by CD4+ T cells.[147] Although mice that did not express SOCS3 were able to mount a strong γδ T cell-driven interleukin 17 response in the lung after *M tuberculosis* challenge, they generally showed reduced survival. Thus, guided, temporal regulation of SOCS3 activity might contribute to controlled and effective antituberculosis immune responses.

Modulation of ion efflux

Clotrimazole and econazole, two over-the-counter antifungals used for treatment of skin infection, were also shown to inhibit growth of *M tuberculosis* in culture.[148,149] Notably, clotrimazole, via inhibition of cytochrome P450,

increases Ca^{2+} current-induced K^+ efflux from the cell cytosol in a range of rodent and human cell types.[95–98] Econazole has also been shown to exert this effect.[150] In this regard, K^+ efflux is the cardinal mechanism that activates the NALP3-inflammasome as a means of early control of *M tuberculosis* replication in macrophages.[151] Nonetheless, re-establishment of the pharmacokinetics of these drugs is crucial for combination therapy with standard antituberculosis regimen.

Antimicrobial peptides

Antimicrobial peptides are highly active molecules located in prokaryotes and eukaryotes, which constitute an important component of the mammalian innate immune system. Although most antimicrobial peptides target the cell membrane of bacteria, DNA replication and protein synthesis can also be disrupted. Cytokine signalling in many cell types governs the production of antimicrobial peptides, which engage a variety of strategies leading to bacterial lysis.[152,153] With regard to tuberculosis, the immunomodulatory antimicrobial peptide cathelicidin (also known as LL-37), whose expression is regulated by vitamin D receptor signalling seems to constitute the major mechanism by which vitamin D3 mediates killing of intracellular *M tuberculosis* in macrophages.

Mannose-capped lipoarabinomannan and phosphatidyl-myo-inositol mannoside, both of which are *M tuberculosis* cell wall components, can trigger production of hepcidin—another immunomodulatory antimicrobial peptide possessing direct antimycobacterial activity.[154] Hepcidin is produced mainly by hepatocytes and plays a major part in physiological iron homoeostasis.[155]

Other antimicrobial peptides with antituberculosis properties include the vitamin D-dependent human β defensin 2, which is produced mainly by epithelial cells,[156] CD8+ T cell and NK cell-derived granulysin,[157,158] and lipocalin 2, which is secreted by macrophages and epithelial cells,[159] although this list is not exhaustive. Because inflammation is the key trigger for production of antimicrobial peptides, regulation of signalling governed by soluble immunomodulators—ie, interferon γ, soluble CD40 ligand, TNFα, CXCL9, and interleukin 6—might contribute to antimicrobial peptide-mediated clearance of intracellular *M tuberculosis*.

Biological interventions with recombinant cytokines

Adjunctive therapy with a range of proinflammatory human cytokines has been explored to augment the Th1 immune response in human beings with tuberculosis, particularly in patients with advanced stages of disease or multidrug-resistant tuberculosis or extensively drug-resistant tuberculosis. Because of the small number of patients treated with cytokine therapies and the paucity of placebo-controlled randomised clinical trials, the full potential of cytokine therapies for the treatment of tuberculosis is unclear at this stage. Of note, the timing of cytokine administration in tuberculosis disease, dose, and mode of administration (inhalation, subcutaneous, or intramuscular) might infer clinically and biologically relevant differences in the method of action, and subsequently, clinical outcomes. Dosing and application modus are therefore covered in detail in this section of the Review.

We do not discuss potential adjunct therapy with monoclonal antibodies that would target central immunological checkpoints in tuberculosis—eg, so-called anergic T cells in patients with tuberculosis[160]—expressing so-called exhaustion markers (eg, Tim-3, LAG-3, CD40L).[161] Some exhaustion marker-positive T cells have been shown to be activated T cells directed at *M tuberculosis*: Tim-3+ T cells that exhibited increased immune effector functions defined by production of Th1 or Th22 cytokines along with cytotoxic T lymphocytes effector molecules. In a preclinical model,[162] these Tim-3+ T cells effectively restricted the growth of *M tuberculosis* in infected macrophages, most likely via binding of Tim-3 to the ligand galectin 9 (expressed by *M tuberculosis*-infected macrophages), which in turn inhibits expansion of effector Th1 cells to prevent further tissue inflammation.[163] Similarly, anti-PDCD1 treatment is effective in a murine model of tuberculosis[164] and blockage of PDCD1, an inhibitory receptor expressed by T cells, has been shown to overcome immune resistance in patients with malignant diseases,[165] and proved to be safe. The advent of therapeutic monoclonal antibodies in malignant disease might therefore pave the way for application of these reagents as a host-directed therapy for treatment of patients with tuberculosis.

