Improving treatment outcomes for MDR-TB — Novel host-directed therapies and personalised medicine of the future☆

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A R T I C L E   I N F O

A B S T R A C T

Multidrug-resistant TB (MDR-TB) is a major threat to global health security. In 2017, only 50% of patients with MDR-TB who received WHO-recommended treatment were cured. Most MDR-TB patients who recover continue to suffer from functional disability due to long-term lung damage. Whilst new MDR-TB treatment regimens are becoming available, conventional drug therapies need to be complemented with host-directed therapies (HDTs) to reduce tissue damage and improve functional treatment outcomes. This viewpoint highlights recent data on biomarkers, immune cells, circulating effector molecules and genetics which could be utilised for developing personalised HDTs. Novel technologies currently used for cancer therapy which could facilitate in-depth understanding of host genetics and the microbiome in patients with MDR-TB are discussed. Against this background, personalised cell-based HDTs for adjunct MDR-TB treatment to improve clinical outcomes are proposed as a possibility for complementing standard therapy and other HDT agents. Insights into the molecular biology of the mechanisms of action of cellular HDTs may also aid to devise non-cell-based therapies targeting defined inflammatory pathway (s) in *Mtb*-driven immunopathology.

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Introduction

The 2018 WHO Global Tuberculosis (TB) Report states that in 2017, the treatment success of multidrug-resistant (MDR) TB using WHO-recommended TB drug regimens cases was a meagre 55% globally (WHO, 2018). With an estimated half a million cases, MDR-TB remains a serious global health threat. Furthermore, most patients who recover from the lengthy and toxic treatment course of anti-TB drugs develop lung damage and long-term functional disability due to aberrant host immune responses to *Mycobacterium tuberculosis* (*Mtb*). Data from clinical trials, retrospective cohort studies and routine follow-up examinations can allow for studying the immuno-physiological status of patients with MDR-TB and designing personalised adjunct host-directed therapies (HDT) based on clinical experience from cancer treatment options (Tiberi et al., 2018).

Drug-susceptible vs MDR-TB: immunopathological differences

A noteworthy difference between patients with MDR-TB and those with drug-susceptible TB is the genetic background of the infecting *Mtb* strain. Resistance to standard antibiotic therapy allows for drug-resistant *Mtb* to linger longer in the host and induce immunopathological changes which may differ to that

caused by their drug-susceptible counterparts. Li et al. (2017) showed that patients with primary MDR-TB tend to exhibit a greater extent of lung cavitation and lower number of calcified nodules compared to patients with drug-susceptible pulmonary TB. A more recent review also shed light on thicker cavity walls in MDR-TB lung lesions further to extensive tissue loss (Wáng et al., 2018), collectively suggesting intense inflammatory processes unleashed by MDR-*Mtb* infection in the lungs. Interestingly, patients with MDR-TB have also been shown to have slightly higher T-cell counts and IgM titres in serum compared to patients with drug-susceptible TB (Sun et al., 2017). Another study showed that the baseline levels of IL-6 and IL-8 produced by peripheral blood mononuclear cells (PBMCs) from patients with MDR-TB are higher than that of PBMCs from patients with drug-susceptible TB (Skolimowska et al., 2012).

Recent data on cellular immune responses and applicability to HDT development

T-cell effector functions are recognised as central to anti-TB immunity. Emerging evidence shows that the 'understudied' natural killer (NK) cells may have an important protective role in preventing active TB disease (Garand et al., 2018; Roy Chowdhury et al., 2018). Vaccination-induced T-cell populations expressing certain surface molecules i.e. CXCR3, CCR7, CD45RA signify specific subsets which can predict desirable clinical responses (Mpande et al., 2018; Stylianou et al., 2018). Next-generation sequencing has revealed differential V-J recombination patterns (at the gene level) among T-cell receptor gamma-delta (TCR-γδ) Vγ9Vδ2 cells in patients with pulmonary TB (Cheng et al., 2018). These translational and clinical studies also capture the different human leukocyte antigen (HLA) backgrounds of patients, an essential determinant of conventional TCR alpha-beta (TCR-αβ) CD4+ and CD8+ T-cell responses. For example, previous studies have shown a correlative link between polymorphisms in HLA class I/II genes in various populations globally and susceptibility or resistance to clinical TB (reviewed by Harishankar et al. (2018)). Collectively, such findings may advance further deconvolution of immune biomarkers with direct clinical relevance to MDR-TB.

