THE POTENTIAL ROLE OF VACCINES IN THE MANAGEMENT/CONTROL OF MDR/ XDR-TB

Keertan Dheda, Jonny Peter, Malika Davids

‘TB drug resistance is a major public health problem that threatens progress made in TB care and control worldwide.’

WHO

‘Can you picture what will be so limitless and free desperately in need of some strangers hand in a desperate land.’

The Doors

High Pressures
Alain Pino
Digital photography
INTRODUCTION

Rifampicin was introduced to treat tuberculosis in the 1960’s. Almost 2 decades later, there was widespread emergence of rifampicin and isoniazid resistance and a burgeoning epidemic of multi-drug resistant tuberculosis (MDR-TB; resistance to isoniazid and rifampicin) (1–3). Even as early at 1992, more widespread resistance emerged, i.e. resistance to rifampicin, isoniazid, fluoroquinolones, and one of the injectable second line drugs (amikacin, kanamycin or capreomycin) (4). This resistance profile, later called extensively drug resistant TB (XDR-TB) because of its poorer prognosis, was already emerging in South Africa in the early 1990’s (4). In 2006 a major and extended outbreak of XDR-TB was reported in the Kwazulu-Natal province of South Africa in HIV-infected persons who are more susceptible to acquiring disease (5). A the time of writing, large numbers of therapeutically destitute XDR-TB treatment failures were being encountered in the Western and Northern Cape provinces of South Africa, and resistance beyond XDR-TB (sometimes called XXDR-TB or super XDR-TB) was being documented in several countries in the world, including India, Iran, Russian and elsewhere (6). The widespread emergence of therapeutically destitute cases of TB has become a ‘game changer’ for vaccinology and immunotherapy related to tuberculosis. Here we describe the burgeoning phenomenon of high-grade drug-resistance to TB that is emerging on a wide scale globally, thus necessitating the need for alternative therapies. We also discuss the poor outcomes in patients with highly drug-resistant TB thus necessitating additional and complimentary therapeutic approaches. We further discuss the relevant immunopathogenesis of drug-resistant TB, and possible immunotherapeutic approaches to this disease.
Epidemiology of Drug-Resistant TB

The Global Project on Anti-Tuberculosis Drug-resistance Surveillance is a reporting platform that networks reference laboratories in a number of countries that annually report on resistance testing of isolates. Serial reports were published in 1994, 2000, 2003, and 2008 (7-10). This forms a basis of what we know about the global epidemiology of drug-resistant TB. Although there are several drawbacks of these reports, including testing of a fraction of prevalent cases, at times sampling only certain sectors such as the private sector, and only covering limited time frames, several trends are apparent. In 2010, it was estimated that there were about 650,000 prevalent cases of MDR-TB, of which 490,000 were new cases and 160,000 were retreatment cases (3.4% of new cases and 20% of retreatment cases). It was further estimated that there were 25,000 XDR-TB cases annually worldwide (10). Although in some parts of the world such Korea, Hong Kong, and Western Europe, the rates of MDR-TB are falling, in other countries such as Peru, South Korea, Botswana and South Africa, the incidence of MDR-TB is increasing (1, 11). Two intervening national surveys in South Africa, i.e. 2002 to 2008 revealed a dramatic 3-fold trebling of MDR rates in this country (12). The burden of MDR-TB in countries of the former Russian Federation, including Kazakhstan and Azerbaijan, is staggering. In such countries approximately 10 to 15% of new cases and more than 50% of previously treated cases have MDR-TB. Almost half the burden of MDR-TB lies in India and China, with a further 10% of cases occurring in Russia (1, 11). Although Africa traditionally had low rates of MDR-TB, opportunistic surveys have indicated alarming rates of disease in several African cities (1, 11). South Africa has a full-blown MDR-TB epidemic with about 80% of MDR-TB being transmitted by the primary route (12). The likely total burden (detected and occult) of MDR-TB in this country is close to 20,000 TB cases. However, less than half these cases are notified, and of these only 50 to 60% are started on treatment. This alarming problem of XDR-TB is now being eclipsed by the spectre of resistance beyond XDR-TB and therapeutically destitute cases of XDR-TB failures (discussed later).
WHY DRUG-RESISTANT TB IS A GAME CHANGER FOR THERAPEUTIC VACCINES AND IMMUNOTHERAPY