Treatment of patients with tuberculosis with biological compounds might also need to take into account the nutritional status[166] of the patients, because systemic metabolic changes affect the developmental profile of proinflammatory or anti-inflammatory immune cell subsets, including regulatory T cells and memory immune formation in CD8+ T cells. Thus, drugs modulating cellular glycolysis or oxidative phosphorylation, and so-called starvation signals,[167] contribute to immune cell plasticity. Metabolic differences are able to dictate whether immune cells will become proinflammatory Th17 or regulatory T cells.[168] The diverse clinical outcome of treatment of patients with tuberculosis with recombinant cytokines might therefore not only indicate a different disease status, or a different genetic background of the individual, but also differences in cellular metabolism associated with concomitant treatment of concurrent other communicable and non-communicable diseases. This situation emphasises the complexity (and potential risks) of host-directed therapies in patients with tuberculosis. Up to now, biologically robust markers that would allow selection of patients who would benefit most from biological therapy have not yet been determined, but are urgently needed.

Interferon γ

In an open-label pilot study, five patients with multidrug-resistant tuberculosis received nebulised recombinant interferon γ three times per week (each dose 500 μg) over 4 weeks with good clinical and microbiological improvement, and only minor adverse events such as cough and muscle aches.[58] In another open-label trial, aerosolised interferon γ at a dose of 2 million units three times per week was also given as adjunctive therapy to six patients with multidrug-resistant tuberculosis after treatment failure, while they continued on identical antituberculosis chemotherapy.[59] All patients tolerated the adjunctive treatment well. Although five of six patients had radiological improvement after aerosolised interferon γ adjunctive therapy, no patients had sputum smear conversion.

In a clinical trial of 89 patients with cavitary pulmonary tuberculosis, patients received either nebulised recombinant interferon γ1β or subcutaneous application of recombinant interferon γ1β at a dose and treatment interval of 200 μg three times per week over 4 months in addition to directly observed treatment (DOTS) or DOTS alone.[60] Nebulised adjunctive treatment with recombinant interferon γ1β resulted in reduction of concentrations of inflammatory cytokines interleukin 1β, interleukin 6, interleukin 8, and interleukin 10 in 24 h BAL supernatants; an accompanied significant difference in the rate of *M tuberculosis* clearance from the sputum smear at 4 weeks compared with DOTS or DOTS with subcutaneous recombinant interferon γ1β was also observed (p=0·03). Four severe adverse events observed during the study were considered by the Data Safety Management Board to be unrelated to the treatment.[60]

A systematic review[61] from 2011 on the role of adjunctive therapy with interferon γ for the treatment of pulmonary tuberculosis includes eight studies from China, of which one is a randomised controlled trial. Meta-analysis of trials with aerosolised interferon γ at doses of 1–5 million units three times weekly over 2–4 months showed statistical benefits on sputum conversion after 1, 2, 3, and 6 months and at the end of treatment.[61] No patients interrupted therapy because of adverse events.

Intramuscular adjunctive application of interferon γ at a dose of 10 million units daily for 1 month followed by three times weekly for 6 months in addition to individualised antituberculosis drug treatment was well tolerated, and resulted in treatment success in seven of eight patients with drug-resistant tuberculosis.[62] Treatment results were better than those for historical controls. In a meta-analysis[61] of three additional trials from China, in which adjunctive application of 1 million units of interferon γ were administered intramuscularly three times per week over 2–4 months, significant benefits on sputum conversion by 2 months were observed; the effect was, however, less prominent than with aerosolised adjunctive interferon γ therapy.

In summary, these results suggest that adjunctive therapy with aerosolised interferon γ is safe and could be effective for patients with non-sputum conversion, especially with multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. The aerosolised route of administration seems to be better than the intramuscular administration; however, additional placebo-controlled studies with large numbers of patients are needed before definitive conclusions can be drawn.

