2b. Emerging resistance and cross-resistance

FREQUENCY OF SPONTANEOUS RESISTANCE MUTATIONS

In vitro, for the standard *M. tuberculosis* strain H37Rv, the frequency of spontaneously-occurring resistance is on the order of 1 in 10^5 for delamanid (European Medicines Agency, 2013) and pretomanid (Haver *et al.*, 2015), suggesting a low genetic barrier to emergence of resistance during treatment unless these drugs are paired with other drugs with substantial activity. By way of comparison, the frequency of spontaneous resistance to isoniazid and rifampin are approximately 1 in 10^6 and 1 in 10^8, respectively.

CLINICAL RESISTANCE

Clinical experience with this drug class is limited, but resistant clinical isolates have been identified.

Delamanid and pretomanid resistance has been observed among clinical *M. tuberculosis* isolates collected in absence of nitroimidazole selective pressure. For example, one analysis of 21 *M. tuberculosis* clinical isolates that were susceptible to all standard first-line tuberculosis drugs found one isolate resistant to delamanid and pretomanid (Doi and Disratthakit, 2006). Among tuberculosis isolates collected from MDR-TB patients at the time of enrollment in a phase II trial of delamanid (Study 204), 2 of 316 evaluated isolates were resistant to delamanid (Stinson *et al.*, 2016).

Resistance also may arise during treatment. During 6 months of treatment with delamanid in combination with other second-line drugs in Study 204, isolates from 4 of 213 subjects developed resistance to delamanid. For all of these patients in whom resistance developed, delamanid either was the only active drug or was paired only with a bacteriostatic active drug among those drugs for which susceptibility testing was performed (European Medicines Agency, 2013). Similarly, since delamanid was approved for clinical use, emergence of resistance has been observed when it is added to a failing regimen (Bloemberg *et al.*, 2015).

RESISTANCE GENES AND MECHANISMS

Both delamanid and pretomanid are prodrugs, and most resistant mutants have loss-of-function mutations in one of several genes related to their activation into active drugs (Haver *et al.*, 2015). Specifically, mutations in the gene encoding the cofactor-F420–dependent nitroreductase Ddn that mediates this reductive activation have been shown to confer resistance to both pretomanid and delamanid (Manjunatha *et al.*, 2006a; Matsumoto *et al.*, 2006). Mutations that affect either the NADP-dependent glucose-6-phosphate dehydrogenase Fgd (required for redox cycling of F420) (Stover *et al.*, 2000) or any of four enzymes (FbiA, FbiB, FbiC, and CofC) involved in the biosynthesis of F420 (Choi *et al.*, 2002) can also confer resistance to pretomanid and likely delamanid; the resulting mutants have high-level resistance, e.g. at least 10 × increase in MIC (Haver *et al.*, 2015; European Medicines Agency, 2013). These resistance-associated genes are nonessential to growth under aerobic conditions (Haver *et al.*, 2015), and this along with the large target size for mutation may allow a low barrier to resistance with little associated fitness cost.

3. MECHANISM OF DRUG ACTION

The bicyclic nitroimidazoles are prodrugs that once activated, appear to have two mechanisms of action. Together, these mechanisms confer activity against both actively-replicating and hypoxic non-replicating *M. tuberculosis*.

ACTIVATION

Delamanid and pretomanid prodrugs are selectively activated by susceptible mycobacteria but not by their human or mammalian hosts (Mukherjee and Boshoff, 2011). Nitroreductive activation of both drugs is catalyzed by the nitroreductase Ddn (Rv3547) in a reaction that requires the deazaflavin cofactor F420 (Manjunatha *et al.*, 2006a). Mycobacteria that lack Ddn (e.g. *M. leprae*) (Manjunatha *et al.*, 2006b) or harbor inactivating mutations in either Ddn or any of several enzymes involved in synthesizing or recycling F420 are resistant to drug action.

ANAEROBIC MECHANISM

A mechanism of action involving nitric oxide-mediated respiratory poisoning allows pretomanid (Manjunatha *et al.*, 2009; Singh *et al.*, 2008)—and likely also delamanid (Singh *et al.*, 2008)—to have bactericidal activity against non-replicating mycobacteria under anaerobic conditions. The reductive activation of pretomanid to the des-nitroimidazole form produces reactive nitrogen species including nitric oxide (Singh *et al.*, 2008). This nitric oxide is thought to mediate anaerobic bacillary killing by interfering with ATP homeostasis and membrane potential, possibly via inhibition of cytochrome bd oxidase (Manjunatha *et al.*, 2009).

AEROBIC MECHANISM

Besides the nitric oxide generation, which is toxic to hypoxic non-replicating mycobacteria (but appears to have little effect on mycobacterial grown under aerobic conditions), both delamanid and pretomanid also have aerobic activity attributed to inhibition of cell wall synthesis. Both drugs inhibit the biosynthesis of mycolic acids, a key component of the mycobacterial cell wall, by inhibiting the oxidation of hydroxymycolic acids to produce ketomycolic acids (Matsumoto *et al.*, 2006; Stover *et al.*, 2000).

This aerobic mechanism, like the anaerobic mechanism, is closely tied to these drugs' F420-mediated activation. The hydroxymycolic acid dehydrogenase Rv0132c that oxidizes hydroxymycolic acids to ketomycolic acids uses the same F420 cofactor that is involved in pretomanid and delamanid activation. Pretomanid has been shown to out-compete Rv0132c for the reduced F420 cofactor, blocking Rv0132c-mediated ketomycolate synthesis and causing accumulation of hydroxymycolic acids (Purwatini and Mukhopadhyay, 2013).