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New tricks for old dogs: countering antibiotic resistance in tuberculosis with host-directed therapeutics

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Summary

Despite the availability of Mycobacterium tuberculosis (Mtb) drugs for over 50 years, tuberculosis (TB) remains at pandemic levels. New drugs are urgently needed for resistant strains, shortening duration of treatment, and targeting different stages of the disease, especially for treatment during human immunodeficiency virus co-infection. One solution to the conundrum that antibiotics kill the bacillus yet select for resistance is to target the host rather than the pathogen. Here we discuss recent progress in so called ‘host-directed therapeutics’ (HDTs), focusing on two general mechanistic strategies: (i) HDTs that disrupt Mtb pathogenesis in macrophages and (ii) immunomodulatory HDTs that facilitate protective immune responses that kill Mtb or reduce deleterious responses that exacerbate disease. HDTs hold significant promise as adjunctive therapies in that they are less likely to engender resistance, will likely have efficacy against antibiotic-resistant strains, and may have activity against non-replicating Mtb. However, TB is a complex and variegated disease, and human populations exhibit significant diversity in their immune responses to it, which presents a complicated landscape for HDTs to navigate. Nevertheless, we suggest that a detailed mechanistic understanding of drug action, together with careful selection of disease stage targets and dosing strategies may overcome such limitations and allow the development of HDTs as effective adjunctive treatment options for TB.

Keywords

tuberculosis; macrophage; drug; innate immunity

Introduction

Why target the host?

Tuberculosis (TB) remains a devastating disease with approximately 2 billion people infected worldwide and 1.2 million deaths in 2010 (1, 2). The pandemic is exacerbated by the rise of Mycobacterium tuberculosis (Mtb) strains that are resistant to some or all available antibiotics, an outcome predicted by Fleming at the beginning of the antibiotic era.

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Several newly developed antibiotics targeting novel bacterial enzymes are currently in clinical trials (4). However, as with existing antibiotics, resistance may ultimately arise against these drugs as well. Drugs that reduce the length or toxicity of existing treatment regimens and targeting non-replicating MTb are urgently needed and remain an important goal for anti-TB therapy. Recent research efforts to develop new antibiotics and vaccines are promising and are reviewed elsewhere within this issue.

Targeting the host rather than the pathogen offers one possible solution to the challenge of antibiotic resistance and killing nonreplicating bacilli. So-called ‘host-directed therapeutics’ (HDTs) affect MTb in two general ways. Some HDTs impair MTb survival and replication by disrupting macrophage (and other myeloid cell) host signaling pathways used by the pathogen during infection, thereby rendering the bacteria more sensitive to host defenses or to antibiotics. Such HDTs include drugs that affect bacterial uptake, trafficking, autophagy, and activation of anti-microbial killing mechanisms [e.g. reactive oxygen intermediates (ROIs), β-defensins, and cathelicidins]. Other HDTs augment the immune response to MTb, and disrupt the equilibrium that the bacteria has established with the host to avoid elimination. Alternately, other HDTs mitigate pathologic inflammatory consequences. Immunomodulatory HDTs may also induce novel responses to MTb that facilitate clearance, which either do not normally occur or are actively suppressed by the bacillus. Such immunomodulatory HDTs include those that affect cytokines, eicosanoid inflammatory pathways, antigen presentation, T-cell activation, cellular trafficking in the lungs, and lung fibrosis. Categorizing HDTs based on mechanism is useful but somewhat arbitrary, because disruption of trafficking in macrophages, for example, can have advantageous immunological consequences, and some HDTs have effects on both pathogenesis and immunity, and some others additionally affect the bacteria directly.

How can we develop HDTs? Most if not all pathogens utilize cellular signaling molecules, either by encoding them directly or by evolving proteins that interface with them, often as a means to enter, move through, or exit a host cell. Indeed, in a remarkable example of evolutionary convergence, many bacterial and viral pathogens use the same host proteins, particularly those associated with the actin cytoskeleton, which govern cellular motility and vesicular trafficking. For example, a wide variety of viruses and bacteria, including Ebola and MTb, utilize host tyrosine kinases (TKs) for pathogenesis, including members of the c-Src and c-Abl TK families, although the mechanisms by which TKs are utilized by each differ (5–17). Importantly, signaling molecules such as TKs are often dysregulated in cancer, and specific inhibitors have been developed to target them. This raises the possibility that cancer therapeutics might be ‘repurposed’ to treat diverse microbial and viral infections, including TB. In general, by understanding the basic cellular mechanism used by pathogens to usurp host signaling, inhibitors targeting these pathways can be developed or repurposed.

There are several advantages to HDTs compared to conventional antibiotics (Fig. 1). First, HDTs make it more difficult for the pathogen to evolve drug resistant variants, as the bacteria would have to develop an entirely new suite of interactions with host factors while still under immune selection. It is not impossible, of course, as evidenced by chloroquine-resistant malaria, but much less likely. Second, HDTs have the potential for activity against MTb strains that are resistant to conventional antibiotics. Such a capacity also raises the
possibility that HDTs may synergize with antibiotics. For example, by directing the organism to a compartment where pathogen defenses are neutralized, HDTs may render bacteria simultaneously more susceptible to antibiotics. In so doing, strains ostensibly resistant to antibiotics may be rendered sensitive.

Several constraints on the use of HDTs exist. Mammalian systems have evolved significant functional redundancy, which pathogens have used to their advantage by interfacing with families of host proteins in ways that are relatively nonspecific, compared to the normal cellular signaling partner. To target such interactions may require that an HDT act somewhat nonspecifically and affect a family of proteins. Several pathogens utilize groups of tyrosine kinases during infection as a means of ensuring replication and spread in a myriad of tissues. Such utilization also necessitates choosing inhibitors that block all of these family members. However, it may not be necessary to inhibit all host molecules with the same stringency nor to the same degree as is required for other diseases. For cancer treatment, any reduction in inhibition of the activity of a target leaves the door open for resistance; however, treating infections may only require that HDTs work well enough to allow development of a protective immune response or enhance killing by anti-Mtb drugs. Finally, the rules regarding toxicity likewise differ. For cancer drugs, the bar is fairly low and toxicity is tolerated in hopes of improving the chances of survival. For TB the bar is higher, as any diminution in immune function or homeostasis can have lethal consequences. Thus, the rules for specificity, target affinity, and toxicity that have guided development of drugs for other diseases such as cancer are different for HDTs targeting infectious diseases. In short, drugs with lower target specificity and affinity, may be both less toxic and more efficacious. Paradoxically, a good source of HDTs for infectious diseases with excellent toxicity profiles may be cancer drugs rejected by the pharmaceutical industry for lack of efficacy.

A continuing conundrum for HDTs that affect the immune response is that the immune status of patients may be heterogeneous due to the stage of the disease or genetic differences, yet such differences may not be easily recognizable. Whereas HDTs with significant immunosuppressive activity are likely to be universally deleterious, those that can modestly enhance protective inflammation or dampen overarching inflammation may be useful against TB, but in different contexts. To some extent dosing becomes a critical factor in determining efficacy, and immunological readouts that determine the choice of dose remain important to define. However, genetic polymorphisms may also be important determinants for both TB susceptibility and, therefore, for sensitivity to HDTs.