The pro-inflammatory cytokines released early after *Mtb* infection include IL-6, IL-1β, IL-12, IFN-γ and TNF-α which, through nitric oxide (NO) production, can also perpetrate DNA damage (Landskron et al., 2014). *Mtb* infection of human macrophages has been shown to induce nuclear membrane blebbing and DNA breakage (Castro-Garza et al., 2018). As such, do patient-specific private mutations (such as the tumour mutational burden in cancer) also develop in TB granulomas and would they govern the course of disease in each individual and influence clinical outcome? Genetic analysis of lung TB granulomas and blood samples from patients with MDR-TB may further enrich personalised, precision medicine HDT strategies for patients with MDR-TB, particularly since *Mtb* mutant target epitopes may prime and expand T cells which are unfamiliar to their wild type counterparts, thereby providing a richer source for potentially protective and non-exhausted cellular immune responses.

Gut and lung microbiomes

The gut and lung microbiomes are inevitably altered in patients with TB who undergo standard antibiotic therapy (Wipperman et al., 2017; Hong et al., 2016). Gut bacterial species producing butyrate and propionate were found to be enriched in patients with TB in conjunction with impaired vitamin and amino acid synthesis compared to healthy controls (Maji et al., 2018). The lung microbiota in patients with pulmonary TB, based on the meta-analysis of 5 independent studies, also appears distinct compared to healthy controls, marked by enrichment of *Rothia mucilaginosa*, *Actinomyces graevenitzii* (opportunistic lung pathogens), *Tumebacillus*, *Propionibacterium* (propionate-producing) and *Haemophilus* spp. in addition to *Mtb* itself (Hong et al., 2018). However, these studies were performed with clinical samples from patients with drug-susceptible TB and not MDR-TB. As an integral part of all mammals including humans, incorporating microbiome profiling (gut and lung) in clinical biomarker studies for MDR-TB would, therefore, be highly valuable.

The types of clinical studies which can contribute to better understanding the host/and pathogen factors modulating immune responses and how this knowledge can help develop new HDTs are represented in Figure 1.

Standardised therapy for MDR-TB

Establishing HDTs as a standardised treatment method would be extremely challenging as the mainstay of antitubercular therapy, including MDR-TB, requires the elimination of *Mtb* from the host. As such, standard forms of future treatment to manage clinical TB would rely on new drug regimens designed based on the patient population, genetics of the infecting *Mtb* strain(s) as well as the host (this affects drug metabolism and bioavailability), *Mtb* drug susceptibility profile(s) and presence of co-morbidities in the patient to name a few (Lange et al., 2018; Gröschel et al., 2018). The combination of these criteria will deliver specific regimens for designated patient groups in various geographical regions, embodying a more personalised and targeted approach compared to classical antibiotic prescribing. Corticosteroids (prednisolone and dexamethasone) are a class of HDT candidates which are already given to patients experiencing severe inflammation during anti-TB treatment. The drugs themselves do not have targeted effects but rather a general dampening of the host immune response to extend survival in the short-term (Critchley et al., 2016). However, emerging evidence from clinical studies involving patients with MDR-TB may help identify more targeted HDT strategies which could be standardised for some patient groups i.e. cytokine neutralisation, metformin therapy.