Granulocyte-macrophage colony-stimulating factor (GM-CSF)

GM-CSF is a cytokine that augments the proliferation of macrophages and granulocytes and has been explored in a clinical phase 2 trial with 31 patients against placebo as adjunctive therapy for patients with pansusceptible pulmonary tuberculosis.[67] In this study, GM-CSF was administered at a dose of 125 mg/m² body surface twice per week for 4 weeks. The clinical outcomes were similar in the GM-CSF and placebo groups, and no difference in sputum culture conversion was observed at the end of the fourth week of treatment. GM-CSF has been widely used as therapy for patients with cancer, either as a sole reagent or as a vaccine adjuvant. However, a recent study suggests that caution needs to be exercised because GM-CSF could subsequently lead to immune suppression and negatively affect disease outcome.[68]

Interferon α

Adjunctive treatment with interferon α in addition to second-line antituberculosis therapy has been explored for patients with multidrug-resistant tuberculosis. In an open-label trial, aerosolised human interferon α was given at doses of 3 million units each, three times a week for 9 weeks, to seven patients with multidrug-resistant tuberculosis, who were not responding to a second-line therapy.[69] The combined therapy was well tolerated and only muscle aches were observed in one patient. All patients showed clinical and microbiological improvements during the course of the aerosol treatment; however, the effect was not sustained when interferon α treatment was discontinued. In an open-label clinical phase 2 study, 3 million units of recombinant interferon α2β were administered subcutaneously every week for 12 weeks together with second line antituberculosis therapy to patients with multidrug-resistant tuberculosis. Only two of five patients had a beneficial clinical course after therapy.[70] Based on the results of these preliminary findings, adjunctive immunotherapy with interferon α does not seem to be a promising treatment option for patients with tuberculosis.

Interleukin 2

Augmentation of the host's immune response in patients with tuberculosis by adjunctive therapy with interleukin 2 has been explored in several clinical trials with conflicting results. Intradermal injection of 12·5 μg twice daily over

30 days of human recombinant interleukin 2 was safe and seemed to be effective to achieve conversion to a sputum-negative smear in an open label non-randomised trial with 20 patients, including patients with multidrug-resistant tuberculosis.[63,64] Daily administration was better to achieve sputum conversion than was pulsed therapy.[65] However, a randomised, placebo-controlled, double-blinded trial assessed adjuvant therapy with interferon 2 in 110 patients with sputum smear-positive, pan drug-susceptible tuberculosis, and could not confirm the results from the preliminary studies.[66] Although additional treatment with 0·225 million units of recombinant interleukin 2 twice daily during the first 30 days of treatment was safe, sputum culture conversion was delayed after 1 or 2 months.[66] After these results, in the past 10 years, no trials have explored the use of interleukin 2 as an adjunctive therapy for tuberculosis.

Possibly, the route of administration and dose might need reassessment for treatment of patients with tuberculosis. Low success rate has been argued to be because of the induction of regulatory T cells, whereas conventional antigen-specific T cells might not have been able to respond adequately. Studies have confirmed this notion; patients with chronic graft versus host disease who did not respond to glucocorticoid therapy received daily low-dose subcutaneous interleukin 2 ($0·3\times10^6$, 1×10^6, or 3×10^6 IU per square meter of body-surface area) for 8 weeks.[169] The numbers of CD4+ regulatory T cells increased in the patients given interleukin 2 with a peak median value at 4 weeks ($p<0·001$) after treatment initiation, without affecting conventional CD4+ T cell counts. Low dose interleukin 2 therapy promotes immune tolerance, whereas conventional interleukin 2 therapy rescues T cells from apoptosis, and induces thymic output and T cell proliferation.[170]

Interleukin 12

Interleukin 12 is essential for protective immunity against *M tuberculosis* in human beings; however, only one case report describes experimental treatment of a patient with pulmonary tuberculosis who did not respond to conventional therapy despite DOTS.[71] Adjuvant treatment with 300 ng/kg bodyweight interleukin 12 administered subcutaneously twice weekly over 3 months significantly improved the clinical condition of this patient. Tuberculosis relapsed on termination of adjunctive interleukin 12 treatment and cure was achieved only after reapplication of interleukin 12 for a further 5 months. Table 2 and the appendix show other candidate cytokines—eg, interleukin 7—that have been assessed clinically in other diseases, or in animal models, within the frame of tuberculosis immunotherapy and host-targeted therapies.

Biological intervention with vitamin D

Vitamin D (1,25-dihydroxyvitamin D) deficiency has been associated with increased risk of tuberculosis.[171] The exact mechanisms have not been determined, but vitamin D seems to effect the gene transcription of antimicrobial peptides DEFB4/HBD2 and cathelicidin.[172] New studies suggest that vitamin D induces interleukin 1β, which leads to reduction of the burden of *M tuberculosis,* via interaction of the NLRP3/caspase 1 inflammasome in infected cells.[51] Vitamin D therapy can therefore be deemed a form of host-targeted therapy; clinical trials are discussed in greater detail in the appendix.