With recent reviews of the TB HDTs in the literature (18, 19), we do not summarize all available drugs here. The compounds with promising pre-clinical and/or clinical TB data with FDA approval or late stage clinical evaluation for other disease conditions are listed in Table 1. Rather, we review recent data on drugs not previously discussed in detail elsewhere and highlight recent advances in the development of some of the previously reviewed drugs. We also prioritize discussion of FDA-approved drugs that offer promise for rapid advancement towards clinical trials. In this review, we organize the drugs into four categories: (i) HDTs that alter trafficking, and in particular autophagy, as a means to facilitate anti-TB immunity; (ii) HDTs that target lipid and carbohydrate metabolism, eicosanoids, and cytokine responses, to augment anti-TB immune responses and mitigate
deleterious inflammation; (iii) HDTs that have dual effects on host as well as Mtb; (iv) HDTs that induce novel anti-TB responses. It is important to note that such categories are arbitrary at best, as some HDTs fall into more than one group. For each we point out both advantages and disadvantages to their use and challenges in moving them forward to clinical testing. Finally, we consider in a general way the criteria and challenges for advancing HDTs into clinical trials and evaluating their efficacy.

**Whetting the cellular appetite: targeting Mtb trafficking and the autophagy pathway with HDTs**

Autophagy is a cellular mechanism conserved from yeast to humans used for recycling of cellular amino acids, phospholipids, and organelles. Autophagy also plays a critical role in the cellular defense against intracellular pathogens including both viruses and bacteria (20–22). For Mtb, autophagy controls bacterial number in macrophages (23), and HDTs that target autophagy represent a potential new class of TB therapeutics. In this section we provide an overview of how Mtb induces autophagy, how HDTs alter autophagy in the context of Mtb infection, and suggest additional means to target the pathway.

Multiple stimuli, including growth factors, amino acids, and pathogens, activate autophagy via mammalian target of rapamycin (mTOR). mTOR acts upstream of ULK1, or ATG1, which induces autophagosome maturation, a process mediated by several autophagy-related (ATG) proteins and the phosphoinositide-3 kinases (PI3K, Vps34, PI3KC3), and modulated by beclin-1. Structurally, autophagosomes sequester cytoplasmic material and traffic along microtubules toward the microtubule organizing center, where they fuse with lysosomes, which results in proteolysis of their cargo (7). The cell then recycles the residual amino acids. This work has been reviewed comprehensively elsewhere (22, 24–26). Fig. 2 identifies key members of the autophagy pathway, along with the mechanism of action of HDT candidates mentioned in this section.

Autophagy plays a critical role in limiting Mtb replication within the macrophage and contributes to killing of Mtb within macrophages, antigen presentation to T cells, and cell death (23, 27). Genome-wide short interfering RNA (siRNA) screens suggest that several proteins that regulate autophagy are critical for control of Mtb (28–31). Accordingly, mice lacking critical autophagy-related genes, such as atg5, are highly susceptible to Mtb infection (32). Following phagocytosis, Mtb resides within the phagosome, where it is targeted for xenophagy by ubiquitination. The ubiquitin ligase parkin (PARK2) has recently been identified as a key protein in the recruitment of Mtb to the autophagosome (33). Accordingly, mice deficient in park2 remain unable to control Mtb replication in macrophages and are highly susceptible to Mtb infection. Furthermore, polymorphisms in the PARK2 gene have been associated with an increased risk of leprosy (34, 35). Thus, the PARK2 axis is critical for delivery of Mtb to the autophagosome as well as the activation of autophagy in response to infection by mycobacteria. Mtb-induced autophagy also depends on Tank-like binding kinase (TBK-1) and STING, which may sense bacterial DNA (32, 36). Mtb actively resists phagolysosomal fusion via several virulence mechanisms. Thus, cellular mechanisms or HDTs that activate autophagy may tip the balance in favor of clearance.
Targeting autophagy with HDTs

One of the best-studied molecules for inducing autophagy is rapamycin, which inhibits mTOR kinase activity. Rapamycin has been used for over 30 years to counter transplant rejection due to its global immunosuppressive effects (37). Blockade of mTOR by rapamycin retards dendritic cell maturation and inhibits antigen uptake and presentation by dendritic cells (38), and attenuates T-cell proliferation by restricting cell cycle progression in G1 phase (39). Furthermore, rapamycin enhances the generation and function of regulatory T cells, which may account for its immunosuppressive effects during transplantation (40). However, rapamycin can also display immunostimulatory effects, particularly at low doses. Araki et al. (41) showed that treatment with rapamycin increased the quantity and quality of antigen-specific memory T cells in a Lymphocytic choriomeningitis virus (LCMV) infection model. Moreover, in mice and rhesus macaques, rapamycin enhanced immune responses to live virus or vaccination in an mTOR-dependent manner (42). Thus, rapamycin acts in a cell intrinsic manner to enhance the quantity and quality of antigen-specific T cells. More recent data suggest that rapamycin can exert differential effects on particular T-cell populations: whereas rapamycin can augment antigen-specific CD8+ T-cell response to *Listeria*, it did not do so against an allograft, suggesting that rapamycin can specifically activate anti-pathogen responses (42).

As with LCMV, rapamycin reduces Mtb replication in macrophages and enhances the capacity of Mtb-infected dendritic cells to induce IL-12p70, IFN-β, and IFN-γ (23). Additionally, rapamycin improves the capacity for murine dendritic cells to present BCG antigen to T cells (43). Its potential immunosuppressive activity has precluded the use of rapamycin against Mtb in humans to date. However, as with LCMV, it may be possible to enhance its anti-pathogen activity by adjusting the dose. Alternatively, it may be possible to use other related molecules that are more effective stimulators of autophagy. For example, whereas rapamycin does not maximally inhibit mTOR complex 1, the small molecule mTOR inhibitors PP242 and Torin 1 inhibit both mTOR complex 1 and complex 2 (44) and are more effective stimulators of autophagy than rapamycin (45).

Several other signaling factors in the autophagy pathway besides mTOR may be targetable. For example, Beclin-1 is a component of a complex that marks membranes for phagophore formation downstream of UNC51-like kinase-1 (ULK1; ATG1) complexes (26). Multiple oncogenes and tumor suppressors positively and negatively affect beclin-1 (27). Recently, a peptide containing 6 amino acids of the beclin-1 protein (Tat-beclin) was identified to induce autophagy in cells and to have anti-bacterial properties against *Listeria monocytogenes* as well as antiviral activity against West Nile virus, Chikungunya, and Sindbis virus (46). Although drugs that specifically target beclin-1 do not exist, others targeting several members of the autophagy pathway have been identified by screening. Sarker et al. (47) identified several small molecules that enhance rapamycin activity (SMERs), two of which had antimicrobial activity against Mtb in human macrophages (48). Likewise, Balgi et al. (49) screened over 3500 drugs for their capacity to induce autophagy. Of those tested, perhexiline, niclosamide, amiodarone, and rottlerin all increased numbers of intracellular autophagosomes (49). Finally, by screening Food and Drug Administration (FDA)-approved compounds that inhibit Mtb growth in macrophages, Stanley et al. (50)
identified two drugs, fluoxetine and gefitinib, that significantly enhanced autophagy, as measured by LC3-II proteolysis. In short, several drugs or natural products that are known activators of autophagy or which have been identified in the context of other diseases and are now recognized as modulators of autophagy may be effective against TB. We briefly review several of these molecules next.