Small-molecule and soluble HDTs for MDR-TB

Studies to date have shown that several clinically approved drugs used in non-TB modalities may have therapeutic value in managing patients with TB. The adjunctive use of metformin, an anti-diabetic drug which activates the 5′ AMP-activated protein kinase (AMPK), was shown to complement the antitubercular properties of rifampicin and reduce the pulmonary load of an MDR-*Mtb* strain while potentiating *Mtb*-directed CD8+ T-cell responses in mice (Singhal et al., 2014). Clinical studies implemented afterwards showed that patients with diabetes mellitus who were also taking metformin had shorter hospital stays due to a diagnosis of active pulmonary TB concomitant with a reduced risk of relapse and lower rates of mortality (Marupuru et al., 2017; Degner et al., 2018; Lee et al., 2018a). Metformin may also help enhance control of the high mycobacterial burden in patients with cavitary pulmonary TB, based on improved sputum culture conversion rates and reduce the culture conversion time in patients with MDR/XDR-TB in the first 2 months following therapy initiation (Lee et al., 2018b). However, further randomised clinical trials are necessary to determine whether metformin therapy is able to extend the lifespan of patients with MDR/XDR-TB while subduing tissue pathology as previously shown in the first preclinical study (Singhal et al., 2014).

Similarly, the non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen and indomethacin have shown remarkable anti-TB and pathology-limiting effects in murine models of TB
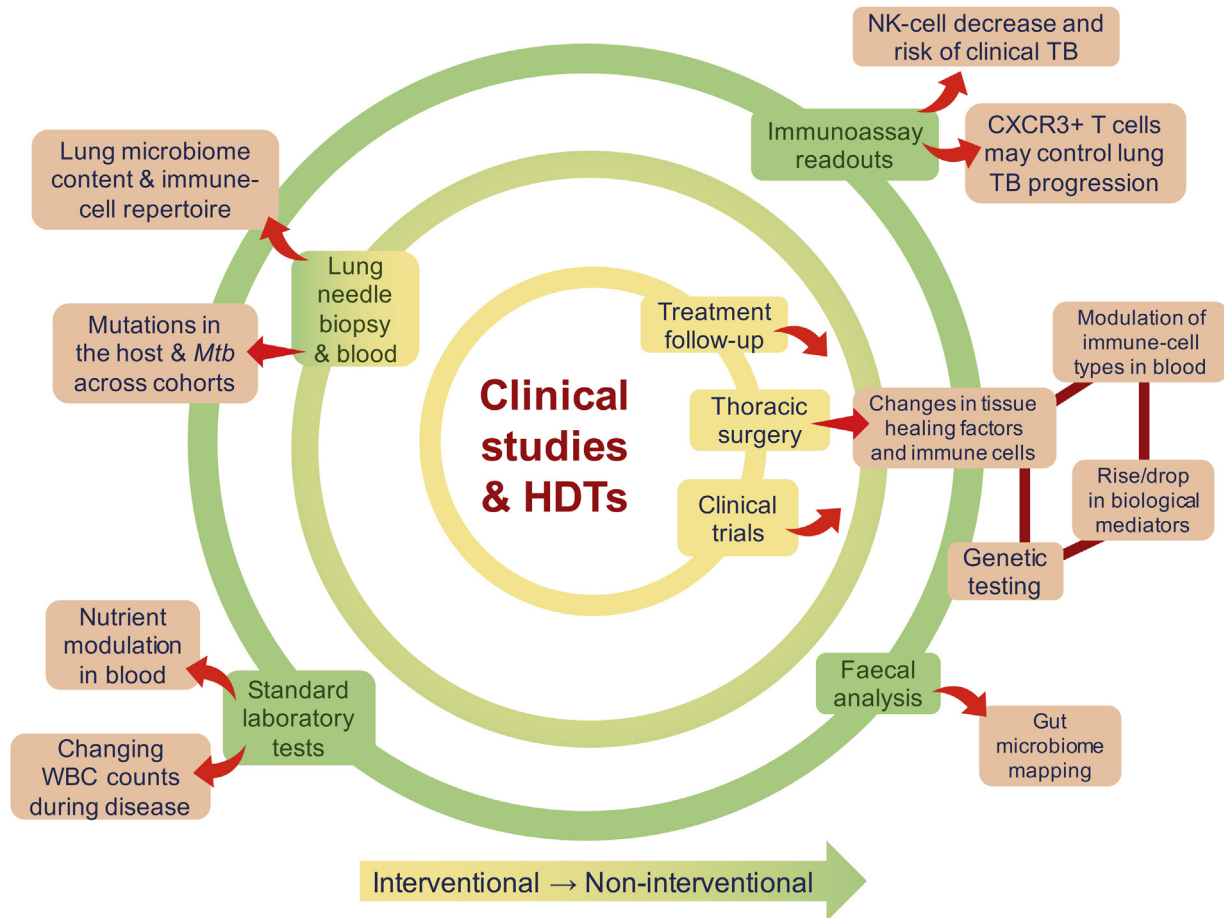
Figure 1. Clinical studies in the context of HDT development for MDR-TB.

