Chapter 6

Discussion & Future Perspectives

In this thesis, we describe the global and Dutch MDR-TB problem and we discuss treatment strategies to combat MDR-TB with a special focus on aminoglycosides and ertapenem, using pharmacokinetic / pharmacodynamics modeling to optimise outcome while minimizing dose-dependent toxicity and adverse effects.

Current WHO strategies to combat the TB epidemic by identifying and treating individuals with the most infectious forms of TB using sputum microscopy for case detection, followed by the DOTs strategy [1] have failed to curb the emergence of DR-TB, in particular MDR- and XDR-TB. In this thesis we do not focus on detection but describe several methods and approaches that we have used in The Netherlands to combat and even to prevent DR-TB, and conclude with some recommendations for low-resource, high-burden TB countries.

In **Chapter 2**, we discuss immunological aspects of TB. Improved knowledge of the biology of *M. tuberculosis* might help in the discovery of novel molecules to target *M. tuberculosis*. We argue that a better understanding of the host immune response may help in distinguishing between paradoxical reactions / IRIS on the one hand, and on-going active TB as a result of failed treatment on the other hand. We also discuss immunotherapeutic and immuno-prophylactic strategies, by designing improved vaccines, to replace or improve the currently only vaccine available – BCG. BCG has been around since almost a century and currently, it is globally the most widely used vaccine. BCG vaccination is however still controversial. It is particularly tragic that, despite much progress in recent years, the use of scarce resources to administer BCG must still be based mainly on blind faith [2].

Paradoxical reactions, IRIS or active TB

During treatment for TB, either for drug-susceptible or drug resistant TB, differentiation between a paradoxical reaction / IRIS and on-going TB disease reflecting treatment failure – is extremely important. Obviously, the two entities need a dramatically different approach. Only follow-up can distinguish the two entities with certainty, and biomarkers or surrogate parameters have not been identified to differentiate these entities, perhaps with the exception of sputum culture status at month 2-3 after start of treatment [3]. Symptoms can be completely overlapping and paradoxical reactions or IRIS, even when the mycobacteria are no longer viable, can also cause significant morbidity and mortality due to an exacerbated immune response. During treatment for drug-susceptible TB, a return of symptoms may either reflect a paradoxical reaction / IRIS but if treatment failure is the cause of recurrent symptoms, acquired drug resistant TB has to be taken into account, while treatment failure in MDR-TB might be caused by acquisition of additional resistance. Excluding active disease requires culture and drug susceptibility testing that

is not only time consuming, but also necessitates considerable infrastructure and incurs huge costs. Even currently available DNA-based molecular testing platforms may not differentiate between the presence of DNA of killed and viable bacilli.

Novel vaccination and immune modulating strategies for both active and latent TB

Boosting or immunomodulation of the immune system may have important future implications in eradicating TB. It may help to prevent reactivation of latent TB and also to combat active TB [4].

Pre-exposure vaccines are designed to prevent TB infection while post-exposure vaccines are either meant to enhance immunity following exposure to viable TB bacilli by boosting immunity, to prevent subsequent TB, or to curb/combat on-going latent TB infection by enhancing lysis of dormant bacilli in macrophages. Therapeutic vaccination is designed to help treating active TB, in combination with antibiotic drug treatment.
Although BCG is impressive with respect to its low cost and its high safety [5], the present regimen of BCG (pre-exposure) vaccination soon after birth provides limited protection in children against disseminated and meningeal TB and has had no impact on adult pulmonary TB, and hence, no impact on the epidemiology of TB in the population. This finding probably reflects a lack of effectiveness in adults, especially in the context of early exposure to environmental mycobacteria [6].

To reduce the global burden of disease, new vaccination strategies against TB need to induce a far better immunity than that achieved with BCG vaccine, not only in infants but also in adolescents and adults. In infants, an optimal vaccine would fully prevent initial infection. Present vaccine candidates are intended to only reduce the initial bacterial burden with containment of remaining *M. tuberculosis* organisms and will therefore neither eradicate the pathogen, nor prevent stable infection [7].

Immunologically contained *M tuberculosis* is thought to transform from a metabolically active replicating form into a dormant form with minimal metabolism and replication that can lead to disease reactivation at a later stage [1,8,9,10]. To prevent this reactivation, appropriate post-exposure or therapeutic vaccines are needed to target dormant and persistent *M. tuberculosis*. Post-exposure vaccines should also prevent reinfection of individuals with latent infection, notably in regions with high TB prevalence [11]. The rationale behind post-exposure vaccine strategies, as part of TB treatment is by enhancing cellular immune response eventually to enhance bacterial killing, thereby shortening treatment duration and preventing TB relapse [12]. These strategies have recently been listed among host-directed therapies [13,14,15].