**Antipsychotics, antihistamines, and lithium**

Many neurodegenerative diseases, including Huntington's disease, Alzheimer's disease, and Parkinson's disease, involve the deposition of abnormally folded proteins (51). Antipsychotic medications such as lithium provide some clinical benefit for these patients, but the mechanisms by which they act are not completely understood. Recent studies suggest that lithium, valproic acid, prochlorperazine, and haloperidol all induce autophagy (50, 52). Moreover, Sundaramurthy et al. (29) identified nortriptyline and prochlorperazine in a screen of approved compounds that activate autophagy via inhibition of mTOR inhibition, and a third, haloperidol, which accelerated endosomal progression. Notably, all these drugs enhance killing of Mtb in culture. Furthermore, lithium has been shown to act via inhibition of TBK-1 to inhibit growth of *Mycobacterium kansasii*, promote host resistance to *Pseudomonas aeruginosa* keratitis, and attenuate IFN-β production and antiviral responses to Sendai virus (52). Another psychiatric medication with anti-MTB activity, desipramine, inactivates acid sphingomyelinase and, in conjunction with alisporivir (cyclophilin D inhibitor) reduces TNF-mediated tissue damage so as to facilitate control of *M. marinum* infection in zebrafish (53). These data suggest a potential role for neurocognitive medications as anti-TB HDTs that alter autophagy and endosomal trafficking as well as other macrophage pathways. These medications are particularly attractive as many have been in clinical use for ~50 years, nearly all are sold as generics, and all are readily available at low cost. The side effects of these medications vary from minimal to quite burdensome, but it remains to be determined at which dose and for what duration they will prove useful *in vivo* and whether some of the side effects would be mitigated under such regimens. Nevertheless, they remain viable as candidates as adjunctive HDTs.

**Got balance? Establishing effective anti-TB immunity by targeting lipids, sugars, eicosanoids, and cytokines**

**Targeting lipid metabolism**

Host cells and Mtb both rely extensively on lipid and carbohydrate metabolic pathways for homeostatic functions. Drugs targeting these metabolic pathways offer promise because they can target pathways in both host and pathogen. Importantly, dysregulation of host lipid synthesis and sequestration plays a critical role in formation of foamy macrophages, which can sustain persistent bacteria and contribute to the tissue pathology that leads to cavitation and release of infectious bacilli in patients who have progressed to active disease. Mtb induces ketogenesis, which activates GPR109A and an anti-lipolytic pathway, resulting in accumulation of lipid bodies that may protect Mtb (54). Lipid body formation in infected macrophages is mediated by host lipid-sensing nuclear receptors, which include peroxisome proliferator-activated receptor γ (PPARγ), liver X receptors α and β (LXRα, β), and testicular receptor 4 (TR4) (55, 56). Finally, a recent study has linked the antimicrobial
compound Vitamin D and its receptor the Vitamin D receptor (VDR) to lipid metabolism and PPARγ (57). The details of how these receptors regulate steps in Mtb pathogenesis has been recently summarized elsewhere (19); however, it is important to note here that at least two stages of the pathway have been targeted by HDTs. First, inhibition of the anti-lipolytic pathway with mepenzolate bromide (MPN) favors the host and restricts Mtb growth both \textit{in vitro} and \textit{in vivo}. Second, targeting PPARγ with HDTs such as thiazolidinediones may regulate Mtb replication in macrophages as well as inflammatory pathways and formation of lipid bodies. Fig. 3 identifies several of these metabolic pathways along with the candidate HDTs and their mechanism of action.

More recent studies suggest that targeting cholesterol metabolism with statins may have utility against Mtb. Using the \textit{apoE}^{−/−} mouse model, Martens et al. (58) demonstrated that hypercholesterolemia causes increased mortality after aerosol infection with Mtb. The hypercholesterolemic mice had increased pulmonary inflammatory infiltrates, increased cytokine production, and impaired priming of the adaptive immune response (58). In addition to the direct effects of statins on cholesterol synthesis, several lines of evidence suggest that these drugs also have immunomodulatory properties. In accordance with this idea, coronary artery disease has recently been shown to have a strong inflammatory component, and initial clinical studies suggest that statins exert their beneficial effects on coronary artery disease outcomes early after initiation of therapy via mechanisms unrelated to their effects on lipid levels (59). In support of this hypothesis, statins are now recognized to have pleiotropic effects that include modulation of both myeloid and T-cell responses. Also, early studies indicated that statins inhibit IFN-γ induction of MHC class II expression by repressing the inducible promoter of the transactivator CIITA as well as subsequent T-cell responses. In a functional context, the effect of statins on T-cells has been investigated in experimental autoimmune encephalitis (EAE), a T-helper 1 (Th1)-dependent central nervous system (CNS) demyelinating disease that is often induced with Freund’s adjuvant, which contains inactivated \textit{Mycobacteria} (61, 62). Treatment with atorvastatin prevented and reversed demyelination, induced STAT6 phosphorylation and Th2 cytokines (IL-4, IL-5, IL-10), and inhibited STAT4 phosphorylation and Th1 cytokines (IL-2, IL-12, IFN-γ) (61). In a separate study, statin treatment of DCs upregulated GATA-3 and downregulated T-bet expression in co-cultured T cells through a mechanism dependent on the chitinase Ym1 (63). Together, these data indicate that statins promote Th2 responses and inhibit Th1 responses.

Studies aimed at defining the effect of statins on myeloid inflammatory responses appear contradictory. Initial studies indicated that lovastatin had an anti-inflammatory effect with inhibition of LPS-induced nitric oxide, TNF, IL-6, and IL-1β in rat astrocytes, microglia, and peritoneal macrophages (64). In contrast, Montero et al. (65) reported that fluvastatin treatment of human peripheral blood mononuclear cells increased IL-1β, IL-18, and IFN-γ secretion after stimulation with heat-inactivated Mtb H37Ra. Fluvastatin also stimulated caspase-1, an effect blocked by geranylgeraniol. The basis for why statins have seemingly opposite effects on cytokine secretion from myeloid cells in these studies is unclear, but methodologic factors including species, cell type, or subtle differences in how particular statins affect signaling may contribute to the complexity of this system. Nevertheless, these
data suggest that statins regulate myeloid cell cytokine secretion in a manner that could alter the immune response to Mtb.

What effect do statins have on Mtb growth in vitro and in vivo? Parihar et al. (66) examined Mtb growth in PBMCs and MDMs in humans with familial hypercholesterolemia and found twofold lower Mtb sputum CFU levels in individuals treated with statins compared to those left untreated. Simvastatin had a similar effect on Mtb growth in murine bone marrow derived macrophages (BMDMs) (66). In a murine aerosol infection experiment, pre-treatment of mice for two weeks with simvastatin or rosuvastatin decreased Mtb CFUs in the spleen at four weeks post infection and reduced the numbers of microabscesses in the lung, though the differences were small. Interestingly, in BMDMs infected with Mtb, simvastatin increased autophagy, as measured by LC3-II puncta formation. Though simvastatin did not impact Mtb lung CFUs when delivered on the day of infection without TB drugs (67), it did facilitate clearance of Mtb when given in conjunction with rifampin, isoniazid, and pyrazinamide. Together, these data suggest that statins may enhance treatment of TB as an HDT. However, although the murine in vivo data is intriguing and suggest that statins could be effective HDTs for TB, the mechanistic data from the autoimmune models predict that statins could worsen TB outcomes. The autoimmune models suggest that statins inhibit Th1 responses and promote Th2 responses, both of which might be predicted to benefit Mtb rather than the host. In myeloid cells, stimulation of the inflammasome and autophagy represent two potential mechanisms that could benefit the host, however, statins reportedly have the opposite effect in LPS-induced macrophages. In short, further dissection of these pathways remains critical for understanding how to maximize the potential for this class of drugs as a TB therapeutic in humans. Given the number of humans taking statins worldwide, data from observational studies would be useful and may provide insight into whether and how statin therapy impacts rates of TB infection, morbidity, and mortality.