MDR-TB has a high mortality, particularly in developing countries. For example, in South Africa, only ~50% of MDR cases have a successful treatment outcome, and default rates are high (13). The poor MDR treatment outcomes have been documented in several resource poor settings (14-16). In a recent study covering 4 provinces in South Africa, in a non-outbreak setting, the mortality of XDR-TB was found to be almost 50% and culture conversion rates were poor (less than 20%) (17). The initial optimism in successfully treating XDR-TB (18) has now been replaced, even in HIV uninfected persons or in a non outbreak context, by the reality of much poorer outcomes in several studies in high burden settings (17, 19, 20). A recent study from the Eastern Cape province of South Africa showed culture conversion rates to be less than 10% (19). The fact these were outcomes in hospitalised patients who received the full duration of second line injectable therapy is even more alarming. MDR and XDR-TB disproportionately affect economically active young adults. In our studies of XDR-TB in South Africa, the median age of patients was 34 years. Besides the substantial morbidity and mortality, management of these patients is accompanied by unacceptably high costs. For example, in South Africa despite drug-resistant TB comprising less than ~2% of the total caseload, it consumes over a third of the total national TB budget, and over 60% of the national TB drug budget (21). Clearly, this is not sustainable and therefore drug-resistant TB has the capability of destabilising functional national TB programmes. The problem of XDR-TB has now been eclipsed by further resistance (termed super XDR-TB, XXDR-TB, pan-resistant TB, totally drug-resistant TB). The latter two labels are somewhat misleading as no isolate is resistant to every known drug and newer drugs are emerging. Nevertheless, the bottom-line is that there are hardly any remaining second line drugs to treat these patients with. Although linezolid remains largely underutilised in most parts of the world, a recent study using this drug for XDR-TB documented a high rate of emerging resistance when used in the context of XDR-TB failure (22). Other drugs such as TMC 207 (Bedaquiline), SQ109, PA-824, and OPC67683 (Delaminid), are on the horizon, but it will be several years before they become widely available in new anti-TB regimens. Even when these drugs become do become available, there is equipoise and an on-going ethical debate on whether such drugs should be reserved for widespread treatment of TB.
or for the treatment of drug-resistant TB, i.e. ‘benefit to the majority at the expense of the minority’. We have, therefore, now come a full circle and once again we are encountering patients in large numbers for which there are no therapeutic options. Given that new multi-drug regimens for TB are some years away means that alternative therapeutic options are urgently required. This has made the need to perform phase 2 and phase 3 studies of several immunotherapeutic options even more urgent. These agents include therapeutic vaccines and immuno-modulators (discussed in detail later).

EMERGENCE OF THERAPEUTICALLY DESTITUTE CASES OF DRUG-RESISTANT TB

Our unpublished data indicate that since October 2008 there were almost 50 culture positive patients who failed XDR-TB treatment and were discharged back into the community as they were therapeutically destitute. In Cape Town, South Africa, patients with XDR-TB are deemed, based on our preliminary published data (17), to have failed treatment if they still have 2 consecutive positive cultures after 12 months of intensive therapy in the absence of mal-adherence, with appropriate treatment with second line injectable drugs such as capreomycin. Such treatment failures are discussed at a multi-disciplinary monthly review committee meeting, which includes physicians experienced in treating drug-resistant TB, social workers, nurses, representative of patient advisory groups, a legal expert etc. In cases where treatment, after careful consideration and when surgery is not an option, is deemed to be futile all further treatment is withdrawn, and the patient is discharged. However, in many cases such patients go back to single roomed dwellings in a resource poor environment, and are often unemployed. There is no systematic programme to assist such patients to deal with home-specific structural infection control measures that may be needed, and there are no no long stay community facilities or palliative care facilities that such patients may utilise. Like in the pre-chemotherapeutic era, about 20 to 25% of these patients continue to have chronic on-going disease and continue to remain culture and/or smear positive. The continuing pressures of housing, food, and seeing to their family commitments means that such patients will often seek employment, and travel for social or other reasons. Patients are advised to wear masks at all times, but adherence is often incomplete. These patients act as sentinels for the transmission of highly resistant strains of TB that have a high mortality and very poor treatment outcomes. These patients are also usually not amenable to surgery and often have bilateral cavitary disease. Given that the
XDR treatment regimen invariably contains a backbone of PAS and capreomycin and several other drugs, including clarithromycin, augmentin, moxifloxacin and clofazimine, are used there are virtually no therapeutic options left for the patient. Linezolid is not currently available in the government sector in South Africa, but even in patients who can procure this drug, constructing a multi-drug regimen is virtually impossible. Immunotherapeutic approaches, including the use of therapeutic vaccines and immuno-modulatory agents such as M. vaccae, IVIG, and other alternatives are now urgently needed.

