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Delamanid and Bedaquiline Resistance in Mycobacterium tuberculosis Ancestral Beijing Genotype Causing Extensively Drug-Resistant Tuberculosis in a Tibetan Refugee

To the Editor:

International efforts in fighting tuberculosis (TB) have achieved a drop in global TB mortality and prevalence rates by 45% and 41%, respectively. Now, the yearly emergence of 450,000 cases of multidrug-resistant (MDR)-TB, defined by resistance to at least rifampicin and isoniazid, is endangering TB control, particularly in Asia and Eastern Europe (1). The approval of bedaquiline (Siriruro; Janssen-Cilag International NV, Beere, Belgium) by the U.S. Food and Drug Administration (2012) and the European Medicines Agency (2014), and of delamanid (Deltaby; Otsuka Novel Products GmbH, Munich, Germany) by the European Medicines Agency (2014), as new anti-TB drugs raised hope for getting the MDR-TB epidemic under control (2). These drugs have been proven to accelerate culture reversion and to improve clinical outcome in MDR-TB (3, 4).

By the end of 2014, the first TB case infected by an isolate with bedaquiline resistance was reported (5). This patient, a 38-year-old Tibetan refugee, was diagnosed in January 2011 with MDR-TB. He received a combination treatment with cycloserine, capreomycin, and para-aminosalicylic acid (PAS) and ethambutol plus bedaquiline under compassionate use for 6 months. After 24 months, treatment was terminated. Five months later, the patient experienced a relapse. The new isolate exhibited resistance to clofazimine with a corresponding mutation in the Rv0678 regulator gene (2 T>C leading to M1A), which had been previously demonstrated to result in cross-resistance to bedaquiline (6).

On the basis of these findings, a new treatment regimen was started in August 2013, with PAS, capreomycin, ethambutol, cycloserine, clofazimine (until October 2014), co-trimoxazole, and inhaled amikacin, but sputum smears remained positive for acid-fast bacilli. In March 2014, therapy was changed to a combination of PAS, ethambutol, cycloserine, co-trimoxazole, and levofloxacin plus delamanid, supplied under compassionate use as last resort. Acid-fast bacilli became undetectable by microscopy on three consecutive sputum smears performed in the Swiss national reference laboratory in Zürich, Switzerland, in June 2014, but 1 month later, sputum smears reverted back to positive. The decision was made to perform a surgical lobectomy. A Mycobacterium tuberculosis isolate was recovered from the resected tissue in August 2014 and sent to the World Health Organization supranational reference laboratory in Munich-Gauting, Germany, for susceptibility testing of delamanid.

There, 10^4 bacteria of the patient’s isolate (GB4492-175) inoculated in duplicate on Middlebrook 7H11 agar containing delamanid at the critical concentration of 0.2 mg/L grew on average 10^3 cfu after 4 weeks of incubation at 37°C, whereas a mean of 98 cfu were counted on the control plate with 1% of the bacterial inoculum (Table 1). Another isolate (CH-2014186009) from the same patient recovered by the Swiss national reference laboratory in February 2014 (spumum collection in January 2014), that is, right before the start of the delamanid-containing regimen; the delamanid-susceptible M. tuberculosis reference strain H37Rv; and a delamanid-resistant control strain obtained by selective plating (kindly provided by Otsuka Novel Products GmbH) yielded 0, 0, and >10^5 cfu, respectively, on drug-containing medium. The minimal inhibitory concentrations (MICs) of the two clinical isolates were determined in mycobacteria growth indicator tubes (Becton Dickinson, Heidelberg, Germany), following the protocol of Keller and colleagues (7) with slight modifications, including the use of pure substance powder (kindly provided by Otsuka Novel Products GmbH) dissolved in dimethylsulfoxide. MIC was confirmed in the resazurin microtiter assay following a modified protocol of

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**References**


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Prothionamide Resistant

Ethambutol Resistant

Isoniazid Resistant

Rifampicin Resistant

KatG S315T

Table 1. Results of Delamanid Susceptibility Testing, Minimal Inhibitory Concentrations, and Resistance Genotype of \( fbiA \)

<table>
<thead>
<tr>
<th>Isolate ( )</th>
<th>Time of Recovery</th>
<th>Delamanid</th>
<th>( 1% ) Control</th>
<th>( 0.2 \text{ mg/L DLM} )</th>
<th>MIC*</th>
<th>( fbiA ) Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH-2014186009</td>
<td>Feb 2014 (before DLM therapy)</td>
<td>Sensitive</td>
<td>143 cfu</td>
<td>0 cfu</td>
<td>( \leq 0.016 )</td>
<td>( fbiA ) R175H</td>
</tr>
<tr>
<td>GB4492-175</td>
<td>Aug 2014 (after DLM treatment failure)</td>
<td>Resistant</td>
<td>98 cfu</td>
<td>10(^6) cfu</td>
<td>( \geq 2.0 )</td>
<td>( fbiA ) D49Y R175H</td>
</tr>
</tbody>
</table>

Definition of abbreviations: cfu = colony-forming units in the proportional method; DLM = delamanid; MIC = minimal inhibitory concentration. *Determined in mycobacteria growth indicator tubes, confirmed by resazurin microtiter assay (for details, see text). The isolate recovered before prescription of the delamanid-containing regimen was susceptible with very low MIC values, whereas the one recovered after treatment failure of the respective regimen was resistant with at least 125\( \times \) higher MIC values.