Vaccine candidates in clinical trials

Vaccine candidates can be segregated into different groups based on the following criteria: 1) according to their target population, either therapeutic or preventive vaccines; 2) according to their composition, preparations of killed mycobacteria, viable recombinant mycobacteria, or viral-vectored and adjuvanted subunit vaccines; 3) according to time of administration with regards to natural infection with *M tuberculosis,* pre-exposure or postexposure vaccines; and 4) according to their relation to the conventional BCG vaccine, either BCG replacement or heterologous prime–boost vaccines. Figure 3 shows important vaccine candidates according to their target population and time of administration, and will be discussed here.

Therapeutic vaccines target patients with severe forms of tuberculosis in adjunct to chemotherapy—notably, in cases of multidrug-resistant tuberculosis, extensively drug-resistant tuberculosis, or *M tuberculosis* and HIV co-infection.[174] The vaccines include RUTI, a semi-purified preparation of *M tuberculosis* grown under stress to induce expression of relevant stress or dormancy antigens;[175] killed preparations of *Mycobacterium vaccae*, an atypical mycobacterial species;[176–178] and killed *Mycobacterium indicus pranii*, which was originally developed as an antileprosy vaccine, but retrospective data analysis showed its potential to provide benefit against tuberculosis.[179]

Preventive pre-exposure vaccines are intended to replace BCG because of increased efficacy or safety, or both, for the target newborn, and hence are given before exposure to natural infection with *M tuberculosis*.[180] These vaccines include recombinant BCG mutants, such as VPM1002,[181] and recombinant *M tuberculosis* double-deletion mutants, such as MTBVAC.[182] VPM1002 (rBCGΔUreC::hly), developed by Max Planck Institute for Infection Biology, Berlin, Germany, expresses listeriolysin (*Hly*) from *Listeria monocytogenes*. Deletion of urease C (*UreC*) allows for acidification of the phagosome in which the vaccine resides and therefore provides an optimal pH for biological activity of listeriolysin, facilitating perforation of the phagosomal membrane.[183] Because of the proline, glutamic acid, serine, and threonine (PEST) sequence, listeriolysin is rapidly degraded once it arrives in the cytosol.[184] This vaccine is both safer and more efficacious than is parental BCG in preclinical models. The recombinant

	Biological effects	Treatment details	References
Interleukin 24	Expression promotes CD8+ T-cell priming, interferon γ secretion, and antituberculosis activity	Vaccination with DNA-encoding human interleukin 24 on day of *M tuberculosis* infection and 7 days pi	Ma et al (2011)
Interleukin 22	Administration promotes T-regulatory cell decrease, antigen-specific T-cell expansion	2 ng recombinant interleukin 22 with α-NK1.1	Dhiman et al (2012)
Interleukin 22	Administration reduced *M tuberculosis* intracellular replication	10 ng/ml recombinant interleukin 22 in cell culture	Dhiman et al (2014)
SOCS1	Early inhibition improved interferon γ-dependent antituberculosis activity; SOCS1-deficient mice succumbed to hyperinflammation 4 wpi	SOCS1-deficiency/conditional silencing in mice	Carow et al (2011)
Interleukin 17	Blockade reduced lung pathology after *M tuberculosis* infection followed by triple BCG vaccination of mice	100 μg α-interleukin 17	Cruz et al (2010)
Interleukin 11	Blockade abrogated early pulmonary inflammation in tuberculosis-susceptible mice	50 μg α-interleukin 11 in IFA; 1 day before infection and 2, 14, 17, 20, and 22 days pi	Kapina et al (2011)
Interleukin 10	Dampens interleukin 17-associated immunopathology	Neutrophil-derived interleukin 10 in chronic BCG infection of mice	Doz et al (2013)
Interleukin 10	Involved in maintenance of granuloma integrity, in equilibrium with TNFα concentrations	In-silico modelling	Cilfone et al (2013)
Interleukin 10	Blockade induces multinucleate giant cell formation; implications in granuloma formation	Addition of 5 μg/mL α-interleukin 10 to PBMC culture	Shrivastava et al (2013)
Interleukin 10	Blockade reduced pathology in lungs and spleen	350 μg α-interleukin 10R weekly; 250 μg α-CD20 every other week	Torrado et al (2013)
Interleukin 10	Blockade reduced *M tuberculosis* replication in lungs and spleen of susceptible and resistant mice	1 mg before BCG vaccination; 0·35 mg weekly for 6 weeks	Pitt et al (2013)
Interleukin 10	Early blockade improved Th1 responses	Addition of 15 μg/mL α interferon 10R to cell culture	Jeyanathan et al (2013)
Interleukin 4	Blockade reduced lung pathology and *M tuberculosis* replication, enhanced granuloma area, NO, and lung chemokine levels	500 μg α-interleukin 4 administered 5 wpi (2nd week post HR treatment) with 100 000 U recombinant interferon γ and 37 μg α-Acr	Buccheri et al (2009)
Interleukin 4	Blockade promoted 40x reduced bacterial proliferation up to 8 weeks in lungs and spleen	500 μg α-interleukin 4 administered 3 wpi with 10 000 U recombinant interferon γ and 50 μg α-Acr	Buccheri et al (2007)
TGFβ	Blockade with LAP increased mycobactericidal activity in the lung and mediastinal lymph nodes	12·5 μg rLAP at infection and 14 days pi	Wilkinson et al (2000)
TGFβ	Blockade increased DHEA levels, leading to activation of antimycobacterial Th1 responses	Addition of 61 μg/mL α-TGFβ to forskolin-containing adrenal cell culture	D'Attilio et al (2012)