**PATHOGENESIS OF DRUG-RESISTANT TB**

Several factors drive drug resistance. The traditional hypothesis is that low-level *Mtb* resistance arises spontaneously and with inappropriate drug exposure or intake, resistant mutants are selected out over several cycles and become the dominant strain. Once drug resistance has developed (as shown in Figure 5.13.1) person-to-person spread occurs amplifying the overall disease burden, i.e primary resistance as opposed to secondary resistance develops. Given that drug-resistance develops even when adherence is excellent it is likely that differential drug penetration into different micro-compartments of the lung, where differential degrees of tissue damage is present, may be driving drug-resistance.

Whole genome sequencing studies have shown that mutations encoding resistance to specific drugs are associated with numerous compensatory mutations elsewhere in the TB genome (23–26). These compensatory mutations may likely impact structural and physiological pathways because analysis has shown differences in proteomic expression depending on the virulence of a strain (27), with lack of certain proteins being associated with hypo-virulence. It has also been demonstrated that XDR-TB organisms have structural changes such as increased cell wall thickness, and different budding characteristics (28, 29). Collectively, these observations raise the possibility that drug-resistance may impact antigenic structure and specificity and hence the immune response. Thus, the compensatory mutations may alter physiological pathways and structure, and hence antigen presentation and the T-cell response. We recently evaluated the immunophenotype in patients with drug-resistant TB (published in conference proceedings). These data indicated that, compared to drug-sensitive TB and those with latent TB infections (LTBI), the immunophenotype of XDR-TB patients was characterised by a very high frequency of CD4 + Foxp3 + regulatory T-cells (T-reg). The extraordinarily high levels of T-regs (up to 25% of CD4 T-cells in several patients) could not be explained by disease chronicity, and was evident even in patients with primary XDR-TB. We further demonstrated in an *in vitro* killing assay that depletion
Figure 5.13.1 Pathogenesis of drug-resistant TB

Note: Pathogenesis of drug-resistant TB. Sequential drug resistance may develop through fragmented treatment (1) and this may be fuelled by several programmatic and socioeconomic factors. However, resistance may develop despite excellent adherence. Several factors, including efflux pumps, drug-specific high metabolic rates in some individuals, and extensive immuno pathology in the lung resulting in differential drug penetration into granulomas and cavities (2), may all drive site-specific drug levels below MIC thus likely facilitating drug resistance. There may be an interaction between strain-specific genotype, newly acquired drug-encoding mutations, and compensatory mutations that may impact fitness cost and hence transmission (3). Once acquired drug-resistance develops, person-to-person transmission, may constitute the major route of spread. Compensatory mutations may be associated with changes in structure and physiological pathways and we speculate that this may impact host immune response and thereby also potentially subvert protective responses and drive progressive disease (4).
of regulatory T-cells, in drug-sensitive (99) and drug-resistant TB (unpublished data), abrogated TB-specific mycobacterial killing suggesting an important biological role for these cells. This raises the possibility that modulation of the immune profile, and particularly regulatory T-cells, may impact disease progression and cure. This is noteworthy given that there are no drug-specific therapeutic options, as already outlined, currently available. Several immunotherapeutic interventions proposed in the next section, including \textit{M. vaccae}, vitamin D, IVIG, etc have T-reg-modulating properties. In vitro studies are now on going to determine the potential role of these agents in both drug-sensitive and drug-resistant TB. Preliminary evidence for some of these agents already exist, and phase 2 and phase 3 studies using these agents are now warranted (discussed later).

The term short-course chemotherapy is curious. Despite the massive reduction in organism load within the first 2 weeks of anti-tuberculous therapy, it remains unclear why treatment is required for 6 months in those with drug-sensitive TB. By contrast almost all other infectious diseases only require therapy for days. By contrast, therapy for drug-resistant therapy lasts two years. The traditional view is that prolonged therapy is required to deal with long term persisters. These special sub-group of organisms have adapted to external stresses, replicate slowly, and take on alternative including cell wall free forms (30). Accumulation of fat droplets and loss of acid fast staining is a feature of such organisms (31). Introducing immunosuppressive therapy, paradoxically, may enhance and potentially shorten treatment as these long-term persisters enter a state of enhanced replication and upregulate their metabolic status in the face of an attenuated host response (32). This phenomenon has been well demonstrated with steroids and human TNF antagonists (33). Thus, it is entirely possible that immunosuppressive agents may enhance chemotherapeutic effect. Indeed a murine model showed that a TNF antagonist (Eternercept) accelerated bacterial clearance when chemotherapy was used (34).

