Molecular Aspects

Transcriptional profiling of a laboratory and clinical Mycobacterium tuberculosis strain suggests respiratory poisoning upon exposure to delamanid

An Van den Bossche, Hugo Varet, Amandine Sury, Odile Sismeiro, Rachel Legendre, Jean-Yves Coppee, Vanessa Mathys, Pieter-Jan Ceyssens

1. Introduction

The most recent WHO report ranks tuberculosis (TB) above HIV as a leading cause of mortality by infectious diseases, with 1.6 million attributed deaths in 2017. A major concern is the worldwide spread of multidrug resistant Mycobacterium tuberculosis (MDR-TB) strains, lowering treatment success rates from 82% to 55% for infected patients [1]. In the pursuit to reduce TB incidence and counter the spread of multidrug resistant TB, the discovery and characterization of new drugs is essential. In this study, the transcriptional response of two Mycobacterium tuberculosis strains to a pressure of the recently approved delamanid is investigated. Total RNA sequencing revealed that the response to this bicyclic nitroimidazole shows many similarities with pretomanid, an anti-tuberculous drug from the same class. Although delamanid is found to inhibit cell wall synthesis, the expression of genes involved in this process were only mildly affected. In contrast, a clear parallel was found with components that affect aerobic respiration. This demonstrates that, besides the inhibition of cell wall synthesis, respiratory poisoning plays a fundamental role in the bactericidal effect of delamanid. Remarkably, the most highly induced genes comprise poorly characterized genes for which functional characterization might hint to the target molecule(s) of delamanid and its exact mode(s) of action.

Transcriptional profiling of MTB in the presence of a drug can reveal more insights into its mode of action. Previous microarray studies on cells exposed to pretomanid (PA-824), another new anti-tuberculous bicyclic nitroimidazole that is activated by Ddn, identified a dual effect [7,8]. At low drug concentration, a change of the transcriptional levels of genes involved in cell wall synthesis was detected, corresponding to the hypothesis that inhibition of cell wall synthesis is thought to be the main mechanism of killing in aerobic conditions [7,8]. In parallel, there was a clear change in gene expression for genes that respond to respiratory poisoning. This observation is correlated to the release of toxic reactive nitrogen species (like nitrogen oxide (NO)) during the activation of the drug, which are probably the main effectors of anaerobic killing by interfering with the electron flow and ATP homeostasis.
[7,8]. In line with this hypothesis, it was reported that the reduced desnitro-from of pretomanid on itself has no anti-TB activity [9]. Due to re-oxidation by oxygen in aerobic conditions, this NO-release is thought to be insufficient for bactericidal activity in actively replicating cells.

Here, we performed transcriptional analyses on two MTB strains in the presence of delamanid using total RNA sequencing (RNA-seq). Changes in expression levels were found to be comparable to the responses induced by pretomanid. Especially the response to respiratory poisoning was detected, while cell wall synthesis genes were less affected.

2. Methods

2.1. Cultures and sample preparation

Three replicates of the pan-susceptible strains M. tuberculosis H37Rv and ITM-04-1195 (lineage 2 Beijing, BCCM™ Collection, TB-TDR-0078, country of origin South-Korea) were cultured in 140 ml Middlebrook 7H9 broth supplemented with 10% OADC (Oleic acid-Albumin-Dextrose-Catalase) at 37 °C until early-exponential phase (optical density 0.1). Each culture was split and supplemented with dimethyl sulfoxide (DMSO) or 3 μg/ml delamanid (Otsuka). Samples of 20 ml were collected (15 min, 4000 g) at the start and 6 h and 24 h after addition of the drug.

2.2. RNA isolation and RNA sequencing

Cell pellets were re-suspended in TRIzol reagent and subjected to three cycles of 1 min bead-beating (0.5 mm silica/zirconia beads) followed by RNA extraction using the Direct-zol RNA Kit (Zymo research) according to the manufactures' protocol. DNA was removed by TURBO DNase (Ambion) until no genomic DNA was detected by PCR. The libraries were prepared using the TruSeq Stranded mRNA Sample preparation kit following the manufacturer’s instructions (Illumina). Cell pellets were re-suspended in TRIzol reagent and subjected to three cycles of 1 min bead-beating (0.5 mm silica/zirconia beads) followed by RNA extraction using the Direct-zol RNA Kit (Zymo research) according to the manufactures' protocol. DNA was removed by TURBO DNase (Ambion) until no genomic DNA was detected by PCR. The libraries were prepared using the TruSeq Stranded mRNA Sample preparation kit following the manufacturer’s instructions (Illumina). Libraries were checked for quality on Bioanalyzer DNA chips (Agilent). More precise and accurate quantification was performed with the fluorescent-based quantitation Qubit dsDNA HS Assay Kit (ThermoFisher). 65 bp single read sequences were generated on the HiSeq 2500 sequencer according to manufacturer’s instructions (Illumina). The multiplexing level was 10 samples per lane.