Targeting sugar metabolism

Epidemiologic studies indicate that diabetes mellitus (DM) is a significant risk factor for both acquisition of TB disease and increased disease severity (68, 69). The original observations were noted more than half a century ago, but concerns have been heightened recently due to the rising rates of Type II DM worldwide (70). In a systematic review of observational studies with age-adjusted estimates of the association of DM with active TB disease, 13 studies were identified with 1,786,212 participants and 17,698 cases of TB (68). The summary finding was that DM increased the risk of TB by approximately threefold. Although there was substantial heterogeneity of effect and magnitude of risk, the findings were consistent, with a positive correlation evident in 12 of 13 studies. In a separate systematic review of the association of DM with TB treatment outcome, 33 studies were identified with various types of outcomes data (culture conversion failure, death, relapse, and relapse with resistant Mtb) (69). In twenty-three unadjusted studies, the risk ratio of death during TB treatment was 1.69 (95% CI 1.36–2.12). This risk ratio increased to 4.95 (95% CI 2.69–9.10) in four studies that adjusted for age and other variables. DM was also associated with relapse, but not relapse with drug resistant strains. Although some studies found an association of DM with increased sputum culture positivity at 2–3 months, these results were not consistently observed across nine studies in the review. Together, these
epidemiologic studies suggest a consistent finding that DM is associated with the risk of acquiring TB disease and worse treatment outcomes compared to those without DM.

What impact does chronic hyperglycemia have on TB pathogenesis? Chronic hyperglycemia can modulate both the innate and adaptive immune responses, a topic that has recently been summarized comprehensively elsewhere (71, 72). Gomez et al. (73) found that monocytes from humans with DM had decreased numbers of Mtb associated in an uptake/phagocytosis assay compared to those without DM. Furthermore, uptake of serum opsonized sheep red blood cells via the complement and Fc receptor was impaired in DM subjects compared to those without DM (74). The adaptive immune response to Mtb has also been examined in DM patients. In humans presenting with active pulmonary TB, in vitro whole blood cytokine responses to PPD stimulation were examined. Subjects with DM had higher levels of IFN-γ, IL-2, and TNF compared to those without DM (75). Although the cellular source of the cytokines was not evaluated, these results suggest that Th1 T-cell responses remained intact but were somewhat elevated in DM subjects. Other possible cellular sources of cytokines include NK cells and NKT cells for IFN-γ and myeloid cells for TNF. The analysis was not stratified by disease burden, however, so the data may be confounded because the DM subjects have a higher antigen load, which could augment T-cell responses. This issue was partially addressed in low dose aerosol infection studies in a diabetic mouse model [streptozotocin (STZ)-induced pancreatic B-cell death]. DM mice with chronic hyperglycemia had higher lung CFU, increased pulmonary inflammation, and higher levels of IFN-γ at later time points (16 weeks) (76). In contrast, IFN-γ levels were lower at earlier time points in the DM mice. A follow-up study demonstrated that STZ-treated mice had delayed development of Mtb-specific T cells in the lymph node and lung (77). Recruitment of lung leukocytes at the earliest stages of infection was decreased, an effect accompanied by decreased levels of the chemokines CCL5 and CCL2. These data demonstrate that innate immune responses in DM mice shape the timing and magnitude of the adaptive immune response. The human studies indicate higher Th1 T-cell responses at the time of disease presentation. When interpreted together, the two lines of evidence are consistent with an innate immune system defect, which in turn results in a deleterious adaptive response that results in an increased bacterial burden.

If chronic hyperglycemia is an important risk factor for TB, then what implications does this have for HDTs? An interesting possibility is that medications used to treat DM might also have efficacy against TB. In this regard, recent studies suggest that metformin may have utility as an HDT for TB. Metformin has pleiotropic effects on cells that include activation of the energy sensor kinase adenosine monophosphate-dependent protein kinase (AMPK), a kinase that phosphorylates UNC51-like kinase-1 (ULK1), or ATG1, which initiates early events in autophagy. ULK1 associates with GABARAP, ATG13, and mTOR and is inhibited by mTOR (78–80). In addition to autophagy induction, ULK1 regulates two cytokine pathways that are important for Mtb pathogenesis. First, ULK1 phosphorylates STING and negatively regulates IRF3-dependent expression of IFN-β (81). Second, ULK1 negatively regulates the NLRP3 inflammasome and inhibits IL-1β secretion (82, 83). Together, these studies suggest that metformin may decrease levels of IFN-β and reduce caspase-1 activation as well as IL-1β secretion in macrophages infected with Mtb. Thus,
metformin could have beneficial effects for TB treatment by increasing autophagy and decreasing IFN-β secretion. However, its effects on IL-1β could be detrimental for the host. The importance of a balance in IFN-β and IL-1β signaling is critical in achieving optimal outcomes for TB and is discussed in more detail below.

Are there any data about TB and metformin in humans? Recent studies from Amit Singhal (Singapore Immunology Network) indicate that metformin decreases in vitro growth of Mtb in macrophages by inducing maturation of phagolysosomes and production of reactive oxygen radicals (A. Singhal, personal communication). In an acute TB mouse model, treatment of Mtb-infected mice with metformin led to decreased Mtb CFUs and improved pathology in comparison to untreated mice. Furthermore, metformin enhanced the efficacy of anti-TB drugs in Mtb-infected mice. Retrospective observational studies of humans with pulmonary TB and DM showed an association of metformin treatment with decreased disease severity and improved outcomes compared to those treated with other oral diabetes medications (A. Singhal, personal communication). Together, these data in mice and humans suggest a potential beneficial effect of metformin as an HDT for TB.

Targeting eicosanoids

Eicosanoids are signaling molecules derived from fatty acids that serve as precursors to prostaglandins, thromboxanes, and leukotrienes (84). This class of small molecules exerts significant control over the immune response to TB via manifold immune mechanisms that include the cytokines TNF, IL-6, and IL-1β, and cell death pathways in macrophages and T cells. This pathway has been reviewed elsewhere (85, A. Sher and K. Mayer-Barber, this volume) and is only briefly summarized here. A balance of LXA4 and LTB4 results in a level of inflammation for the host that maximizes killing of Mtb but minimizes tissue damage, which can facilitate the spread of the bacteria (Fig. 4). Using a forward genetic screen, Tobin et al. (86) found that mutations in leukotriene A4 hydrolase, which catalyzes the final step in the synthesis of an inflammatory eicosanoid leukotriene B4 (LTB4), are associated with increased susceptibility to M. marinum in zebrafish. Hypersusceptibility from these mutations results not only from reduction of LTB4, but also from redirecting eicosanoid substrates to anti-inflammatory lipoxins, which limits production of TNF. Extending their study to humans, Tobin and Ramakrishnan (87) found that for TB, loss of heterozygosity at the LTA4H locus was associated with increased mortality amongst TB meningitis patients. Furthermore, white blood cell (WBC) counts in the cerebrospinal fluid (CSF) were highest among the major allele homozygotes, lower in heterozygotes, and lower still in the minor allele homozygotes (87). Moreover, the benefits of steroid (dexamethasone) treatment were genotype-specific. Subjects with higher CSF WBC counts without dexamethasone treatment had a poor prognosis, but responded better to dexamethasone treatment compared to those with lower counts. These data suggest that genetic determinants of the host immune state can influence the outcome to immunomodulatory HDTs and provide a constraint on their application. Because the immune status of patients can vary with both the stage of disease and with genetic background, some immunomodulatory HDTs may require assessment of the immune status of a patient for their optimal and safe application.
Cytokines that regulate signaling through the leukotriene and prostaglandin pathways also play a critical role in TB infections. In particular, studies with mice deficient in IL-1R and IL-1B Type II IFNs, (88, 89), and the observation that the Mtb virulence gene Rv0198c (zmp1) encodes a metalloprotease that inhibits the inflammasome and IL-1β production (90) indicate that IL-1β, IFN-γ, and Type II IFNs are required to control Mtb infections in vivo and in macrophages. By contrast, other cytokines appear to do the opposite and increase sensitivity to infection. Thus, the hyper-virulent clinical strain HN878 causes increased Type I IFN production (91, 92). Moreover, mice with deficiencies in Ifnar−/−, which cannot respond to IFN-α or IFN-β, are less susceptible to Mtb infection, and display decreased CFUs and increased survival compared to WT strains (92–95). Likewise, Irf3−/− mice, which have a deficient IFN-β signaling, are protected against infection, and display decreased CFUs and increased survival (36). Finally, mice treated with polyinosinic-polycytidylic acid, a potent inducer of IFN-β, have increased susceptibility to Mtb (96). Thus whereas IL-1β and Type II IFNs are protective, Type I IFNs are not.