See appendix for the full references. pi=postinfection. IFA=incomplete Freund's adjuvant. TNFα=tumour necrosis factor α. wpi=weeks postinfection. PBMC=peripheral blood mononuclear cells. Acr=*tuberculosis* α-crystallin. NO=nitric oxide. LAP=latency-associated protein. DHEA=dehydroepiandrosterone. TGFβ=transforming growth factor β.

Table 2: **Examples of promising host-directed therapies under preclinical evaluation**

M tuberculosis double-deletion mutant MTBVAC developed by the University of Zaragoza, Zaragoza, Spain, is deficient in the transcription factor PhoP and in FadD26, thus fulfilling the demand of the WHO for two independent mutations in *M tuberculosis*-based constructs to prohibit reversion to wildtype.[185] PhoP regulates expression of numerous genes involved in virulence and persistence of *M tuberculosis*, whereas FadD26 is a crucial enzyme in glycolipid cell-wall synthesis.

The heterologous booster vaccines are either viral vectors expressing *M tuberculosis* antigens or formulations of *M tuberculosis* antigens in adjuvants. The MVA85A/Aeras-485 vaccine developed by the University of Oxford, Oxford, UK, is composed of a modified vaccinia Ankara (MVA) construct expressing the immunodominant antigen shared between BCG and *M tuberculosis*, antigen Ag85A (encoded by Rv3804).[186] The vaccine is intended for pre-exposure administration in infants and postexposure administration in adults. The vaccine has completed a phase 2b efficacy trial, but unfortunately without positive outcome;[186] the vaccine given as boost of BCG prime failed to confer better protection than did BCG prime alone in infants that were vaccinated. Moreover, increases in the frequency of *M tuberculosis*-specific interferon γ-producing mononuclear cells; frequency of *M tuberculosis*-specific CD4+ T cells producing interferon γ, interleukin 2, and TNFα; and frequency of *M tuberculosis*-specific Th17 T cells were observed in the group of children that were vaccinated with a heterologous boost on top of BCG compared with control group vaccinated with BCG alone, and thus failed to function as measurable correlates of immune protection.[186]

At present, two human adenovirus-based vaccines are passing through the clinical trial vaccine pipeline; Ad5 developed by McMaster University, Hamilton, Canada, only expresses Ag85A (Rv3804), whereas Crucell Ad35/Aeras-402, coexpresses a total of three antigens—namely Ag85A (Rv3804), Ag85B (Rv1886), and TB10·4