Shown is a schematic representation of the different types of clinical studies and their contribution to understanding biological pathways and targets in patients with MDR-TB. The innermost circle (yellow) represents interventional clinical studies which include small-molecule and vaccine clinical trials, routine thoracic surgery (lung resections) as well as treatment follow-up. These scenarios yield ample patient material i.e. biopsies, resected lung tissue and blood that can be used for extensive immunological and genetic assessments of the patients. The readouts from these studies will inform of the mutations in the host — both naturally occurring and pathogen-induced, the modulation of immune cells in blood and tissue and how this is directly affected by the disease process and in combination with therapy i.e. antibiotics and/or surgery, where applicable. The middle circle (yellowish-green) represents semi-interventional studies, where blood draws and lung biopsies are obtained. Here, similar studies to those achievable via clinical material from interventional studies can be implemented i.e. lung tissue specimen and blood but the target groups would also include household contacts of patients with MDR-TB who may have LTBI and could be treated with prophylactic moxifloxacin and/or isoniazid, for example. This measure allows for tracking of biological pathways that become aberrant upon constant exposure to MDR-TB and how this might affect initially healthy household contacts to eventually contract clinical disease. Importantly, these data could lead to identification of druggable host targets. Finally yet importantly, the outermost circle (green) represents non-interventional studies which incorporate the use of faecal material and results from standard laboratory data i.e. sputum and blood, and if possible, bronchoalveolar lavage fluid in some instances. This circle accounts for the largest collection of biological samples since these studies are usually observational and prospective in nature and can be performed in large cohorts of participants across various geographical regions, involving patients and clinically healthy individuals alike.

(Kroesen et al., 2017). There is currently one clinical trial in the recruitment phase to test the adjunctive potential of ibuprofen in patients with XDR-TB in Georgia (ClinicalTrials.gov identifier: NCT02781909).

Vitamin D has also been tested in many clinical trials involving large patient cohorts although there still appears to be no decisive effect on the clinical outcome of TB in treated individuals (reviewed by Wallis and Zumla, 2016). However, further clinical trials are warranted due to a clinical benefit observed in some of the studies described in the review. There is also clinical evidence of cytokine neutralisation using antibody-based drugs i.e. anti-TNF-α to reduce mortality and immunopathology during severe clinical TB (Wallis et al., 2009). Another important biological mediator, IL-6, can be measured at TB diagnosis and during antibiotic treatment to guide the selection of patients who are likely to succumb to severe pulmonary tissue damage despite achieving microbiological cure (Nagu, 2017), thus making it a possible HDT target. Further clinical trials are nevertheless

required to weigh the benefit of pursuing these approaches as adjunct HDTs catering for large patient groups.