An alternative explanation, rather than these organisms being therapeutically resilient because of low replication rates, is that the immune system is rendered ineffectual and polarised to a non-mycobactericidal state. It may be that these organisms are immunologically undetectable or that TB deliberately drives a response that corrupts protective Th1-associated pathways. It remains unclear what this corrupting influence is, but it could include regulatory T-cells (35–37) and Th2-like cytokines etc (38). Thus, TB may not only need drug therapy, but also
realignment or redirection of the immune system to deal more effectively with mycobacteria. The observation, in therapeutically destitute patients and in the pre-chemotherapeutic era that approximately 20% of patients self-cured, indicates that it is possible for immune-mediated sterilisation, even in the face of active disease. This suggests that immuno-modulatory treatments or immunotherapeutic interventions have the capability, in synergy with the immune system, to possibly effect sterilisation and cure. Thus, although speculative, what may be required, either alone or in combination with drug therapy, are immuno-modulators that are able to redirect the corrupting influence by \( \text{MTB} \), and thus realign the immune system (39, 40). The next section further describes putative immunotherapeutic agents that might be potentially useful in patients with drug-resistant TB.

**POTENTIAL IMMUNOTHERAPEUTIC INTERVENTIONS INCLUDING THERAPEUTIC VACCINES FOR DRUG-RESISTANT TB**

Rationale and types of potential immunotherapeutic approaches for DR-TB

As outlined already, our understanding about the immunology of drug-resistant TB, and hence immunotherapeutic vaccines and other strategies are in their infancy, but now warrant consideration and increased research efforts (39,40). The potential rationale and role for adjunctive immunotherapeutic interventions in MDR- and XDR-TB include: i) potential improvement of poor cure rates and decrease in the time to culture conversion, ii) possibly minimising tissue damage and lung remodelling associated with an excessive host immune response, iii) potential improvement of overall health status by mitigating the systemic impact of long-standing chronic inflammation, and iv) possibly reducing transmission of TB. It is encouraging that, in contrast to the lengthy development and regulatory process associated with new TB drug development, a number of potentially useful immune-modulatory vaccines and cytokines are already in clinical use, and thus potential therapies for TB or DR-TB could, in appropriate cases, move directly into phase II and III clinical trials.

Potential immunotherapy for DR-TB can be considered in three main groups:

i) immunomodulatory vaccines and biologic agents to restore or re-align towards a more favourable immune response e.g. \( \text{M. vaccae} \) or anti-IL-10, which may exert their effects by modulating Treg function.
ii) immunosuppressive agents e.g. thalidomide or steroids to suppress excessive host inflammation thereby limiting tissue destruction and potentially improving the access of TB drugs to site of infection.

iii) adjunctive cytokine therapy to enhance the mycobactericidal activity of effector cells or improve patient health status by reducing the deleterious effects of chronic inflammation (39, 40).

Table 5.13.1 highlights some of these immunotherapeutic approaches and their mechanisms of actions. Unfortunately, to date, the majority of studies investigating these agents have involved drug-susceptible TB patients. Nevertheless, a number of these agents may have potential application to drug-resistant TB and are discussed below.

**Immunomodulatory vaccines**

Immunomodulation or ‘realignment’ of the host response in a chronic infection like drug-resistant tuberculosis is theoretically an appealing option. Evidence of self-cure, even in the pre-chemotherapeutic era, amongst therapeutically destitute patients indicates that a curative immune response is a possibility. Exactly what underpins or typifies this response is unclear but driving Th1 and CD8-specific responses (41), and down-regulating potentially deleterious responses such as necrosis, or modulating regulatory T cell receptor or Treg responses is likely to be important. By the same token, driving the wrong type of regulation may provoke bacteriostatic or excessive local and systemic inflammatory responses, thereby potentially causing harm. The role of therapeutic vaccines is largely limited to studies in animal models or patients with drug-sensitive TB (summarised in Table 5.13.1).