Latent infection with *M. tuberculosis* is actively controlled by the immune response and once immunity fails or wanes, TB may reactivate. Better understanding of immunological mechanisms can form the basis for rational design of new vaccination strategies against TB [16,17]. Immunotherapy (such as DNA vaccines) may reduce the duration of chemotherapy (being an adjunct to chemotherapy) and may also reduce reactivation TB [18]. One problem in designing novel vaccines is that current immunogenicity markers have differed among different research groups [19]. Most researchers believe that enhancing certain cytokines e.g., interferon-gamma responses and TNF-alpha by CD4+ and CD8+ T cells reflects enhanced autophagy in macrophages translating into enhanced protection against TB or TB infection [20]. This paradigm has recently been challenged in experimental animals studies [21,22,23] as well as in human vaccine trials [24,25]. Immunotherapy though it may contribute to TB treatment, should first of all be safe [26]. Although a large multicenter trial of *M. indicus-pranii* – one of five candidate therapeutic vaccines [27] – has not shown efficacy for the end point chosen, safety appeared excellent [28].

Conclusions and recommendations: we advocate that new vaccines are urgently needed to reach the WHO Sustainable Development Goal of substantially reducing and eliminating TB as a public health problem by 2030 [29,30,31]. Long-term vaccination strategies need to target these more ambitious goals. Even though vaccine development will have a price, the return of investment will greatly exceed original costs [32].

In **Chapter 3** we describe the treatment results of two observational studies of all patients in the Netherlands diagnosed with drug resistant TB (DR-TB). We demonstrated a high success rate: 86% of those started on treatment had a favourable outcome thereby reaching targets set for DR-TB.

The optimal composition and duration of currently available MDR-TB treatment regimens are still uncertain [33,34]. In a large individual patient data meta-analysis of 9,153 patients, overall treatment results were less favourable than the Dutch data —treatment success was achieved in around 60% of all patients. Treatment success was significantly associated with the specific durations, the number of likely effective drugs for the initial intensive and continuation phases of therapy, and with the use of later generation quinolones. However, because of important limitations in the included studies, cautious interpretation of these results is needed [35]. The evidence of efficacy of the former WHO class 5 drugs is still limited [36] and especially drug resistance emerging during treatment is associated with poor outcome [37]. Our strategy to prevent emergence of resistant clones during therapy is to individualize treatment. An important aspect of this approach is to optimise treatment

results while at the same time minimising drug concentration-dependent adverse effects. Optimising treatment results was based on pharmacokinetic (PK) measurements relative to pharmacodynamics (PD; i.e., drug susceptibility measures). Based on in vitro modelling, efficacy end points following PK/PD calculations would then need to exceed certain cut-off ratios in order to be considered adequate [38]. Dosage adjustment according to PK/PD, the latter based on minimal inhibitory concentration (MIC) test results would provide efficacy for each drug component in the regimen chosen [38,39,40]. The impact of drug susceptibility testing (DST) on outcome has been shown [41]. Molecular identification of resistance genes translating in phenotypic resistance should shorten time to a tailored treatment regimen, but until these molecular tools have been fine-tuned, phenotypic DST results will remain indispensable for definitive PK/PD calculations.

The 2016 update of WHO treatment guidelines for drug-resistant TB advocates a shorter treatment duration for selected patients, i.e. 9-12 months. In our retrospective analysis of our MDR-TB patients we show that nearly half (85/172; 49.4%) of our patients would meet the criteria for the new shorter regimen. Only 4 of our eligible patients still had positive sputum smear microscopy after 4 months of treatment; 81/172 (i.e., 95% of those eligible for shorter treatment duration) would therefore be eligible for 9 months treatment only, according to the new guideline. We have treated patients in whom intuitively, treatment was extremely long, considering the fact that their lesions were limited, as well as their bacterial loads [42]. Therefore we warmly welcome these new recommendations; obviously, shorter regimens should not jeopardize excellent outcomes earlier achieved and therefore vigilance is warranted. A shorter regimen is only applicable in MDR-TB patients without previous exposure to, or resistance to 2nd line TB drugs.