Martin and colleagues (8). Eight delamanid test concentrations were applied in both systems (i.e., 0.016, 0.032, 0.063, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/L). The MICs of isolates CH-2014186009 and GB4492-175 were \( \leq 0.016 \) and \( \geq 2.0 \) mg/L, respectively, in both mycobacteria growth indicator tubes and resazurin microtiter assay, confirming an at least 125-times higher MIC of the resistant isolate compared with the susceptible one, which was well below the epidemiological cutoff values and in the range of MICs reported elsewhere for susceptible \( M. tuberculosi \) isolates (0.006–0.024 mg/L) (7, 9, 10).

The genomes of isolates CH-2014186009 and GB4492-175 were sequenced at the Research Center Borstel, Germany, using next-generation sequencing, as previously described, employing the Nextera XT library preparation kit and the NextSEQ 500 instrument (both from Illumina, Eindhoven, the Netherlands), reference mapping to the \( H37Rv \) genome (GenBank ID: NC_000962.3), and variant detection using in-house perl scripts (11). Whole-genome sequence data were submitted as fastq files to the European Nucleotide Archive at the European Bioinformatics Institute (Cambridge, UK) sequence read archive (accession number ERP011090). For a detailed phylogenetic classification of the Beijing genotype isolates, identified single nucleotide polymorphisms were cross-referenced to a representative global dataset of \( M. tuberculosi \) Beijing strains. The maximum likelihood analysis classified GB4492-175 as part of the “Asian ancestral 3” Beijing subgroup, which we recently defined as not highly associated with drug resistance (11). Further comparative analysis to the most closely related, fully susceptible Beijing strain within this cluster allowed us to differentiate 1,782 genetic background polymorphisms from 384 variants specific for the patient’s isolates. Among those, we found a series of mutations known to cause resistance to various drugs, including the previously described mutation \( Rv0678 \) \( T>C \) conferring clofazimine/bedaquiline cross-resistance (Table 2) (5, 6).

Direct genome comparison of the two isolates from our patient revealed that phenotypic delamanid resistance arose together with 11 new genetic polymorphisms in isolate GB4492-175, which were absent in the delamanid-susceptible isolate recovered before initiation of delamanid treatment. The most likely variant conferring delamanid resistance was mutation \( \text{FbiA D49Y} \) (Table 1); the other variants were supposedly coselected after applying the

Table 2. Drug Resistances, Minimal Inhibitory Concentrations, and Resistance Genotypes for Isolate GB4492-175 Recovered in August 2014 after Treatment Failure of the Delamanid-Containing Regimen

<table>
<thead>
<tr>
<th>Antituberculosis Drug</th>
<th>DST Phenotype</th>
<th>MIC (mg/L)</th>
<th>Resistance-associated Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>Resistant</td>
<td>—</td>
<td>KatG S315T</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Resistant</td>
<td>( \geq 4.0^* )</td>
<td>RpoB S450L</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>Resistant</td>
<td>( \geq 4.0^* )</td>
<td>EmbB D328Y</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Resistant</td>
<td>( \geq 1.0^* )</td>
<td>S12 RpsL K88R</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>—</td>
<td>—</td>
<td>PncA E107Stop(^1)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>Resistant</td>
<td>( \geq 2.0^* )</td>
<td>GyrA D94Y</td>
</tr>
<tr>
<td>Amikacin</td>
<td>Susceptible</td>
<td>—</td>
<td>TyA A17E(^7)</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>Resistant</td>
<td>—</td>
<td>EthA 1047fs(^1)</td>
</tr>
<tr>
<td>Prothionamide</td>
<td>Resistant</td>
<td>0.5(^6)</td>
<td>( Rv0678 ) M1A</td>
</tr>
<tr>
<td>Bedaquiline</td>
<td>NA</td>
<td>2.814 g/t(^5)</td>
<td></td>
</tr>
<tr>
<td>Clofazimine</td>
<td>Resistant</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Linezolid</td>
<td>Resistant</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Definition of abbreviations: DST = drug susceptibility testing; MIC = minimal inhibitory concentration; NA = not applicable because of missing thresholds. Dash indicates not determined.

\(^*\) Determined by resazurin microtiter assay after the protocol of Martin and colleagues, with minor modifications (8).

\(^1\) Several amino acid exchanges of the enzyme known to be associated with resistance phenotypes, but this particular variant has so far not been further characterized or reported.

\(^6\) Determined with pure substance in mycobacteria growth indicator tubes after the protocol of Keller and colleagues, with minor modifications (7).

\(^5\) Detected as heteroresistance.
delamanid-containing regimen (see Table E1 in the online supplement). Of note, both isolates also harbored variant FbiA R175H, and we cannot completely exclude possible synergies between both FbiA variants.