2.3. Data processing

Bioinformatic analyses were performed using the RNA-seq pipeline from Sequana [10]. Reads were cleaned for low-quality and adapter sequences using Cutadapt version 1.11. Only sequences of at least 25 nt in length were considered for further analyses. Bowtie version 0.12.7 (parameters –chunkmbs 400 -m 1) was used for the alignment on the reference genome (M. tuberculosis H37Rv, from NCBI). Genes were counted using featureCounts version 1.4.6-p3 from the Subreads package (parameters: t gene -g ID -s 1). Count data were analyzed using R version 3.4.1 [11] and the Bioconductor package DESeq2 version 1.18.1 [12]. The normalization and dispersion estimation were performed with DESeq2 using the default parameters and statistical tests for differential expression were performed applying the independent filtering algorithm. Two independent analyses have been performed to study the H37Rv and ITM-04-1195 strains separately. For each of them, a generalized linear model was set in order to test for the differential expression between the time points and/or treatments. For each pairwise comparison, raw p-values were adjusted according to the Benjamini and Hochberg (BH) procedure [13] and genes with an adjusted p-value lower than 0.001 were considered differentially expressed.

RNA-Seq data have been deposited at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) under the accession number GEO ID: GSE123294.

3. Results and discussion

3.1. Genome-wide impact on gene expression levels

To investigate the transcriptional impact of MTB to exposure to delamanid, triplicates of a laboratory (H37Rv) and a DLM susceptible clinical (ITM-04-1195) MTB strain were spiked during 6 h and 24 h with the drug or its solvent DMSO. The viability of the cultures was followed post-sampling by measuring the optical density over time (Fig. S1). Illumina sequencing was performed on total RNA extractions, leading to the detection of transcripts for 3927 and 3918 genes for H37Rv and ITM-04-1195, respectively. Variance Stabilizing Transformation (VST) and subsequent Principal Component Analysis (PCA) showed a good correlation between the biological replicates for both strains (Fig. S2).

Global differential analysis of the transcriptional data showed that delamanid significantly (log₂FoldChange > [1], P-adjusted < 0.001) influences the expression level of a large number of genes (Tables 1 and S1). In total, 288 genes were significantly induced in both strains at both time points and 317 genes were significantly repressed (Table 1). Overall, strain ITM-04-1195 responded slightly more pronounced than H37Rv. Moreover, the responses seem to be time-dependent with more genes affected after 24 h than after 6 h of drug pressure.

Genes that were induced by delamanid are mainly part of the functional categories virulence, detoxification, adaptation (42 and 38 genes, respectively after 6 h and 24 h of incubation), cell wall and cell processes (57 and 79 genes), intermediary metabolism and respiration (72 and 94 genes) and conserved hypotheticals (98 and 151 genes) (Table 1). Additionally, about a quarter of the regulatory proteins were induced (20.3% and 25.4%, after 6 h and 24 h). This last observation might explain the large number of genes that has a modified expression pattern. The repressed genes are primarily classified as genes involved in information pathways (78 and 86 genes), cell wall and cell processes (73 and 99 genes), intermediary metabolism and respiration (67 and 101 genes) and conserved hypotheticals (71 and 91 genes).

3.2. Delamanid induces a response similar to pretomanid

To gain further insights in the transcriptional response of MTB to this drug class, we compared our data with the outcome of microarray recordings of cells treated with pretomanid, potassium cyanide or isoniazid, which represent respectively another anti-tuberculous bicyclic nitroimidazole, an inhibitor of aerobic respiration and an inhibitor of cell wall synthesis [7,8]. For these data, Boshoff and co-workers sampled replicates of H37Rv cultures after 6 h of incubation with multiple concentrations of the respective compounds, under aerobic conditions.

A heat map of the transcriptional profiles of genes with a significant response to delamanid (log₂FoldChange > [1], P-adjusted < 0.001) shows that, globally, delamanid modifies the transcriptional expression levels in a way similar to pretomanid (Fig. 1). For example, after 6 h of incubation, an increasing number of 356 (37.5%), 502 (66.9%) and 582 (77.6%) of the significant up- and down-regulated genes were found to have equally higher and lower expression levels in the presence of respectively 0.2 μg/ml, 0.4 μg/ml and 2.0 μg/ml pretomanid (log₂FoldChange > [0.5] in the microarray dataset [7]) (Table 2). This effect of increasing similarity at higher concentrations correlates with the use of high concentrations of delamanid during our experimental setup. In addition, the heat map of transcriptional profiles demonstrates similarity with the responses induced by potassium cyanide (KCN). Using 5 μg/ml and 20 μg/ml of this inhibitor of aerobic respiration yielded respectively 420 (56.0%) and 441 (58.8%) genes (log₂FoldChange > [0.5]), which were up- and down-regulated in a same manner as for delamanid (Table 2) [7,8]. On the other hand, global expression changes induced by isoniazid look different from
Table 1
Functional categories of the significantly induced and repressed genes.