Conclusions and recommendations: we realize that the high success rate in our study is the compound result of many different factors. The relative contribution of each individual component in our approach cannot be determined. All components may ultimately prove essential: selection of adequate empirical initial drug combinations; DST performed in a well-coordinated fashion in a central reference laboratory; adjusting drug treatment to DST, MIC and PK measurements, using certain cut-offs for PK/PD ratios; a committed treatment team that applies the principles of close supervision, under the umbrella of a well-organised national TB program; and TB centres of excellence, thereby merging a developing expertise with a DOTs strategy.
In close cooperation and with support of high resource countries, methods like drug treatment adjusted to DST and PK measurements could and should be made available in high TB burden countries as well.

In **Chapter 4** we describe the efficacy and side effects of aminoglycosides, using PK/PD modeling; see figure.

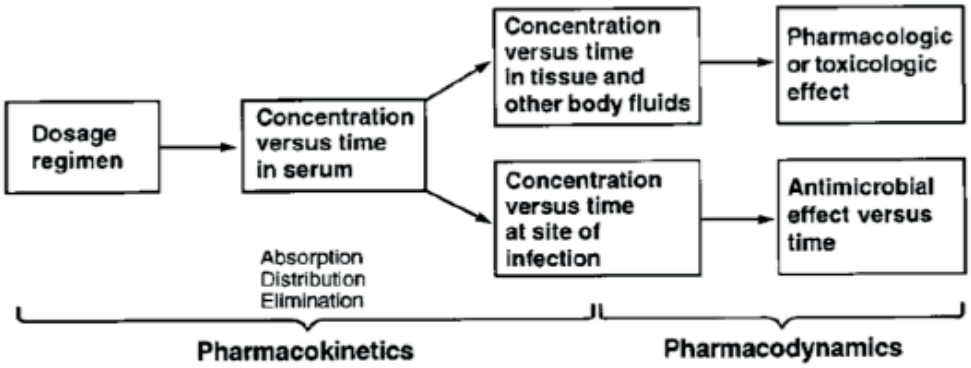

FIGURE 1: Overview of pharmacokinetics and pharmacodynamics in antimicrobial therapy
From: Craig WA. Pharmacokinetic / pharmacodynamic parameters:
rationale for antibacterial dosing of mice and men. Clin Infect Dis 1998; 26: 1–12

The assessment of efficacy of TB treatment is challenging. Typically, using only one single drug is bound to fail, as no single drug is able to kill all different clones within the bacterial population present in one single individual that suffers from TB. No single drug, however effective in most (95, or 99% of organisms present in the specimen isolated from the host) can be dosed high enough to prevent drug-resistant mutants to replace susceptible organisms. Invariably, drug combinations with at least 3 active drugs are required to effectively kill the bacterial population. Although actively replicating, metabolically active organisms are killed rapidly in a log-wise fashion within the first three weeks of treatment, the population of slowly replicating, metabolically inactive phenotype organisms – the so-called persister organisms – require long-term treatment in order to obtain sterilising treatment. Rifampicin in combination with isoniazid and pyrazinamide; and perhaps, clofazimine have the largest activity against persister phenotype organisms. In vitro models like culture may predict efficacy reasonably accurately, but single drug susceptibility assays using agreed breakpoints have been based on consensus rather than robust evidence in clinical trials with patient outcomes as the reference test. These breakpoints have been challenged [43] using more accurate in vitro models like the hollow fiber model that more closely mimics PK variations over time than the traditional solid and liquid culture media [44]. Even the hollow fiber model still simplifies the reality as only variations of drug concentration in the blood stream over time are modelled; drug penetration in sanctuary sites may further complicate matters [45,46,47].

For most drugs in TB treatment, the optimal dosage has not yet been established. Even with excellent compliance, simulation studies show that up to 1% of patients will develop drug resistance due to variability in drug concentrations [48,49,50,51,52]. Amikacin and kanamycin were classified until 2016 as WHO group 2 (injectable agents) and in the new classification in group B for the treatment of MDR-TB and are administered in a dose of 15 mg/kg/day with a maximum of 1000 mg daily in MDR-TB treatment [53]. The pharmacodynamic index of aminoglycosides is usually quantified as the ratio of the maximum blood concentration (Cmax) to the MIC. The area under the concentration–time curve might be a more appropriate pharmacokinetic parameter in comparison with the Cmax or Cmin [54]. Cmax / MIC is a key predictor of the antibacterial activity of aminoglycosides; in gram-negative and gram-positive bacteria there is consensus that it should reach a value of > 10 in order to prevent the emergence of resistant clones, and to ensure clinical efficacy [55,56].