**Mycobacterium vaccae**

The heat-killed *Mycobacterium vaccae* (*M. vaccae*) has been extensively studied as a immunotherapeutic adjunct to standard chemotherapy in drug-sensitive TB patients (42). Immunologically, *M. vaccae* vaccination drives a Th-1 phenotype, likely through modulation of T reg function (40, 43, 44). Meta-analytic outcome data for drug-sensitive TB is conflicting (42, 45). De Bruyn *et al* included eight, English language only, randomised or quasi-randomised trials and found that *M. vaccae*, using a single dose of ~10^9 heat-killed organisms, when used as an adjunct to standard dose chemotherapy offered no benefit (45). By contrast, a more recent meta-analysis by Yang *et al*. included fifty four predominantly Chinese studies and suggested that *M. vaccae* offered a small but significant improvement in smear conversion rates, radiological improvement, and cavity closure (42). Notably, the
### Table 5.13.1 Potential immunotherapeutic agents, their putative mechanisms of action, and relevant animal or human outcome data targeting drug-sensitive and/or drug–resistant TB.

<table>
<thead>
<tr>
<th>Product/Agent</th>
<th>Theoretical mode(s) of action</th>
<th>Outcome(s), reference(s) and comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Therapeutic vaccines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subunit vaccines e.g. with fusion protein ID93</td>
<td>Alters and boosts T cell responses toward a Th1 profile</td>
<td>Improved survival and decreased bacillary load in a murine MDR-TB model (53)</td>
</tr>
<tr>
<td>DNA vaccine e.g. with HSP65 of Ag85A</td>
<td>HSP65 vaccine enhances cytotoxic T-cell activation and decreases Th2 responses</td>
<td>In murine models is effective against TB and MDR-TB, alone and in combination with chemotherapy (55, 56, 89)</td>
</tr>
<tr>
<td><em>Mycobacterium vaccae</em> (heat-killed environmental mycobacterial preparations)</td>
<td>Alters T cell response towards Th1 profile, modulates Treg function, and directs CD8 + T cell against epitopes common among mycobacterial strains</td>
<td>No or limited benefits seen in double-blind randomised trials for DS-TB (42, 45, 88); Possible small clinical benefit in DR-TB (46); Multiple dose <em>M. vaccae</em> studies needed</td>
</tr>
<tr>
<td><strong>RUTI (Mycobacterium tuberculosis liposomal preparation)</strong></td>
<td>Used together with initial period of chemotherapy the idea is to reactivate dormant <em>M. tuberculosis</em> and make them more susceptible to killing by vaccine-generated antigen-specific T cells</td>
<td>In murine or guinea pig model showed some efficacy in controlling tuberculosis after initial chemotherapy (48); Phase I and Ila clinical trial confirmed safety and immunogenicity and phase IIdb trials under way in DS-TB (52)</td>
</tr>
<tr>
<td><strong>2. Other immunotherapeutic approaches for DS-TB and DR-TB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2.1 Immunomodulatory agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesenchymal stromal cells (MSCs)</td>
<td>Down regulation of inflammation-related genes and up regulation of phagocytic genes (64); tissue regenerative capacity</td>
<td>9 patient study in MDR-TB with favourable outcomes (40)</td>
</tr>
<tr>
<td>Product/Agent</td>
<td>Theoretical mode (s) of action</td>
<td>Outcome (s), reference (s) and comment</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>High-dose intravenous immunoglobulin</td>
<td>Multiple mechanisms not all clear; fully sialylated Fc oligosaccharides in IVIg preparations down-regulate inflammatory genes</td>
<td>Marked therapeutic effect in a murine model when administered early in infection (66) No studies in DR-TB</td>
</tr>
<tr>
<td>16a-bromoepiandrosterone (HE2000)</td>
<td>Unknown mechanism of action</td>
<td>In murine model TB had therapeutic effect even in absence of chemotherapy (90)</td>
</tr>
<tr>
<td>Dzherelo (plant extracts)</td>
<td>Unknown mechanism of action</td>
<td>Small human studies in MDR- XDR-TB from the Ukraine show possible benefits (91, 92)</td>
</tr>
</tbody>
</table>

### 2.2. Immunosuppression to reduce inflammation

<table>
<thead>
<tr>
<th>Product/Agent</th>
<th>Theoretical mode (s) of action</th>
<th>Outcome (s), reference (s) and comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids</td>
<td>Predominant action is suppression of TNF-α</td>
<td>Phase II clinical trial in HIV-infected TB patients improved 1 month culture conversion but with side-effects (93)</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>Suppression of TNF-α, increases IL-2 and IL-12, co-stimulatory effects on T cells</td>
<td>Beneficial effects demonstrated for severe inflammatory reactions associated with TB (61, 62) No studies in DR-TB</td>
</tr>
<tr>
<td>Etanercept</td>
<td>Anti- TNF-α, neutralises TNF-α</td>
<td>Not yet systematically studied as adjunctive TB therapy</td>
</tr>
</tbody>
</table>