A crucial factor to consider when adding small molecules to MDR/XDR-TB regimens is the possible drug-drug interactions which may eliminate the HDT potential. For instance, CYP3A4, which in addition to the *bona fide* enzymes CYP2R1 and CYP27A1 can hydrolyse cholecalciferol (vitamin D3) to calcifediol (25-hydroxy-vitamin D) prior to conversion to its active form calcitriol (1,25-dihydroxy-vitamin D), is reversibly inhibited by isoniazid (Robien et al., 2013). Existing pharmacological experience will guide dosing and timing of the intervention, importantly with assistance from clinical biomarker studies in patients with TB (as described in Figure 1).

Cell-based therapeutics as personalised HDTs to treat MDR-TB

Clinical studies provide ample information for patient selection and stratification to develop immune-based personalised HDTs.

Much can be learned from personalised cancer immunotherapy, where clinical guidelines for managing metastatic, chemotherapy-refractory disease are available and undergo constant renewal. Routine clinical diagnostics may also incorporate extended immunological tests to report circulating lymphocytes (including NK cells), cytokines and antibodies to pre-screen for patients who may most benefit from personalised HDTs.

Adoptive cell therapies: learning from cancer management

The workflow used in anti-cancer adoptive cell therapy programmes using tumour-infiltrating lymphocytes (TIL) provides an excellent template for developing personalised cellular HDTs for MDR-TB. Whole-exome sequencing of genomic DNA isolated from MDR-TB lung granulomas would identify all host- and pathogen-associated mutations, from which mutated protein targets (neo-antigens) recognised by the host's circulating and lung-derived lymphocytes can be screened using *in vitro* immunoassays. Lung granuloma tissue specimens from patients with MDR-TB as well as those with LTBI or healed/calcified lesions (i.e. tissue from lobectomy procedures), peripheral blood, pleural effusion (PE) and bronchoalveolar lavage (BALF) represent highly valuable sources of TB-specific lymphocytes. Flow cytometric analysis can further characterise specific T-cell populations that may be selected for targeted therapy based on (i) surface marker expression (i.e. CXCR3, CCR7, CD45RA) which indicates access to target tissue — a prerequisite for successful protective immune responses, (ii) intracellular cytokine expression/production profiles and (iii) multimer-based recognition of specific epitopes which promote *Mtb* eradication without exacerbating existing immunopathology. These initial screening assays will also reveal specific TCRs – $\alpha\beta$ and $\gamma\delta$ alike – and B-cell receptors (BCRs and immunoglobulin profile) correlating with clearance (sterilising immunity) or optimal control of *Mtb* infection. The most promising and non-cross-reactive Mtb target-specific TCRs can also be transfered into appropriate recipient effector (T or NK cells) either by lentiviral infection or transient CRISPR-Cas9 technology to develop a genetically modified therapeutic cellular product. The most promising TB-specific T cells – possibly also mutation-directed subpopulations – can be expanded *in vitro* with gamma-chain cytokines i.e. IL-2/IL-15/IL-21 (Rao et al., 2018) and assessed further for biological activity and re-infusion into patients with MDR-TB.

Mesenchymal stromal cells

Mesenchymal stromal cells (MSC), known for their anti-inflammatory, immunomodulatory properties and safety in several clinical modalities (Mizukami and Swiech, 2018), are excellent candidates for treating patients with MDR-TB. Adjunctive therapy with a single infusion of bone marrow-derived autologous MSC has been clinically evaluated in two separate studies involving patients with MDR/XDR-TB. In the first study, 30 patients were enrolled, comprising 17 MDR-TB cases and 13 XDR-TB cases. 21 patients showed radiological improvements in the lungs at 6 months post-MSC therapy (MDR-TB = 12 patients; XDR-TB = 9 patients) while stable disease was observed in 2 patients with MDR-TB and 3 patients with XDR-TB (Skrahin et al., 2014). This was accompanied by approximately 80% reduction in viable *Mtb* load in sputum, with only 20% of patients displaying positive culture conversion. A total of 16 patients who received MSC therapy achieved microbiological cure, indicated by at least 18 months of *Mtb*-negative cultures. Only 3 patients with MDR-TB and 1 patient with XDR-TB experienced disease progression, with grade 3 adverse events recorded in only 2 patients throughout the study period. In comparison, only 5 out of 30 patients with MDR/XDR-TB in the control arm who did not receive adjunctive MSC therapy experienced microbiological cure,