In our approach in using aminoglycosides for MDR-TB treatment, [57,58] we have applied individualised treatment based on the Cmax/MIC ratio using a limited sampling strategy. The importance of individual dosing in relation to toxicity and effectiveness of aminoglycosides was stressed by Zhu et al [59]; the AUC of streptomycin in 19 patients varied from 124 – 680 µg•hr/ml while the Cmax varied from 9 – 107 µg/ml. Since the pharmacokinetics of aminoglycosides like streptomycin, amikacin and kanamycin are assumed to be comparable, variations in AUC and Cmax of amikacin and kanamycin can be expected.

Dosages of aminoglycosides in patients with MDR-TB in our observational study (a median dose of 400 (IQR; 350.0 – 500.0 mg) were more than two-fold lower than the dose recommended by WHO, while nonetheless, outcome (clinical efficacy) was favourable in the vast majority of our patients. Besides, no treatment failures or documented relapses were observed using this lower dose of aminoglycosides in an analysis of all MDR-TB patients diagnosed and treated in the Netherlands [60]. Like in earlier studies treatment duration and the cumulative dose were correlated with side effects, but not with the dose or the dosing frequency [61,62,63].

This limited sampling strategy provides a good estimate of the AUC_{0-24h} and is therefore suitable for daily patient care and use in outpatient clinics, but also during therapeutic drug monitoring (TDM) in prospective clinical trials [64]. TDM using standard or alternative sampling techniques, such as dry blood spot (DBS) sampling or oral fluid sampling, could improve acceptability by doctors and patients, thereby contributing to limiting adverse effects by tapering the TB-drug dose to the lowest possible effective dose [65]. Therefore ultimate goals should perhaps be: either optimizing doses for specific populations by

taking into account pharmacokinetic variability or, better still, individualization of each patient's doses if resources are available [64].

WHO recommendations underscore that countrywide, comprehensive and effective implementation of the WHO-recommended Stop TB strategy (developed from the DOTS framework), is an important approach for preventing drug-resistant TB [66]. However there are suggestions that monitoring the levels of TB drugs in a patient's blood could be as important as monitoring compliance with therapy -- in contrast to current WHO guidelines [50,53].

Conclusions and recommendations: to monitor efficacy of the aminoglycosides in treating MDR-TB, in future studies the use of the AUC/MIC ratio instead of the Cmax/MIC ratio to monitor efficacy and the use of a limited sampling strategy needs to be validated in an in vitro model for infection and subsequently tested in a prospective clinical trial [67]. Nevertheless, evidence in animal models suggest that the $AUC_{0\text{-}24h}$/MIC ratio predicts the efficacy of aminoglycoside therapy [54], and we speculate that this ratio can also be applied to humans [68]. This needs to be confirmed in a hollow fiber model as has already been done for moxifloxacin [49].

Chapter 5: Ertapenem.
In the battle against TB, the development of new and/or the exploration of repurposed drugs are urgently needed, with a quest for few(er) drug-drug interactions, high(er) efficacy, few(er) side effects and low(er) cost. This is not only required for DR-TB but also for *M tuberculosis* sensitive for the first line drugs in order to facilitate and shorten therapy as much as possible. In particular, as treatment of DR-TB is costly and requires prolonged use of many drugs, administration of safe and effective drugs is crucial [69]. All this will improve patients' compliance even for patients with drug susceptible *M tuberculosis*, after all a so-called standard "short" course treatment for TB is lasting 6 months. In regard to treatment duration in patients with MDR-TB, the shortest treatment with acceptable outcome and minimal side effects still lasts 9 months [70,71,72]. This is a considerable improvement compared to the 20-24 months recommended in the former guidelines by WHO for DR-TB, while the new 2016 WHO guidelines recommend a period of 12 months for a shorter regimen for selected patients. However 9 months is still asking a lot from the patient and the supporting, providing system. Generally speaking, many patients do not experience clinical signs of their disease anymore after several weeks of treatment, and this will contribute to 'treatment fatigue' and ultimately non-adherence. Therefore: the shorter the duration of the therapy, the better. Additionally, it would be most useful when these therapies are beneficial for drug-susceptible as well as for DR-TB [73].

In adding new drugs to a TB regime there are 2 possibilities: (a) repurposed drugs 74,75,76 and (b) new drugs, like bedaquiline (TMC 207), delamanid (OPC 67683) and pretomanid (PA-824) [77].