### 2.3 Effector cytokine therapy to enhance microbicidal effect

<table>
<thead>
<tr>
<th>Product/Agent</th>
<th>Theoretical mode (s) of action</th>
<th>Outcome (s), reference (s) and comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>Increased Th1 responses, increased IP-10/decreased IL-17 with decreased neutrophil-mediated inflammation (40)</td>
<td>Decrease in constitutional symptoms and increased rate of sputum conversion but this was not sustained and not significant at 2 months (87, 94) Decreased tissue damage improved time to <em>Mtb</em> clearance (95) Small studies in DR-TB (96, 97) The results of phase 2 commercial trials remain unpublished due to lack of efficacy.</td>
</tr>
<tr>
<td>Product/Agent</td>
<td>Theoretical mode (s) of action</td>
<td>Outcome (s), reference (s) and comment</td>
</tr>
<tr>
<td>---------------</td>
<td>--------------------------------</td>
<td>--------------------------------------</td>
</tr>
</tbody>
</table>
| IL-2          | Theoretically drives Th1 responses. Modulates Treg function. | RCT showed slightly prolonged time to sputum conversion in treatment group likely due to Treg expansion (75) 
Aerosolised IL-2 may offer compartmentalised utility (40) 
No studies in DR-TB |
| IL-7          | Helps to improve immune responsiveness in “immune-exhaustion” states by protection from apoptosis, improving dendritic cell activation and immune memory | Successful use post stem cell transplant and in HIV infection and similar effects maybe useful for longstanding TB infection (80, 81) 
No studies yet in DR-TB |
| IL-15         | Helps to improve immune responsiveness in “immune-exhaustion” states by protection from apoptosis and improvements in immune memory function | In murine model showed survival advantage in TB infection if co-administered with BCG (78, 79) |
| IL-24         | Direct activation of CD8 + T-cell in mice, increased IFN-γ production; activates neutrophils and their IL-12 production | In a murine model had a protective effect against M.tb infection (98) |

Chinese studies have involved multi-dose regimens of a different (Chinese) *M. vaccae* preparation administered by intramuscular injection (42). Thus, dosing frequency and route of administration may account for outcome differences between the meta-analyses. A meta-analysis in drug-resistant TB evaluated the effect in seventeen small predominantly Chinese studies and found a minimal but clinically significant benefit (46). Collectively these data indicate that single dose *M. vaccae* has a negligible impact on TB treatment-related outcomes. It has been suggested that a single dose is probably inadequate to sustainably drive favourable responses. The findings of the DARDAR study may add some credence to this hypothesis. This randomised controlled trial involved the administration of five intradermal doses of *M. vaccae* or placebo to HIV-infected patients with CD4 ≤200 cells/ml, a BCG scar and a negative Tuberculin skin test. *M. vaccae* reduced...
the hazards ratio of definite and disseminated active TB by ~50% (47). However, the role and benefit of multiple dose *M. vaccae* for drug-sensitive or resistant TB, if any, remains unclear. Phase III studies of multiple dose *M. vaccae* are planned or currently underway in drug-sensitive and MDR-TB patients.

**RUTI**

RUTI®, manufactured in Spain by Archivel Farma 48, is a therapeutic vaccine that was developed for use as an adjunct to shorten chemotherapy for latent TB infection (LTBI). RUTI® comprises fragmented cells of MTB bacilli grown under conditions of low oxygen concentration and pH to replicate growth condition in a human granuloma (49). In a mouse model, inoculation with RUTI® and a short period of concurrent chemotherapy induces a strong Th1-specific poly-antigenic immune response including against secreted antigens (ESAT-6 and Ag85 complex) (50). Efficacy has been demonstrated in several animal models of drug-sensitive TB. A phase 1 clinical trial demonstrated safety and immunogenicity in humans using different subcutaneous doses (48, 50-52) and phase Iia and Iib evaluations are underway or been completed. A phase III study in HIV-infected persons is being planned in South Africa. Phase II and III studies in MDR-TB patients are warranted.