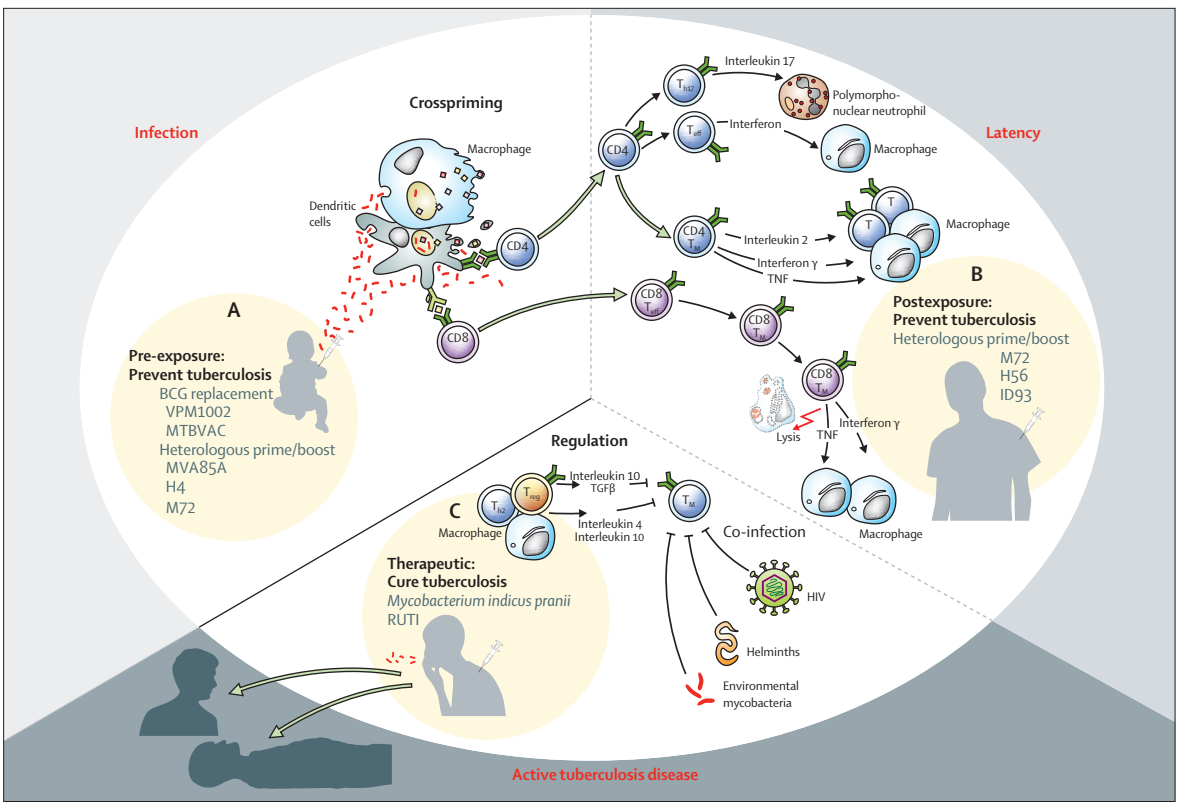

Figure 3: **Different types of vaccination protocols in the context of immunity to tuberculosis**
(A) Pre-exposure vaccines are given before *M tuberculosis* infection generally to newborn babies or infants and are intended to prevent active tuberculosis. These vaccines are either replacements to BCG, such as VPM1002 or MTBVAC, or heterologous boost vaccines given after BCG prime such as MVA85A or H4 vaccines. Infection with *M tuberculosis* causes the stimulation of CD4+ and CD8+ T cells and both contribute to protection. (B) Postexposure vaccines are intended to prevent tuberculosis, but are given to already infected individuals, typically adolescents and adults. Present candidates are all heterologous prime-boost vaccines including the viral-vectored MVA85A and protein–adjuvant formulations, such as M72, H56, and ID93. CD4+ T cells, which produce type 1 cytokines, activate increased antimycobacterial capacities in mononuclear phagocytes. Helper T (Th) 17 cells that produce interleukin 17, which attracts and activate neutrophils probably also contribute to protection at an early stage. CD8+ T cells produce a similar cytokine pattern as CD4+ T cells, thus activating antimycobacterial capacities in mononuclear phagocytes, and in addition, secrete cytolytic molecules, which can directly kill *M tuberculosis*. These mechanisms are crucial for protective immunity against tuberculosis. (C) Therapeutic vaccines are given in adjunct to chemotherapy to patients with active tuberculosis—typically those patients with further complications such as multidrug-resistance or HIV and tuberculosis co-infection. Therapeutic vaccines include *Mycobacterium indicus pranii* and RUTI. Protection is the result of complex interactions between different immune mechanisms. Perturbation of the protective immune response through co-infection with HIV, helminths, environmental mycobacteria, or endogenous regulatory immune mechanisms that suppress protective immunity, result in progression to active tuberculosis disease. Adapted from Ottenhoff and Kaufmann,[173] with permission. T_{eff}=T effector cells. TGFβ=transforming growth factor β. Th1=T helper 1 lymphoctes. Th17=T helper 17 lymphocytes. T_M=T memory cells. TNF=tumour necrosis factor. T_{reg}=T regulatory cells.