further to 5 other patients who died while receiving second-line antibiotic treatment. No deaths were reported in the MSC-treatment arm. Importantly, MSC infusion induced immune re-focusing of *Mtb* antigen-directed T-cell responses, resulting in improved recognition of Ag85B (Rv1886), an immunodominant *Mtb* protein, as well as increased sensitivity to IL-2 and IL-7 stimulation. The second study showed that 81% of 36 patients with MDR/XDR-TB experienced a successful clinical outcome (radiological improvements) 6 months after MSC infusion as opposed to only 39% of 36 control patients who did not receive adjunctive MSC therapy (Skrahin et al., 2016). No immunological analysis was performed in the second study. Further large, controlled cohort studies are required to confirm the usefulness of adjunct MSC therapy in MDR-/XDR-TB, and the immunological correlates therein.

Building capacity for HDT evaluation in low-resource and high TB endemic settings

Patients with MDR-TB, further subdued by HIV-co-infection in countries where MDR-TB/HIV is a major issue i.e. South Africa, India, Indonesia, China to name a few (WHO, 2018) (although the rates of HIV-co-infection differ across the countries) are generally very weak and frequently anaemic, which poses a major challenge to obtaining blood, and worse still, bone marrow aspirates. In addition, allogeneic NK, TCR $\gamma\delta$ T cells and MSCs cells from immuno-competent individuals, as opposed to patients with MDR-TB, may be physiologically fitter, exhibit uncompromised biological activity and able to expand more readily with cytokine conditioning. Similarly, TCRs which can promote disease amelioration in patients with MDR-TB – initially discovered from *in vitro* screening of patient material – can be transduced into allogeneic NK or Vγ9Vδ2T cells. The use of allogeneic sources of NK cells, MSCs and Vγ9Vδ2 T cells for therapy should, therefore, be encouraged in resource-limited and high TB burden settings since technological advances must be translated to areas of greatest clinical need. This approach is feasible and will also reduce the number of biological samples i.e. blood, tissue as well as the frequency at which they are obtained from patients with MDR-TB (Jarry et al., 2016; Veluchamy et al., 2017; Galipeau and Sensébé, 2018). Figure 2 is a schematic representation of how personalised cellular HDTs, as a future possibility, may be incorporated into the standard MDR-TB treatment programmes in practice in high-burden countries. The strategy proposed here is also explained in detail in the accompanying legend and considers that resource limitation is a crucial factor governing the provision of cutting-edge personalised medical care. As previously mentioned, personalised HDTs are a suggestion to complement more stratified/generalised HDTs for suitable patients in combination with conventional drugs and not a replacement for standard therapy at this juncture.