**Subunit and DNA vaccines**

A number of novel subunit and DNA vaccines are currently in various stages of development and clinical evaluation for the primary prevention of TB and to prevent progression from latent to active TB. In addition, some may have application as therapeutic vaccines for use in patients with active drug-sensitive, MDR- and XDR-TB. To date though, none of these subunit or DNA vaccines have been evaluated in humans with drug-resistant TB. A few have, however, shown promising preliminary studies in animal models. Bertholet *et al.* described the utility of boosting BCG-vaccinated animals (guinea pigs and mice) with the recombinant fusion protein ID93 (consisting of four *M. tuberculosis* antigens, including members of the virulence factor families PE/PPE and EsX) to reduce death and bacillary loads after challenge with MDR strains (53). Liang *et al.* showed the utility of an Ag85A DNA vaccine alone or in combination with rifampicin and pyrazinamide chemotherapy to reduced pulmonary and splenic bacterial loads in mice challenged with an MDR clinical isolate (HB361) (54). Similarly, DNA vaccines encoding hsp65, with or without incorporation of additional immunostimulatory motifs, have been shown to drive Th-1 responses in murine models thereby improving survival and decreasing bacillary load (55, 56). Further studies, with consideration of the growing knowledge-base about MDR-TB immunology, will need to select the most appropriate novel vaccine candidate to study in these patients.
Other potential immunotherapeutic approaches for MDR-TB

Other immunotherapeutic agents with potential application to MDR-TB are outlined in Table 5.13.1. The majority of these agents has been evaluated in human MDR-TB studies (exceptions are highlighted). Some of these agents and their possible use in MDR-TB is discussed below.

Immunosuppressive therapy that may lessen immunopathology and/or excessive tissue damage and improve mycobactericidal drug activity have been shown to offer benefit in the clinical management of certain types of drug-sensitive TB. For instance, the use of corticosteroids as adjunctive treatment for TB meningitis forms part of routine clinical practice. However, recent data suggests that the therapeutic benefit is only in certain patient groups (57). Tobin et al. show that an intronic single nucleotide polymorphism (SNP) at the LTA4H locus could predict steroid responsiveness in drug-sensitive TB meningitis patients (57). The mechanism relates to the association between certain LTA4H genotypes and dysregulated, pathologically high TNF levels, which although initially beneficial for mycobacterial killing, lead to increased tissue damage in established infection (58). In animal models of TB infection, the utility of using other anti-TNF agents e.g. Thalidomide has been shown to improve bacillary clearance and increase survival (59). However, clinical studies of thalidomide suggest benefits in adults with intracranial tuberculomas, but deleterious outcomes in childhood TB meningitis (60-63). Nevertheless, the availability and recent licensing of oral anti-TNF agents e.g. Tofacitinib makes further study of these agents for TB appealing. Nevertheless, a clinical benefit from anti-TNF agents will likely only be found in certain patient groups with dysregulated TNF inflammation. In MDR-TB patients, given the chronic inflammation and likely high numbers of slowly replicating bacilli, anti-TNF agents may offer benefit both to enhance mycobactericidal drug activity and to mitigate the negative systemic effects of chronic inflammation. However, the use of immunosuppressive therapy in the context of drug-resistance and hence ineffective chemotherapy might also be hazardous. Proof-of-principle studies are first required.

Two other immunomodulatory agents limiting excess inflammation and aiding tissue repair warrant brief discussion. The infusion of bone-marrow-derived mesenchymal stem cell has been shown, in a proof-of-principle study, to be of potential benefit in nine MDR-TB patients (Skrahin et al. unpublished) (40). Of the nine treated patients, 5 were cured and 4 showed disease stabilisation. Proposed mechanisms include a direct lung regenerative effect or an overall down-
regulation of inflammation-related genes, but an upregulation of genes involved in phagocytosis (demonstrated in a murine sepsis model) (40, 64). The coupling of cytokines e.g. IL-7 or MTB antigens to modified MSCs homing to areas of maximal tissue damage is a potentially novel strategy that maybe of use for MDR-TB patients (65). Intravenous immunoglobulin (IVIG), already in routine clinical use for immunodeficiency patients and for those with autoimmunity disorders, has shown safety and proof-of-principle in a murine model of drug-sensitive TB (66). A subset of immunoglobulin G (IgG) found in high-dose IVIg have fully sialylated fragment crystallizable (Fc) oligosaccharides which are anti-inflammatory and antagonise the pro-inflammatory agalactosyl (also asialic) IgG found in TB (39, 67-69). Proof-of-principle human studies in MDR- and XDR-TB patients are now required.