These cytokines must be synchronized with signaling in the leukotriene and prostaglandin pathways, and thereby determine the degree of inflammation and control over Mtb during infection. Recent work indicates that in controlled disease, IL-1α and β drive COX-2 synthesis and PGE2 production, which limits Mtb infection (Fig. 4). Under these conditions, balanced production of another arachadonic acid metabolite, LTA4 (lipoxin), the product of 5-lipoxygenase (5-LO), is critical for control of Mtb (97) (Fig. 4A). By contrast, in hyperinflammatory environments characteristic of late stage disease and uncontrolled bacterial growth, IFN levels are elevated and IL1α/β levels are insufficient to activate PGE2 production, and an eicosanoid signaling imbalance occurs that favors lipoxin production (Fig. 4B). This state can be recapitulated in latent mice with the COX-2 inhibitor valdecoxib, which decreases production of PGE2, or with loss of IL1, both of which render animals more sensitive to Mtb (98). Augmenting synthesis of PGE2 levels, by treatment with PGE2 or with the 5-LO inhibitor zileuton, restores the inflammatory equilibrium and reduces IFN levels, which reduces bacterial burden and significantly improves survival in mice with uncontrolled active disease. Together these data indicate that that alteration of the leukotriene balance can have critical impact on the immune response to Mtb in vivo and suggest new HDTs against TB. However these data indicate that drugs affecting the eicosanoid balance may be most effective in different stages of disease. Active uncontrolled infections resulting from high LTA4 and low PGE2 may be best controlled with zileuton, whereas those with high PGE2 and low LTA4 may best be controlled with drugs such as aspirin or valdecoxib (Fig. 4C).

These data also raise the possibility that the most common pharmaceutical modulator of the prostaglandins, aspirin, may have potential as HDT for TB. Aspirin covalently inhibits cyclooxygenase and has a long history of safe administration. In an RCT in South Africa, 146 TB patients were randomized to placebo or two doses of aspirin along with standard drug therapy and prednisolone. Although there was no difference in survival among the three arms, none of the children who received high dose aspirin developed hemiplegia (compared to 9 in the other 2 arms) (99). Another small (N=119) randomized trial of aspirin (along with standard TB drug therapy) in TB meningitis was performed to test for stroke
reduction in children. Even though the drug did not impact stroke risk (P=0.18), TB mortality was significantly decreased in the aspirin group compared with the placebo group (P=0.03) (100). Although these data are intriguing, the use of prednisolone was not standardized in the protocol, and it was used inconsistently. With regards to other COX inhibitors, murine studies suggest a possible benefit of ibuprofen (101). However, there have been other studies of non-steroidal anti-inflammatory drugs (NSAIDs) in murine TB models with inconsistent results (102–105). Together, the human data on aspirin and murine studies with COX inhibitors suggest a possible beneficial effect as a TB HDT strategy. Investigators in Vietnam are testing this concept further with a Phase II RCT (N=120) comparing placebo, low dose aspirin (81 mg daily for 60 days), and high dose aspirin (500 mg twice per day for 60 days) (Guy Thwaites, personal communication, ClinicalTrials.gov Identifier NCT02237365). Primary objectives of the trial include safety with the primary endpoint a clinically significant upper gastrointestinal or cerebral bleed by 60 days, and the acquisition of preliminary data on efficacy with the primary endpoints of an MRI–verified brain infarct or death by 60 days. Similar to the LTA4H data, individuals with inflammation induced by excessive activation of the prostaglandin pathway may benefit from interventions such as zileuton or aspirin; others who have inappropriately low inflammation may not and may even suffer deleterious effects. Developing biomarkers to predict the expected outcome of immunosuppressive or immunostimulatory HDTs in the context of arachidonic acid metabolism may be a critical step in the application of these HDTs for TB. Alternatively, drugs that affect the prostaglandin/leukotriene balance may be most useful against patients with late stage disease with diagnosed hyperinflammatory responses.

Targeting vitamin D

Vitamin D has been associated with TB prevention and treatment since the 1800s (106). Sunlight, which induces formation of vitamin D, and foods such as cod liver oil and eggs, which are rich in the molecule, were long used as treatments for TB. Following its isolation from cod liver oil, the molecule was used briefly as an HDT (107, 108). However, interest in sunlight and vitamin D waned with the advent of antibiotics. Recently that interest has been rekindled with data suggesting that vitamin D deficiency is a risk factor for TB and that its replacement may prove beneficial (109, 110). Mechanistically, vitamin D was found to boost the antimicrobial activity of human macrophages against Mtb. Schauber and colleagues (111) found that activation of TLR1 and TLR2 by mycobacterial lipids induces expression of CYP27B1 (25-hydroxyvitamin D-1α-hydroxylase) in monocytes and macrophages, which converts vitamin D into a bioactive form called 1,25(OH)2D, and upregulates the vitamin D receptor (VDR). Vitamin D stimulates the VDR to induce cathelicidin expression, which has both immunoregulatory and direct antimicrobial activity (112–116).

The benefit of vitamin D as an HDT in clinical studies is uncertain. Two small, randomized trials suggested that vitamin D could provide a benefit for treatment of TB (117, 118). Also, administration of a single oral dose of vitamin D (2.5 mg) to individuals limited BCG growth in whole blood (119). However, in a larger double blind, randomized, placebo-controlled trial, vitamin D treatment (2.5 mg D3 × 4 doses over 42 days) provided no detectable improvement in clinical outcome or mortality among patients with TB (110). The
authors speculated that the dose used was insufficient, because both the placebo and treated groups had comparable serum levels at the start of the trial and months later. A fourth trial in Guinea Bissau (N=365) randomized PTB patients to vitamin D3 (100,000 units x 3 over 8 months) versus placebo (120). There was no difference in the primary outcome of clinical improvement or of secondary outcomes including sputum culture conversion and mortality. Comparison of the results of the four human vitamin D TB trials, indicate variation in study design that includes dose, duration, formulation (D2 versus D3), genetic background, geographic location of study (and sunlight exposure), and nutritional status. It is worthwhile noting that dose-escalation studies on normal populations might also resolve whether the levels of 1,25-OH vitamin D required to augment immunity to Mtb may be too high for to avoid significant side effects. Such side effects include elevated serum calcium, which can cause potentially life-threatening complications when given in high doses (600,000 IU over 2–3 months) (121). Indeed, such a result might suggest that future TB trials be restricted to patients with low vitamin D levels. Alternatively, vitamin D signaling pathways that alter autophagy and immune signaling may be separable from those affecting calcium signaling by using analogs that affect one but not both of these pathways. In this regard, Sato et al. (122) have developed vitamin D receptor ligand, VDRM2, which preferentially modulates the bone remodeling effects of vitamin D in a calcium-independent manner. Further work in this area may lead to immune-specific activators of the VDR pathway allowing for a more potent antimicrobial effect with fewer deleterious side effects.