Public-private partnerships (PPPs) are key in fuelling modern infrastructure for healthcare provision, the Biovac Institute in Cape Town, South Africa and the African Health Markets for Equity (AHME) project in Ghana and Kenya (Suchman et al., 2018) serve as good examples. Cellular therapies may also be developed and administered at certain centres with suitable infrastructure i.e. Alberts Cellular Therapy (Gauteng, South Africa), and once the mechanisms of protection and correlates of beneficial clinical responses in patients with MDR-TB treated with personalised HDTs are better defined, the HDT treatment regimen could potentially be replaced with more generalised biologicals to reach more patients. Here, timing and dosing of intervention with the appropriate HDTs is of paramount importance, based on lessons from cancer (Rothschilds and Wittrup, 2019) and inflammatory diseases i.e. ankylosing spondylitis, arthritis and IL-17A blockade (Baraliakos and Braun, 2018). Also, MSCs in GVHD work via reduction of IL-6,
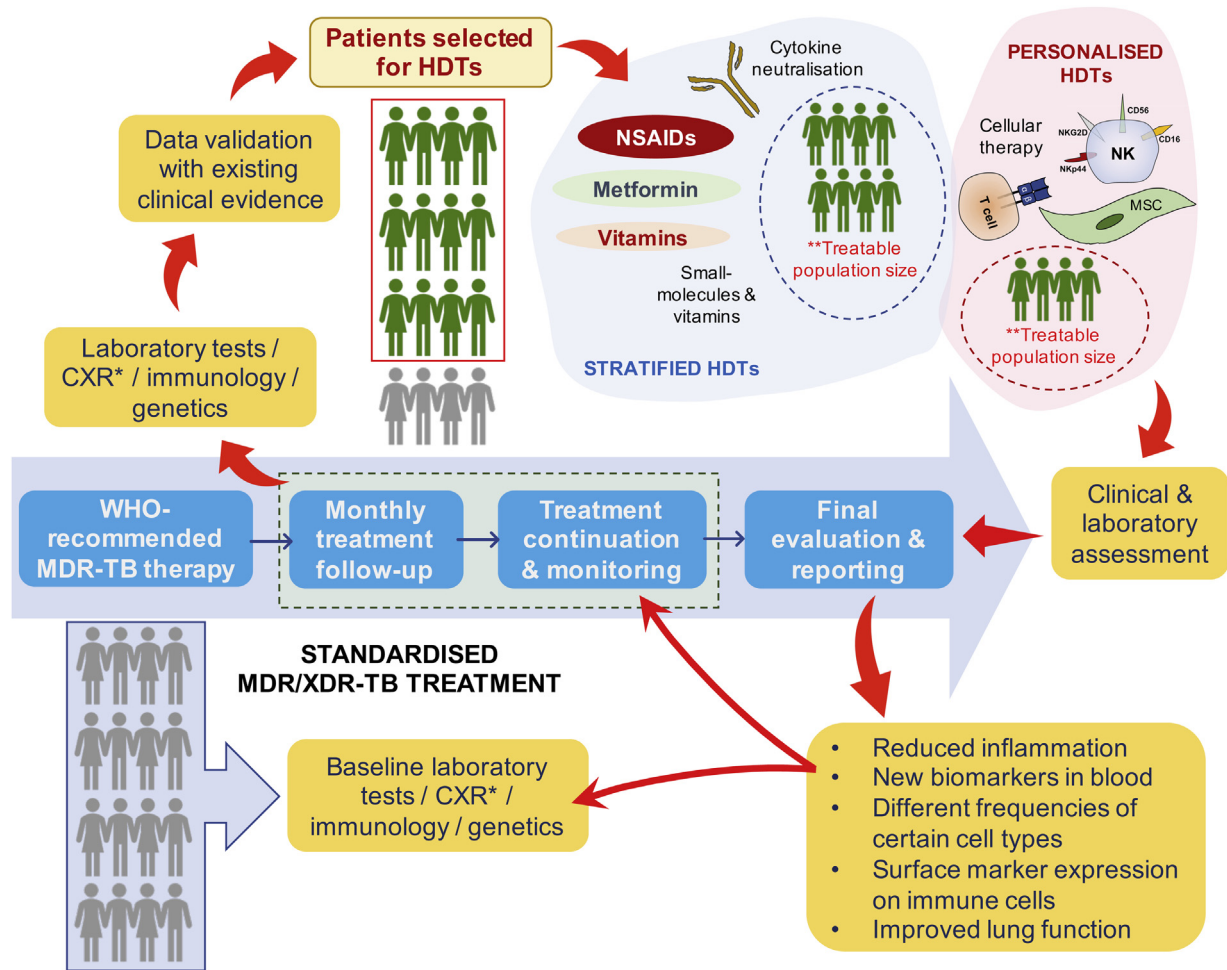
Figure 2. Possible HDT strategies for inclusion into future anti-MDR-TB therapy programmes.