(Rv0288).[187,188] Both vaccines could be candidates for postexposure vaccination. A crucial issue with these vaccines is the high prevalence of antiadenovirus antibodies—notably to Ad5—in healthy individuals in tuberculosis-endemic areas, which could affect efficacy of adenovirus-based vaccines. Furthermore, adenovirus 5-based vaccines against HIV have caused increased infection rates and exacerbated risks of AIDS, and have thus stimulated discussions as to whether adenovirus-based vaccines should be further pursued against any type of disease (including tuberculosis) in areas with high HIV incidences.[189–191]

The M72 vaccine developed by GlaxoSmithKline in Rixensart, Belgium, comprising two antigens shared by *M tuberculosis* and BCG (Rv1196 and Rv0125) in the adjuvant AS01E (a liposome-based mix of the saposin QS21 and the Toll-like receptor 4 ligand monophosphoryl lipid A) is intended for use as a pre-exposure and postexposure vaccine.[192]

The Hybrid 1 (H1) and Hyvac 4/Aeras-404 (H4) vaccines developed by Statens Serum Institutet, Copenhagen, Denmark, are composed of Ag85B (Rv1886) combined with ESAT-6 (Rv3875) or TB10·4 (Rv0288), respectively.[193,194] Ag85B is shared by *M tuberculosis* and BCG, whereas ESAT-6 and TB10·4 are present in *M tuberculosis*, but absent in BCG. This shift from ESAT-6 to TB10·4 was made to avoid cross-reactivity between *M tuberculosis* infection and vaccination, because ESAT-6 is also present as an antigen in diagnostic tests for *M tuberculosis* infection (both active and latent tuberculosis).[195] H4 is likely to replace H1 in the future. Both antigens are adjuvanted by IC31® (comprising a

Search strategy and selection criteria

We searched publications in the English language in PubMed and Google Scholar (1940–2013), the Cochrane Library (2001–12), and Embase (2001–12) with the terms "tuberculosis", "*Mycobacterium tuberculosis*", and "TB", combined with "vaccines', "new vaccines", "vaccination", "immunization", "vaccine safety", "subunit vaccines", "biomarkers", "vaccine development", "vaccine trials", and the terms "host-directed therapy" combined with "TB", "tuberculosis", "*Mycobacterium tuberculosis*", "adjunct therapy", "adjunct treatment", "drug resistance", "MDR-TB", "XDR-TB", and "immunotherapy". We complimented the search with publications from the WHO Global TB Department, the International Union Against Tuberculosis and Lung Disease, Treatment Action Group, Stop TB Partnership, and the George Institute for International Health. We also reviewed studies cited by articles identified by this search strategy and selected those we identified as relevant. We focused on completed and ongoing clinical trials of cytokine-based host-directed therapy, and "repurposed" drugs or compounds that have already been tested (for other clinical indications). Most of the work about *M tuberculosis* infection and autophagy has been generated in preclinical models. The appendix describes concepts in *M tuberculosis* and HIV co-infection, cytokines in *M tuberculosis* immunotherapy, preclinical models of host-directed therapies, and biological intervention with vitamin D.

cationic polypeptide and an oligodeoxynucleotide as the Toll-like receptor-9 ligand developed by Intercell).[196] H1 has also been formulated in the adjuvant CAF01 developed at Statens Serum Institutet, and comprising dimethyldioctadecyl-ammonium bromide and trehalose 6,6'-dibehenate in a liposome-based adjuvant.[197] These vaccines are intended for pre-exposure immunisation.

While H1 and H4 are being developed for pre-exposure use, the best suited candidate for post-exposure immunisation in this group is Hybrid 56/Aeras-456 (H56) in IC31® adjuvant because this vaccine construct includes, in addition to Ag85B and ESAT-6, the antigen Rv2660c, which is claimed to be expressed by *M tuberculosis* under starvation or stress conditions, and is shared by, but apparently not immunogenic in, BCG.[198] Recent findings, however, have cast doubt as to whether Rv2660c can be expressed at all in the host.[199] The presence of ESAT-6 was deemed acceptable for this vaccine because it is intended for postexposure vaccination, that is, for individuals who are already positive for ESAT-6 antigen because of natural *M tuberculosis* infection.

A recent development is the vaccine ID93 by the Infectious Disease Research Institute, Seattle, WA, USA, comprising four different antigens—namely, Rv2608, Rv3619, Rv3620, and Rv1813—all shared between *M tuberculosis* and BCG; Rv1813 is expressed under starvation or stress conditions.[200] These four antigens in the form of a fusion protein have been adjuvanted by a stable emulsion of glucopyranosyl lipid as a Toll-like receptor-4 agonist with squalene. Similar to H56, this vaccine is intended for postexposure vaccination of adolescents and adults.