Repurposing redux: HDTs that target the bacillus and the host

Several HDTs have been described recently that affect not only the host, in particular trafficking pathways, but also the bacteria. Such dual purpose drugs offer the capacity to not only direct Mtb to compartments where it is less able to counter host antimicrobial defenses but also to directly kill the bacteria. As with other autophagy activators, such molecules also have the capacity to render antibiotic-resistant Mtb strains sensitive.

Calcium channel blockers

Calcium channel blockers have been used extensively to control hypertension and tachycardia. However, recent work suggests that these medications may also induce autophagy as well as inhibit mechanisms used by Mtb to evade antimicrobial therapy. Verapamil neutralizes efflux pumps used by Mtb to develop tolerance to antibiotic within macrophages (123–127). However, the concentrations used in vitro to block Mtb efflux pumps would likely prove toxic in humans, and the drug is also toxic to the bacteria itself at these concentrations, complicating inferences about synergy. Additionally, recent evidence suggests that L-type calcium channel blockers such as verapamil or nifedipine alter intracytosolic calcium and raise cAMP levels, which in turn activates autophagy (128). A study of drug-resistant Fasciola hepatica showed that co-administration with verapamil reduced pathogen-induced clearance of the medication, due to inhibition of pathogen efflux pumps as well as induction of autophagy within host cells (129). The multiple effects of this class of medications make it attractive for future drug trials for TB, though proper dosing will likely prove to be a critical factor in its success.
Nitazoxanide

Nitazoxanide (NTZ) is an anti-parasitic drug of the thiazolide class. The FDA has approved NTZ for gastrointestinal infections caused by Cryptosporidium parvum and Giardia lamblis, but the drug may have broader applicability against viral and bacterial infections. Tizoxanide, the active metabolite of NTZ, inhibits replication of hepatitis C virus (HCV) within infected cells (130), and patients coinfected with cryptosporidiosis or HIV, together with hepatitis B (HBV) or HCV, displayed decreased liver damage while on NTZ therapy. In addition, combining NTZ with ribavirin and pegylated IFN-α improves virologic responses to HCV (131). Finally, NTZ improves clinical outcomes in patients suffering from acute influenza (132). Several studies also suggest that NTZ may be an attractive anti-Mtb therapy. NTZ directly inhibits growth of Mtb, including against clinical isolates, and strongly stimulates autophagy via the mTORC1 complex, which results in decreased Mtb replication in cultured macrophages (133, 134). NTZ also appears to have direct clinical activity against Mtb clinical isolates, making it particularly attractive as an anti-Mtb drug (135). Together with its extremely benign side effect profile and extensive clinical use, the dual efficacy against both the bacteria and the autophagy pathway make NTZ an extremely attractive adjunctive HDT for TB.

Creating an anti-infective milieu: HDTs that alter Mtb trafficking and mimic emergency innate immune responses

The involvement of tyrosine kinases in microbial pathogenesis was first recognized by Martin (136) who identified v-Src as the transforming activity within Rous Sarcoma Virus (RSV), a result that led to the discovery by Mike Bishop and Harold Varmus (137) of the cellular homologue c-Src. Notably, c-Src and related tyrosine kinases, such as c-Abl, regulate a variety of cellular functions, including motility and trafficking. Importantly tyrosine kinases such as Abl are dysregulated in human cancers such as chronic myelogenous leukemia (CML) via a chromosomal translocation (9:22), also known as the Philadelphia chromosome (138–142), which has enabled the development of pharmacological inhibitors. The Abl tyrosine kinase inhibitor imatinib mesylate (marketed as Gleevec), developed by Brian Drucker and others, remains a front-line therapy for CML and related leukemias caused by translocations and mutations that activate c-Abl1, and was the first and crowning achievement of this effort (143). Incorporating a tyrosine kinase into its genome as RSV does is a specialized feature of integrating retroviruses, but it turns out that many pathogens make use of such key signaling molecules by evolving adapters. Thus, bacteria, including Mtb, viruses, and even parasites utilize these enzymes for pathogenesis. These data raised the possibility that therapeutics developed to target tyrosine kinases dysregulated in cancer, such as imatinib might be ‘repurposed’ to treat diverse infections, including TB. This therapeutic strategy has now been validated for a variety of pathogens: imatinib and related inhibitors block release of poxviruses and Ebola in vitro (14, 16), and the drug restricts dissemination of poxviruses and protects from an otherwise lethal infection in vivo (14, 144). Recent data indicate that cAbl inhibitors may likewise affect polyomavirus replication in humans.
What happens with mycobacteria? Imatinib and related inhibitors are effective in acute mycobacteria infections in mice whether administered prophylactically or therapeutically and cause clearance of the pathogen in up to half of the infected animals (17). Imatinib is effective against diverse mycobacteria, raising the possibility that the drug may also prove useful in treating other diseases caused by members of this genus, such as leprosy (17). Perhaps most importantly, imatinib is effective against a rifampicin-resistant strain, and, when co-administered, imatinib acts synergistically with rifampicin or rifabutin against drug-susceptible strains of mycobacteria in vitro and in vivo (17).

Two non-mutually exclusive mechanisms of action appear to account for imatinib effects on Mtb (Fig. 5). First, at therapeutic concentrations, imatinib inhibits entry and promotes vesicle acidification, which results in enhanced antimycobacterial activity in macrophages (17, 145). Accordingly, imatinib has also been found to induce autophagy in some tumor lines (146). Experiments with cell lines lacking Abl1 and Abl2, suggest that this antimycobacterial effect depends on inhibition of c-Abl1 and c-Abl2 as well as other redundant tyrosine kinases (17). However, a second immunomodulatory mechanism appears to mediate effects on Mtb in vivo. Notably, doses required to maximally reduce CFU counts in mice appear far lower than those used in tissue culture. Peak inhibitory effects in mice are achieved with doses of 60mg/kg/d, which results in serum concentrations of ~100–150ng/ml (or 200–250nM). Such concentrations are ~10 fold lower than those achieved at the current clinical dose for CML in humans. Importantly, however, higher doses of drug proved less, rather than more, efficacious. Such a ‘U-shaped’ dose response curve is a hallmark of an immunomodulator (17). Recent studies by one of us (D.K.), suggest a molecular basis for such dosage effects (Napier et al., manuscript submitted). Imatinib at low doses induces differentiation of hematopoietic stem cells and progenitors in the bone marrow, augments myelopoiesis but not lymphopoiesis, and increases numbers of myeloid cells in blood and spleen (D.K. et al., manuscript submitted). Importantly, these effects are not simply inflammatory, but rather an effect on all myeloid lineage cells. Progenitor differentiation depends on partial inhibition of the tyrosine kinase c-Kit by imatinib. Thus, at low doses, imatinib mimics ‘emergency hematopoiesis’, a physiological innate immune response to infection. These data also raise the possibility that imatinib may have the capacity to overcome of innate responses suppressed by Mtb and thereby facilitate clearance. Moreover, the drug may have activity against a broad range of pathogens, including those that do not utilize imatinib-sensitive tyrosine kinases for pathogenesis. In summary, both the trafficking and immunomodulatory mechanisms may be engaged in vivo depending on the dose.