Cytokine therapy for TB and MDR-TB is another appealing strategy given the clinical availability of a number of recombinant cytokines and cytokine blocking agents with delivery via different routes of administration. IFN-gamma adjunct therapy has been evaluated in both drug-sensitive and MDR-TB patients via either a subcutaneous, intramuscular or aerosolised route of administration (70). In the small studies (n<10 patients) IFN-gamma therapy, administered via different routes, for MDR-TB has shown limited or no overall benefit (71-73). However, it is worth noting that in certain patients IFN-gamma adjunctive therapy resulted in a dramatic clinical and radiographic improvement (71). Nevertheless, overall there have been more than one commercial study showing no benefit of this therapy (these data have never been published).

IL-2 with and without GM-CSF, to stimulate T cell proliferation and enhance IFN-gamma responses has also been evaluated in murine and small human studies of drug-sensitive and resistant TB (74, 75). Johnson et al., in an RCT of 110 drug-sensitive patients, found that IL-2 prolonged the time to negative sputum conversion most likely due to Treg expansion (75). An earlier study suggested benefit in 5/8 MDR-TB patients (76) and a recent murine model of drug-resistant TB found the co-administration of IL-2 and GM-CSF to decrease bacillary loads and improve survival by 30% (77). IL-7 and IL-15 cytokines have also both demonstrated proof-of-principle in mouse models of drug-sensitive TB (40, 78, 79), but given their ability to restore immune responsiveness warrant further investigation in MDR-TB. IL-7 has been successfully used to aid immune reconstitution in HIV-infected patients and after haemopoeitic stem cell transplant (80, 81). Finally, given the recent identification of a type 1 interferon bio-signature able to differentiate active from latently infected TB patients (82, 83), the role of type 1 interferons in the development of active tuberculosis is an area of intensive study. The availability of both aerosolised IFN-
alpha (84) and IFN-alpha antibodies (85) will make further human studies feasible. Interestingly, in contradistinction to murine data demonstrating failure to improve survival in TB infection, a small study of aerosolised IFN-alpha therapy in seven MDR-TB patients showed decreased bacillary loads and improved radiological appearance of TB lesions on high resolution CT scans (84).

**Barriers to use**

Despite the promise of a number of these therapies as well as their ready availability, a number of barriers to the application of immunotherapy to DR-TB remain (40, 86). To date, the success of adjunctive immunotherapy for predominantly drug-susceptible TB has been variable and/or limited. Studies have either shown benefit in small numbers of patients or only non-human models (e.g. mesenchymal stem cells (MSC) (40), IVIg (66) and IL-15 (79); only modest benefits such as a reduction in TB symptoms e.g. (IFN-γ) (87) or no overall benefit in larger double blind randomised trials (e.g. *M. vaccae* for drug-susceptible TB (88). A number of possible explanations may account for the wide variation in therapeutic responsiveness: i) genetic variability in the nature and magnitude of individual immune responses, ii) incorrect matching of therapy in relation to the stage of TB infection e.g. Th17 responses that may be beneficial early in infection but deleterious late in infection, iii) a lack of understanding of the type of immune response in MDR- and XDR-TB and hence the selection of inappropriate therapeutic immune targets, and iv) the failure to deliver therapy to the site of infection (40). Undoubtedly, immunotherapeutic strategies will not be applicable in a “one-size fits all” approach (86). The timing and nature of immunotherapy, if it does work, will need to be tailored to individuals or particular patient sub-groups based on adequate immune-profiling (40). Clearly, research efforts must be accelerated to better understand the nature of the immune response to DR-TB, rapid and affordable immune-profiling that can guide the selection and timing of therapy must be developed, and funding must be made available for phase IIb and III clinical trials in larger groups of MDR- and XDR-TB patients.

**CONCLUSION**

We have now come a full circle from the 1940’s to the early 21st century where, once again, there are large numbers of patients for whom there are no further therapeutic options. Highly drug-resistant forms of TB have a high mortality, mainly afflict economically young active adults, and consume a disproportionate fraction
of national TB resources, which is clearly unsustainable. Therefore, drug-resistant TB has the capacity to destabilise national TB programmes. The unavailability of suitable drug options, and the fact that such options may only be available in several years, and even then mainly in the context of what we know as drug-sensitive TB, makes the need for immunotherapy trials urgent and compelling. Several immunotherapeutic interventions are in phase 1, 2, and early phase 3 trials. There is an urgent need for a co-ordinated effort amongst funding agencies such as the National Institutes of Health, Gates Foundation, UK MRC, the EU Horizon 20/20 programme, and other funding agencies to drive these studies so that the benefits of immunotherapeutic interventions, if any, may be more rapidly realised.

REFERENCES


5. DEVELOPMENT OF NEW VACCINES AGAINST TB