The schematic diagram represents a possible path to including HDTs in standard-of-care programmes for MDR-TB. The WHO-recommended antibiotics regimens are mandatory, thus comprising the standardised therapy regimen for patients based on designated criteria, at which point routine laboratory examinations i.e. microbiology, haematology, chest X-rays (CXR) and drug susceptibility testing (DST) are carried out to form a baseline of the patient's clinical status and amenability to antibiotics. All patients who undergo standard treatment are required to appear for routine follow-up sessions with the attending medical team. In addition to lung function and microbiological examinations, blood draws could be obtained to understand the immunological status of each patient after having undergone at least several weeks of anti-MDR-TB therapy and in comparison with baseline (prior to treatment initiation) using the same set of laboratory analyses. CXR bears an asterix(*) since not all patients may be amenable to exposure to radiation during follow-up. This also applies to the treatment continuation phase since clinical follow-ups are regular. Following consultation with published clinical studies concerning biological mediators and biomarkers in patients with MDR-TB, the individuals who are most likely to respond to HDTs can then be selected. Of note, it is of paramount importance that the timing of immunomodulatory HDT is best planned for after the intensive phase of therapy so that the necessary anti-mycobacterial defence system in the host is not compromised in any way. More generalised HDT options may be administered here, possibly differing from one patient group to another based on their biomarker profile(s) stratification. Herein, HDTs targeting cytokine neutralisation (i.e. anti-IL-6, anti-TNF-α), vitamin supplementation (i.e. vitamin D, vitamin A), metformin therapy and non-steroidal anti-inflammatory drugs (NSAIDs i.e. ibuprofen, aspirin, indomethacin) would more likely cater for a large number of patients at the same time as these are readily available ('off-the-shelf') therapeutics. In contrast, more personalised HDTs such as cellular therapy with T cells, NK cells as well as mesenchymal stromal cells (MSCs) would require a longer preparation time and very importantly, a sufficient amount of material to start with for cell propagation. This also requires a suitable facility for cell expansion and quality control processes i.e. a GMP-compliant laboratory. NK cells and MSCs may be of allogeneic origin, thus curtailing the product generation time compared to isolation, culture and expansion of autologous T-cell products for re-infusion into patients. TCR-transduced T cells could also use allogeneic sources if the patient's native, restricting HLA elements matches that of the donor's. The clinical and laboratory assessment of the data arising from HDTs, further to the continuation of standard treatment for MDR-TB, will give rise to newer information which can contribute to previously unknown therapeutic HDT targets, thus feeding into baseline immunological data collection as well as immune-monitoring.

TNF-α and inducing tissue regeneration and remodelling by vascular endothelial growth factor (VEGF) production (Wang et al., 2016), potentially paving the way for treating more patients with HDTs targeting the IL-6/TNF-α axis and VEGF supply albeit with correct timing of intervention. With tantamount funding, specialised GMP-compliant laboratories within mobile container units such as the BioGo™ Mobile solution (Germfree, USA) – as an alternative to permanent facilities – can be deployed in suitable regions to manufacture advanced cellular therapeutics.

These solutions augment the state-of-the-art under technically challenging circumstances and can be supported by clinical research grants as well as investigator-initiated studies and/or (co-)funded by pharmaceutical companies. Assessment of clinically relevant immune reactivity and general immune-modulation in the host may identify new, biologically relevant HDT targets and ultimately revolutionise the clinical management of MDR-TB.

Conclusions

Conventional MDR-TB drug treatment needs to be complemented with HDTs to reduce tissue damage and improve treatment outcomes. However, the critical question in developing personalised therapies for TB is whether this strategy is routinely applicable in resource-limited settings. This viewpoint proposes to

consider the application of personalised cell-based HDTs to complement promising small molecule-based and soluble HDT agents as adjuncts to conventional anti-TB treatment regimens. Therefore, with the growing number of HDTs, infrastructure for conducting clinical trials of HDTs should be established in resource-limited and high TB endemic settings with international and local support.

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Conflict of interest

The authors declare no conflicts of interest.

Ethical approval

No ethical approval was required for this work.

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