Several lines of evidence suggest that the anti-infective state induced by imatinib in the host, which is mediated by myeloid cells, resembles that seen in patients who are protected from TB (Fig. 5). First, studies of initially IGRA-negative TB household contacts indicate that low baseline neutrophil count is a predictor of subsequent IGRA conversion (147). Thus, protected individuals may have, by virtue of continuous or repeated exposure, a heightened basal myeloid response that provides protection, and which resembles that induced by imatinib. In this regard, Ernst (148) has suggested that neutrophil- and macrophagemediate killing results in apoptosis and engulfment of Mtb by dendritic cells, which facilitates adaptive immune responses. Second, Kaushal’s group (D. Kaushal, personal
communication) has shown in primates that mutants of Mtb, which are cleared by the immune response, induce a strong hematopoietic response, whereas Mtb does not. Thus, Mtb may suppress the emergency response, which may be overcome by imatinib. Third, Fletcher and colleagues (148, H. Fletcher, personal communication) have shown that BCG vaccinees who remain unprotected from TB have transcriptional signatures that may be indicative of either low myeloid responses or hyperactive ones, whereas protected individuals have an intermediate response. Current efforts are aimed at determining whether these protective responses resemble those seen with imatinib (H. Fletcher and D.K., personal communication).

Getting imatinib to the clinic

Imatinib is already approved by the FDA for use in humans and therefore has the potential to immediately impact human health. Imatinib is pharmacologically compatible with antiretroviral therapy after minor dosing adjustments and with all anti-TB antibiotics except rifampicin, though it is compatible with rifabutin (149). Imatinib is remarkably well tolerated in humans, even with long-term exposure, with adverse events only associated with much higher doses than those anticipated for TB and serious adverse events a rarity. Imatinib is not immunosuppressive in humans, nor does it limit anti-pathogen immunity in mice. Such a lack of toxicity may be due to the fact that the drug is neither highly selective nor particularly potent. The drug inhibits c-Ab1 as well as other structurally related tyrosine kinases such as c-Kit (150). Notably, imatinib interacts with c-Ab1 (or BCR-Ab1) in a conformation-specific manner, which causes cyclic binding and unbinding of the drug as enzyme activity changes (151). As such, the drug is neither particularly potent nor selective (152). Lower selectivity ensures activity of a drug against a kinases that redundantly control trafficking. Moreover, partial inhibition of c-Kit appears required for facilitation of myelopoiesis (D.K. et al., manuscript submitted). Finally, cost is also a concern, especially in resource-limited settings. In this regard, imatinib is off patent in 2015 and may be provided cheaply in generic form. Commercially, selling the drug at volume just above cost may be a viable economic model for its distribution.

That imatinib targets the host and not the pathogen (17) makes it unlikely that the pathogen would develop resistance to the drug; to circumvent such a blockade, Mtb would have to alter its entire virulence strategy. Moreover, there is little reason to expect that the drug will prove any less effective on MDR-TB or XDR-TB; experiments to validate this assertion are in progress. In addition, because imatinib acts in synergy with antibiotics (17), it may decrease the likelihood of developing resistance against a co-administered antibiotic. Indeed, with co-administration of imatinib, it may even be possible to use antibiotics against strains that are ostensibly resistant to them. Thus, co-administration of antibiotics with imatinib may both potentiate existing drug-treatment regimens and shorten their duration, thereby mitigating compliance issues. To advance imatinib to a clinical trial will require an initial clinical study to determine the optimal doses for inducing a protective myelopoietic response in humans. For TB, the most effective trial design may be on MDR patients with standard of care drugs and randomization to imatinib or placebo. It is not clear how stage of disease will impact efficacy of imatinib, though experiments to test this are under way. Readouts might include sputum culture conversion, relapse rates, as well as immunological...
measures of innate immune responses. Such a design will allow direct measure of imatinib effects on antibiotic-resistant strains as well as the capacity for synergy with antibiotics.

Getting HDTs through clinical trials

Which clinical trial study designs could be used to assess the efficacy of TB HDTs? A number of choices need to be considered including the following: antibiotic-sensitive versus antibiotic-resistant Mtb, the target population (latent versus active disease, pulmonary versus extra-pulmonary TB, HIV uninfected or infected), end points (cure rates, relapse rates, mortality, early bactericidal activity (EBA) [measurement of fall in sputum colony forming units (CFU) of Mtb early after onset of treatment], culture conversion rates, radiographic and/or immunologic monitoring), and mechanistic goals (e.g. pro versus anti-inflammatory, Mtb replication effect versus host pathology modifier). To assess whether a drug is efficacious for treatment of TB, the optimal trial design includes primary end points of treatment response rates at the completion of therapy as well as relapse rates at later time points. For drug-sensitive Mtb, current first line regimens are highly efficacious with high treatment response and low relapse rates. To discriminate the effects of an HDT added to a standard drug regimen for antibiotic-sensitive Mtb infections, large numbers of subjects and considerable expense are required. Clinical trials with MDR-TB patients may offer an opportunity to design trials with smaller sample sizes at reduced cost and still evaluate the outcomes of cure and relapse rates. An example of this type of trial design was utilized for assessing linezolid efficacy in XDR patients in Korea (153). Forty-one patients with XDR-TB who had failed all available chemotherapy options were randomized and administered linezolid either immediately or with a two-month delay, without a change in the background regimen. The primary end point was two-month culture conversion. Patients were then randomized a second time after smear conversion or 4 months and allowed to continue on linezolid at either 300 mg or 600 mg for at least 18 additional months. This trial design successfully demonstrated that linezolid causes culture conversion in XDR patients (34 of 39 patients) and cured 13 patients. Variations of this type of design could be considered for design of small Phase 2a trials for TB HDTs.

Another major challenge is development of shorter regimens. For HDTs to achieve this promise, secondary endpoint efficacy data would need to be sufficiently robust to justify proceeding with a larger trial with treatment shortening as the randomization variable and cure/relapse rates as the primary outcomes. Secondary measures that might allow such a discrimination include evaluation of EBA and two-month culture conversion rates, a commonly used intermediate readout that has utility in predicting clinical outcomes and is currently used to decide who requires prolonged treatment regimens (duration extended from 6 to 9 months in those who have positive 2m sputum culture). An important limitation of EBA is exemplified by rifampin, which does not show activity by this measure yet is a first line TB drug. Although an HDT with EBA activity might provide impetus for further testing, the lack of response with this measure alone cannot be used to exclude an HDT. Other tools to assess treatment responses include PET scans to measure metabolic activity of lung inflammatory foci. While recent PET studies indicate wide variety in the response of individual lung lesions during therapy (154, 155), this technology could provide early detection of beneficial or harmful effects of HDTs, which do not display obvious clinical
manifestations. One limitation of monitoring with imaging is that radiographic resolution of inflammation (e.g. chest X-Ray abnormalities) often lags behind clinical improvement in patients. Current radiotracers for PET scans do not discriminate between host and bacterial metabolism. However, improvements of Mtb-specific radiotracers may facilitate early detection of pathogen clearance and provide a powerful tool for monitoring responses to treatments. Other less expensive and more readily applied measures of lung function could also be considered, including, for example, spirometry tests to monitor lung function. Potential secondary endpoints include a whole blood bactericidal assay (156–158), which measures the specific capacity of immune cells in the blood to kill Mtb ex vivo. Likewise, immunological assays can directly assess the predicted responses to immunomodulatory HDTs (e.g. zileuton/PGE2 & IL-1/Type I IFNs or PDEi and TNF). Finally, genome-wide exploratory studies with transcriptomic and proteomic profiling of HDT effects could also be included as secondary endpoints to generate insight into mechanism or provide biomarkers to predict treatment response.