The tuberculosis vaccine trial pipeline

Phase 1 trials for vaccine candidates of tuberculosis are mainly done in adults; first in the geographical area of development, and second in a developing country with a high prevalence of tuberculosis. The primary goal of these trials is safety assessment and, generally, first insights into the immunogenicity of these candidates are included. Frequently, clinical trials comprise both tuberculin skin test (TST)$^{+}$ and TST^{-} individuals, that is, individuals who have had previous BCG vaccination or *M tuberculosis* infection, although those patients with *M tuberculosis* infection are generally excluded by additional diagnostic tests that can distinguish *M tuberculosis* infection from BCG vaccination.[196] Two vaccine candidates are not progressing further. First, BCG85-expressing Ag85B developed by the University of California Los Angeles, Los Angeles, CA, USA, is on hold, although it successfully completed phase 1 assessment.[201,202] Second, the phase 1 trial of r-BCG:pfo/Aeras-422 (NCT01340820) had to be terminated prematurely because of severe adverse events—namely, reactivation of shingles in some study participants.[203,204]

Two products (H4 and H56) are being prepared for phase 2 trials and several trials are in clinical phase 2 assessment (H56: NCT01865487 [recruiting]; H4: NCT01861730 [recruiting]). In phase 2a, optimum dose, route, and safety in the target population are assessed. Phase 2a has been completed for the M72 vaccine in several target populations including tuberculin skin test negative[193] and positive[205–208] individuals and in HIV coinfected study participants.[209] M72 is being prepared for phase 2b testing. Crucell Ad35/Aeras-402 has been revised to phase 2a from phase 2b. The VPM1002 (rBCGΔUreC::hly) has successfully completed the core observation of phase 2a in newborn infants after completion of two phase 1 studies in adults.[210] Because in the phase 2a study, VPM1002 was substituted for BCG in one study group, infants will be observed for an additional time in phase 2b. MVA85A/Aeras-485 has completed phase 2b as a heterologous boost of BCG prime in infants without protective efficacy,[186] and is also being tested in a phase 2b study in adults with *M tuberculosis* or HIV infection having successfully completed a phase 2a study.

Therapeutic vaccines that were, or are, under clinical investigation include the following: RUTI, which successfully completed a phase 2a study;[175,211] DAR-901, a new version of the *M vaccae* preparation—the previous *M vaccae* vaccine had been tested in a phase 3 trial in

HIV-positive patients with tuberculosis;[177] and *M indicus pranii*, which is already licensed for restricted use as tuberculosis therapy in India.[179,212] Although the clinical benefit of another *M vaccae* vaccine produced by Anhui Longcom in China (NCT01979900) has been questioned after phase 2b, this vaccine remains in the clinical development pipeline.[176,209] Possibly, some of the preventive vaccines—M72, H56, and ID93—will be repurposed for tuberculosis therapy in addition to their prime aim as postexposure preventive vaccine; a good example of the tight link between vaccination and host-targeted therapies.

Although single vaccine candidates are being clinically evaluated, discussions between different vaccine developers and interested stakeholders must be initiated concerning the launch of combination vaccination strategies comprising improved prime and heterologous boost vaccine candidates for clinical efficacy trials. Worth consideration is combining vaccine candidates that have already successfully completed phase 2b trials, that is, before phase 3 and licensing as part of an adaptive trial design. In the specialty of tuberculosis, the approval of clinical trials testing combinations of novel drug candidates before their individual licensing sets a precedent for such an approach.

Future outlook

In the natural course of tuberculosis in most human beings, *M tuberculosis* can be eradicated by way of innate immune mechanisms.[213] However, our understanding of the complexity of human immune defence against *M tuberculosis* is at present too restricted to augment the immune response in the right direction for most patients who are not naturally cured of the disease to enable control of the growth of *M tuberculosis* and subsequent clearance of infection in the absence of drug treatment. Recent advances in vaccine development and host-directed therapies offer promising perspectives to continue to explore immunotherapy as a treatment option for patients with tuberculosis, especially for multidrug-resistant and extensively drug-resistant tuberculosis. Substantial investments in research and development are still needed to identify adequate immune-based interventions that hopefully can in the future be used for the prevention and cure of tuberculosis.

Contributors

MM, MS, and AIZ initiated the idea. SHEK wrote the first draft of the tuberculosis immunity and vaccines section. MM wrote the first draft of the host-targeted therapies section. All authors contributed to the writing and finalisation of the manuscript. For correspondence about host-directed therapies, contact MM. For correspondence about vaccines, contact SHEK at kaufmann@mpiib-berlin.mpg.de.

Declaration of interests

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