FbiA and the related gene products FbiB and FbiC are involved in the biosynthetic pathway of the flavin cofactor F420. FbiC catalyzes the synthesis of F0, which FbiA and FbiB together modify further to F420 (12, 13). Delamanid constitutes an inactive prodrug and is supposed to be metabolized by the F420-dependent nitroreductase Ddn (Rv3547) into its active form (10). F420 is recycled into the reduced form by a glucose-6-phosphate dehydrogenase (Fgd1).

Any mutation in one of these genes may lead to resistance to delamanid (10). Mutations of ddn and/or fgd1 have already been reported to be associated with resistances to delamanid and PA-824, a closely related nitroimidazole-oxazine drug, respectively, but one that only rarely occurs (9, 14). Likewise, mycobacterial strains with mutated fbiA or fbiB were shown to lack coenzyme F420 and to be resistant to PA-824 (15). FbiA (homolog synonym cofD) is highly conserved among archeal and bacterial organisms that are known to produce F420. Although D49 is not involved in substrate binding, the amino acid position is highly conserved (16). The data support the hypothesis that the identified D49Y mutation of fbiA causes delamanid resistance. In vitro frequencies of spontaneous delamanid resistance are comparable to those of isoniazid, that is, ranging from 1/106 to 1/105 M. tuberculosis organisms (10).

Our case illustrates how rapidly bacterial resistance may counteract the positive impulses given by the advent of new anti-TB drugs, as long as these are not imbedded in efficient concepts of drug, patient, and infection control measures. The surprisingly rapid development of resistance observed even toward the newest compounds available underlines the need of systematic surveillance of drug resistance toward new anti-TB drugs, allowing for immediate actions on the emergence of resistance to prevent rapid neutralization of the potentials that new drugs bring along to help combat the MDR-TB epidemic.

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References


An Important Step Forward, but Still a Way to Go

To the Editor:

Since the publication of the American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association evidence-based guidelines in 2011 (1), significant advances have been made in idiopathic pulmonary fibrosis (IPF), leading to a recently published update (2). Although this represents a major step forward, some important questions remain unanswered.

In our view, the main weakness of the guideline document relates to the same level of recommendation (e.g., conditional recommendation for use) given to antifibrotic therapy (regardless of the presence or absence of symptomatic reflux) as to antibiotic agents (e.g., pirfenidone and nintedanib) despite different levels and quality of evidence. Only observational/retrospective studies and post hoc analysis of patients randomly assigned to placebo in clinical trials of pharmaceutical interventions are available for antifibrotic therapy compared with large phase 3 randomized controlled trials for antibiotic drugs. Despite the editor’s note added to the guideline, we are seriously concerned that the recommendation on antifibrotic therapy may be perceived as equivalent to other conditional recommendations that are based on much stronger evidence. This paradox may be partly a result of the methodology used. According to international standards for guideline development, and to balance the need to minimize bias with the need for expertise to inform decisions, only panel members without conflicts of interest were allowed to discuss, formulate, grade, and vote on the recommendations. Conversely, in the previous version of the guidelines, both conflicted and nonconflicted members participated without restriction in all the key steps of the process. We speculate that many of the conflicted experts would possibly have not given a conditional recommendation for use of antifibrotic therapy because of insufficient evidence.

Furthermore, some crucial questions remain unanswered because of a lack of evidence in several areas in which research is utterly needed. There is no doubt that pirfenidone and nintedanib slow disease progression in patients with FVC more than 50% predicted and diffusing capacity of the lung for carbon monoxide more than 30% predicted, but what the effect is in patients with IPF with more advanced disease is unknown. Pirfenidone and nintedanib did not show an effect on quality of life, probably because of the suboptimal tools available to measure this outcome in IPF. Combination therapy, successfully used in other complex disorders such as pulmonary arterial hypertension, would target multiple profibrotic pathways and is potentially the next step in IPF therapy (3); however, safety, efficacy, and cost issues need to be carefully evaluated as a priority. Furthermore, despite the conditional recommendation against the use of some drugs for treatment of pulmonary arterial hypertension, further research is needed to determine whether this is a useful target for treatment in IPF. Progress is also needed to treat patients with progressive lung fibrosis other than IPF, such as chronic hypersensitivity pneumonitis, connective tissue disease–associated interstitial lung disease, and fibrotic nonspecific interstitial pneumonia, by setting up specific trials to assess whether the efficacy of the novel antifibrotic compounds also extends to these conditions.

Several challenges also remain in the diagnostic process of IPF (not addressed in the 2015 update). The 2011 guidelines provide a useful framework for diagnosis, which, however, is mainly applicable to clinical trials rather than to clinical practice. In many patients, high-resolution computed tomography does not show a definite usual interstitial pneumonia pattern, and according to the guidelines, confirmatory surgical lung biopsy should be considered (1). Yet this is often not possible because of severe functional impairment, comorbidities, or patient preferences. The role of less invasive biopsy procedures such as transbronchial cryobiopsy (4) should also be further evaluated. Alternative/additional criteria are needed to formulate a secure diagnosis in those patients who are likely to have IPF (those with possible usual interstitial pneumonia pattern on computed tomography), but in whom no histologic data are available and whose disease currently remains unclassified (5).