The challenges of demonstrating efficacy for TB drugs include expensive and lengthy clinical trials. Within this landscape, what approach should be taken for evaluating HDTs? Early stage assessment of multiple drug candidates could include endpoints such as two-month culture conversion, EBA, whole blood bactericidal activity, and the correlation of these endpoints with in vivo diagnostic measures such as spirometry or lung PET scans. Although each of these readouts has limitations, a positive signal from any one of them could provide a rationale to proceed with a larger trial with conventional efficacy endpoints. Trials may be most effectively designed with MDR patients to increase the chances of detecting an outcome with a smaller number of patients and at lower cost. From small to large-scale trials, it will be critical to include extensive bacteriologic, immunologic, and functional or radiographic secondary endpoints to maximize our understanding of why a particular HDT succeeded or failed.

Conclusions

HDTs have potential for use against TB and other infectious diseases by interfering with how pathogens utilize host factors, or alternatively, by augmenting protective immune responses or by interfering with deleterious ones. Such drugs may be effective against antibiotic resistant strains, will not themselves easily engender resistance, and may even synergize with existing antibiotics. HDTs affect various cellular pathways including autophagy, lipid and sugar metabolism, eicosanoid signaling, pathogen trafficking, and hematopoiesis, to name a few. Additionally, several candidate HDTs are dual action, affecting both the host and bacteria. A key feature of immunomodulatory HDTs is the requirement to maintain a balanced inflammatory response, without favoring hyper- or hypo-inflammation. Heterogeneity in immune response or disease stage may alter how an individual responds to HDTs. Safety and toxicity are key considerations in this regard. However, careful dosing, treatment at appropriate disease stages, and/or evaluation of diagnostic biomarkers may ensure optimal activity with minimal toxicity. Several promising FDA-approved HDTs await testing in humans, some of which are repurposed and have already shown excellent safety profiles in humans. An additional consideration is the pharmacological compatibility with common coordinately administered drug regimens,
including antibiotics and antiretroviral therapy. For these drugs, early, or even immediate, testing in humans is warranted because further testing in animal models is unlikely to determine their eventual efficacy in humans. For other drugs, further testing in animal models will be required to evaluate safety profiles or dosing requirements in the context of an infection. Importantly, the dose required for optimal activity of repurposed drugs against tuberculosis in humans may not be the same as for the original indication, thereby necessitating additional dose optimization studies on normal human subjects. For TB trials, HDTs will be tested and administered in conjunction with antibiotics. Because antibiotics work extremely well on drug-sensitive Mtb, large numbers of subjects will be required to distinguish an effect of an HDT. MDR patients may give a clearer indication of efficacy with smaller numbers of subjects and reduced cost.

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Fig. 1. HDT effects on Mtb

(A). Advantages of HDTs against Mtb. (B). Mechanisms of HDT action against Mtb. Some HDTs inhibit host factors required for Mtb pathogenesis (1). Others affect the immune response by augmenting innate immune responses such as autophagy, which can facilitate adaptive responses (2). Still others inhibit deleterious immune responses such as hyperinflammation, which facilitate Mtb pathogenesis (3). Some HDTs induce novel innate or adaptive responses, such as myelopoiesis, or responses that are suppressed by Mtb (4). Finally, in addition to their effects on mammalian factors, some HDTs inhibit bacterial functions that render the pathogen more susceptible to host defenses (5). Note that these mechanisms are not mutually exclusive, and some HDTs have pleiotropic effects and carry out more than one action. Green arrow indicates stimulation and red arrow indicates inhibition.
Fig. 2. TB, autophagy, eicosanoids, and HDTs
Autophagy is critical for macrophage killing of Mtb and critical break points in this pathway are shown. Candidate HDTs are included in shaded boxes at their site of action on the pathways. Abbreviations: AMPK, adenosine monophosphate-dependent protein kinase; mTORC, mammalian target of rapamycin complex; PI3K, phosphoinositide-3 kinase; ULK1, unc-51 like autophagy activating kinase 1; TBK1, tank-like binding kinase 1; SMERs, small molecule enhancers of rapamycin.
Lipid and glucose metabolic pathways that modulate TB pathogenesis are depicted. Drugs developed for treating diabetes and hyperlipidemia are included with potential mechanisms of action that could impact Mtb. Abbreviations: AMPK, adenosine monophosphate-dependent protein kinase; LXRα,β, liver X receptors α and β; mTOR, mammalian target of rapamycin; Mtb, Mycobacterium tuberculosis; PPARγ, peroxisome proliferator-activated receptor gamma; TR4, testicular receptor 4; TZD, thiazolidinediones.
Arachidonic acid metabolites are critical mediators of host defense against Mtb. Candidate HDTs that affect this metabolic pathway are shown in shaded boxes at their site of action on the pathway. (A). In controlled TB disease, the arachidonic acid pathway yields balanced levels of TNF and Type I IFNs, which control infection. (B). In uncontrolled TB disease with elevated levels of LTA4, TNF, and Type I IFNs, eicosanoid signaling is out of balance and favors production of LTA4 at the expense of PGE2. (C). Restoration of equilibrium and control of disease may be achieved with zileuton, which block 5-LO. As a consequence LTA4 levels are reduced and, indirectly, PGE2 levels become elevated, which reduces Type I IFNs and TNF, and permits control of the bacteria. Other drugs such as aspirin or valdecoxib, which inhibit PGE2 production, may be useful when the eicosanoid pathway imbalance favors production of PGE2 at the expense of LTA4. Abbreviations: COX, cyclooxygenase; PGE2, prostaglandin E2; 15LO, 15-lipoxygenase; 5LO, 5-lipoxygenase; LXA4, lipoxin A4; LTA4, leukotriene A4.
Imatinib has potent anti-Mtb effects in macrophages and \textit{in vivo}. Imatinib alters trafficking of Mtb into acidified compartments and thereby facilitates bacterial killing by the macrophage. At doses lower than those used clinically for CML, imatinib induces myelopoiesis and mimics the emergency response to infection, thereby tuning the innate response to facilitate clearance.
### TABLE 1

CANDIDATE TB HOST DIRECTED THERAPEUTIC SMALL MOLECULE COMPOUNDS*

<table>
<thead>
<tr>
<th>Compound (host target enzyme)</th>
<th>Host Target pathway</th>
<th>FDA approved?</th>
<th>Ref</th>
</tr>
</thead>
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<tr>
<td>Imatinib (ABL tyrosine kinase)</td>
<td>Kinase</td>
<td>Y</td>
<td>(17), unpublished</td>
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<tr>
<td>Vitamin D (VDR)</td>
<td>Multiple</td>
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<td>(110, 117, 118, 159)</td>
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<td>CC-3052 (PDE4 inhibitor)</td>
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<td>N</td>
<td>(160–162)</td>
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<td>(163)</td>
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<td>(164)</td>
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<td>(98)</td>
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<td>PGE2</td>
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<td>(166)</td>
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<td>Thiazolidinediones (PPARγ agonist)</td>
<td>Lipid-sensing nuclear receptors</td>
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<td>Metformin (AMPK kinase)</td>
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</tr>
<tr>
<td>Nortriptyline</td>
<td>Autophagy</td>
<td>Y</td>
<td>(29, 52)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>Autophagy</td>
<td>Y</td>
<td>(29, 52)</td>
</tr>
<tr>
<td>Desipramine (Acid sphingomyelinase)</td>
<td>Reactive oxygen species, TNF, necroptosis</td>
<td>Y</td>
<td>(53)</td>
</tr>
<tr>
<td>Alisporivir (Cyclophilin D)</td>
<td>Reactive oxygen species, TNF, necroptosis</td>
<td>N, Phase III</td>
<td>(53)</td>
</tr>
<tr>
<td>Verapamil (Ca2+ channel blocker)</td>
<td></td>
<td>Y</td>
<td>(123, 125, 126)</td>
</tr>
</tbody>
</table>

* Molecules in this table have promising data as candidate TB HDT compounds and are either FDA-approved or in late stage human trials for non-TB indications.