There are more than a dozen new or repurposed tuberculosis drugs under clinical investigation. In the revised 2016 WHO guidelines on DR-TB, two new drugs, delamanid and bedaquiline are recommended as add-on agents. The first reported patient with both these add-on agents was described in 2016 [78].

In Chapter 5, we focus on one of the repurposed drugs, ertapenem, one of the carbapenems, labelled for other bacterial infections and potentially useful in the treatment of DR-TB (MDR- as well as XDR-TB) [79].

Ertapenem has not yet been labelled as a group 5 drug to be used as part of MDR-TB treatment. In case of proven efficacy against DR-TB and because of its relative long half-life time which enables once daily dosing, ertapenem may be an attractive carbapenem. The drawback is its parenteral use. Parenteral drug administration has a price, both in terms of logistics and finance as well as in terms of risks – bleeding, nosocomial transmission of drug-resistant organisms, bleeding and thrombosis [80]. An inhaled formulation of injectable TB drugs might be advantageous, provided that such treatment would be tolerated and would result in acceptable and adequate bioavailability.

In our retrospective study among MDR- and XDR-TB patients, safety and pharmacokinetics of ertapenem were evaluated. Ertapenem treatment was well tolerated during MDR-TB treatment and showed a favourable PK/PD profile in MDR-TB patients [81]. Although our study did not focus on efficacy, sputum smear and culture were converted in all patients. In the review study by Sotgiu et al [79] the efficacy/effectiveness profile of the carbapenems was promising.

In another retrospective study of our group the aim was to develop a model to predict the area under the concentration-time curve, measured over the first 24h after ertapenem drug administration ($AUC_{0\text{-}24h}$) during steady state, using a limited sampling strategy in patients with MDR-TB. Whether TB drug concentrations influence clinical outcome is still a controversial issue [82]. Although not yet proven for patients with TB, %T>MIC is expected to be an important pharmacodynamic parameter. According to the population model, most of the patients reached a minimum of 40% time above MIC of 0.5 mg/L and eleven patients exceeded %T>MIC of 0.25 mg/ L. We had previously shown in a population pharmacokinetic model based on healthy volunteers that $AUC_{0\text{-}24h}$ of ertapenem can be estimated in with 2 sampling time points using a limited sampling strategy [83]. This study

shows that $AUC_{0\text{-}24h}$ of ertapenem can be estimated also in patients with MDR-TB with only 2 sampling time points using a limited sampling in a population model and in a linear regression model [82].

Indeed, ertapenem safety and pharmacokinetics in combination with TDM as described above make us believe that ertapenem in combination with clavulanate is a highly promising drug for the treatment of MDR-TB that warrants further investigation [79,82, 84].
Ineffective or incomplete treatment, slow drug responses leading to prolonged infectiousness, acquired drug resistance, treatment failure and early relapse, as well as the emergence of MDR- TB that all thrive in the absence of TDM, call for a change to bring TDM to the forefront [85]. TDM could be cost-effective even in high incidence, low resource settings. With the novel tools and procedures in place, TDM should no longer be a remote possibility but rather be adapted as an integral component of national TB programs similar as TB diagnostics and first- and second-line TB drug supply. We therefore propose that methods like limited sampling strategies and optimized by TDM supported by high resource countries, can already be very useful in high TB burden countries, to optimize outcome, minimize side effects and toxicity, and prevent emerging drug resistance.

Conclusions and recommendations: we advocate a prospective study in patients with DR-TB with ertapenem, dosing by estimating the $AUC_{0\text{-}24h}$ translated in a %T>MIC with 2 sampling time points (a limited sampling strategy); parameters being efficacy and safety.

Future perspectives and conclusions

To defeat TB (including MDR-TB) by 2030, which is the current target set by the United Nations in their Sustainable Development Goals (SDG), a combination of effective vaccination, immunomodulation and improvement of current drug treatment is needed. To achieve the ambitious goals, the recently revised guidelines on MDR-TB (WHO, 2016) have already agreed that in selected patients, treatment duration might be shortened to 9-12 months. This thesis describes several approaches, like effective vaccination, immunomodulation and improving current drug treatment as valuable options in the battle against MDR-TB.

To reach the TB-related SDG, we advocate and emphasize aspects of vaccine development (pre- and post-exposure; and therapeutic), finding ways of boosting the immune system during active disease, the promotion of precision diagnostics and treatment with a combination of active drugs killing rapidly replicating bacteria and of drugs targeting persister organisms. Initial drug treatment with relatively high efficacy and low toxicity

should be guided by a selection based on mutations detected by PCR (Xpert-TB RIF; Haine Line Probe Assay), tailored if possible on phenotypic DST (i.e., below or above the EUCAST breakpoint) and preferably MIC, allowing for optimized by TDM, instead of a shot gun approach with all the collateral damage, like (avoidable) drug-induced toxicity, and preventing the emergence of drug resistance. The proposed strategy is currently not evidence-based; it should be possible to test the hypothesis that individualised, PK/PD driven treatment for MDR-TB yields improved outcome with reduced toxicity compared to programmatic standardized treatment; a study design using wedge-stepped randomisation might be the best way forward.

A close collaboration between all stakeholders in TB is mandatory in reaching the TB-related SDG, both in high-resource and in high-burden countries. This approach should be (made) affordable for national programs in endemic regions and countries. The required resources combatting global TB – both in manpower and in financing - are huge. Solving the TB-problem will perhaps be extremely costly, however in the light of an earlier experience with AIDS, these problems must and should be overcome [86]. Besides, few health interventions have been as cost-effective as fighting TB [87,88].

Last but not least, in order to be able to compare studies on MDR-TB a common set of core research definitions (like on efficacy and safety) is needed to ensure there is comparability in clinical trials on MDR-TB and to maximize policy impact [89].

NOTE

From 2011 until 2016 the aminoglycosides were represented in group 2 of the five groups of drugs of the WHO list for treating DR-TB. These five groups were:

WHO recommended grouping of anti-TB drugs (until 2016)

Group		Anti-TB agent	Abbreviation
1	First-line oral anti-TB drugs	Isoniazid	H
		Rifampicin	R
		Ethambutol	E
		Pyrazinamide	Z
		Rifabutin	Rfb
		Rifapentine	Rpt
2	Injectable anti-TB drugs (the injectables)	Streptomycin	S
		Kanamycin	Km
		Amikacin	Am
		Capreomycin	Cm
3	Fluoroquinolones (FQs)	Levofloxacin	Lfx
		Moxifloxacin	Mfx
		Gatifloxacin	Gfx
		Ofloxacin	Ofx
4	Oral bacteriostatic second-line anti-TB drugs	Ethionamide	Eto
		Prothionamide	Pto
		Cycloserine	Cs
		Terizidone	Trd
		p-aminosalicylic acid	PAS
		p-aminosalicylate sodium	PAS-NA

5	Anti-TB drugs with limited data on efficacy and/ or long term safety in the treatment of drug-resistant TB (This group includes new anti-TB agents)	Bedaquiline	Bdq
		Delamanid	Dlm
		Linezolid	Lzd
		Clofazimine	Cfz
		Amoxicillin/Clavulanate	Amx/Clv
		Imipenem/Cilastatin	Ipm/Cln
		Meropenem	Mpm
		High-dose isoniazid	High dose H
		Thioacetazone	T
		Clarithromycin	Clr

In 2016, WHO changed these groups in Updated guidelines on MDR-TB.

Current Medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant TB (Table 6 in the Updated 2016 WHO Guidelines on MDR-TB) [1]

A. Fluoroquinolones	Levofloxacin Moxifloxacin Gatifloxacin		Lfx Mfx Gfx
B. Second-line injectable agents	Amikacin Capreomycin Kanamycin (Streptomycin)		Am Cm Km (S)
C. Other core second-line agents	Ethionamide / Prothionamide Cycloserine / Terizidone Linezolid Clofazimine		Eto / Pto Cs / Trd Lzd Cfz
D. Add-on agents (not part of the core MDR-TB regimen)	D1	Pyrazinamide Ethambutol High-dose isoniazid	Z E H
	D2	Bedaquiline Delamanid	Bdq Dlm
	D3	p-aminosalicylic acid Imipenem-cilastatin Meropenem Amoxicillin-clavulanate (Thioacetazone)	PAS Ipm Mpm Amx-Clv (T)

1 http://www.who.int/tb/areas-of-work/drug-resistant-tb/MDRTBguidelines2016.pdf?ua=1

The second line injectable agents were and still are an important component of a DR-TB regime.

Of the carbapenems imipenem (in combination with cilastatin[2]) and meropenem were classified as group 5. In the new guidelines they are considered as one of the add-on agents.

Ertapenem is not included yet in the new WHO guidelines.

2 Cilastatin is a chemical compound which inhibits the human enzyme dehydropeptidase. This enzyme is situated in the kidney and is responsible for degrading the antibiotic imipenem. Cilastatin is considered a beta-lactamase.

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