<table>
<thead>
<tr>
<th>Functional Category</th>
<th>6 h H37Rv</th>
<th>ITM-04-1195</th>
<th>both</th>
<th>24 h H37Rv</th>
<th>ITM-04-1195</th>
<th>both</th>
<th>6 h &amp; 24 h H37Rv</th>
<th>ITM-04-1195</th>
<th>both</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Up-regulation (P &lt; 0.001)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virulence, detoxification, adaptation</td>
<td>50</td>
<td>80</td>
<td>42</td>
<td>45</td>
<td>62</td>
<td>38</td>
<td>28</td>
<td>52</td>
<td>24</td>
</tr>
<tr>
<td>Information pathways</td>
<td>24</td>
<td>25</td>
<td>16</td>
<td>31</td>
<td>32</td>
<td>23</td>
<td>21</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Cell wall and cell processes</td>
<td>83</td>
<td>87</td>
<td>57</td>
<td>101</td>
<td>99</td>
<td>79</td>
<td>61</td>
<td>70</td>
<td>45</td>
</tr>
<tr>
<td>Insertion seqs and phages</td>
<td>22</td>
<td>17</td>
<td>15</td>
<td>24</td>
<td>20</td>
<td>15</td>
<td>18</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>PE/PPE</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>15</td>
<td>19</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Intermediary metabolism and respiration</td>
<td>101</td>
<td>136</td>
<td>72</td>
<td>119</td>
<td>167</td>
<td>94</td>
<td>77</td>
<td>104</td>
<td>55</td>
</tr>
<tr>
<td>Unknown</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Regulatory proteins</td>
<td>42</td>
<td>64</td>
<td>40</td>
<td>58</td>
<td>66</td>
<td>50</td>
<td>35</td>
<td>55</td>
<td>33</td>
</tr>
<tr>
<td>Conserved hypotheticals</td>
<td>122</td>
<td>169</td>
<td>98</td>
<td>184</td>
<td>218</td>
<td>151</td>
<td>103</td>
<td>152</td>
<td>84</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>31</td>
<td>38</td>
<td>22</td>
<td>22</td>
<td>36</td>
<td>19</td>
<td>18</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>491</td>
<td>630</td>
<td>372</td>
<td>607</td>
<td>723</td>
<td>482</td>
<td>376</td>
<td>510</td>
<td>288</td>
</tr>
<tr>
<td><strong>Down-regulation (P &lt; 0.001)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virulence, detoxification, adaptation</td>
<td>25</td>
<td>30</td>
<td>21</td>
<td>38</td>
<td>34</td>
<td>33</td>
<td>22</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>Information pathways</td>
<td>82</td>
<td>89</td>
<td>78</td>
<td>92</td>
<td>96</td>
<td>86</td>
<td>76</td>
<td>83</td>
<td>73</td>
</tr>
<tr>
<td>Cell wall and cell processes</td>
<td>101</td>
<td>105</td>
<td>73</td>
<td>132</td>
<td>135</td>
<td>99</td>
<td>88</td>
<td>90</td>
<td>62</td>
</tr>
<tr>
<td>Insertion seqs and phages</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>PE/PPE</td>
<td>39</td>
<td>36</td>
<td>25</td>
<td>36</td>
<td>24</td>
<td>20</td>
<td>27</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>Intermediary metabolism and respiration</td>
<td>95</td>
<td>103</td>
<td>67</td>
<td>150</td>
<td>127</td>
<td>101</td>
<td>73</td>
<td>84</td>
<td>54</td>
</tr>
<tr>
<td>Unknown</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Regulatory proteins</td>
<td>16</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>17</td>
<td>14</td>
<td>10</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Conserved hypotheticals</td>
<td>101</td>
<td>109</td>
<td>71</td>
<td>132</td>
<td>122</td>
<td>96</td>
<td>79</td>
<td>82</td>
<td>57</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>41</td>
<td>40</td>
<td>30</td>
<td>50</td>
<td>46</td>
<td>37</td>
<td>33</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>504</td>
<td>533</td>
<td>378</td>
<td>661</td>
<td>611</td>
<td>496</td>
<td>413</td>
<td>442</td>
<td>317</td>
</tr>
</tbody>
</table>

Number of genes of strain H37Rv and ITM-04-1195 with a significant up- or down-regulation (|log2FC| > 1, p-adj < 0.001) after 6 h and 24 h of incubation with delamanid. Genes are classified using Mycobrowser (https://mycobrowser.epfl.ch/).

Fig. 1. Heat map of significantly induced or repressed genes after incubation with delamanid compared with data of pretomanid, KCN and isoniazid. * Microarray data of pretomanid, KCN and isoniazid were published by Boshoff et al. [7].
these of delamanid, pretomanid and KCN (Fig. 1) [7,8]. A notable smaller portion of genes was equally induced or repressed by both this drug and delamanid, respectively 77 (10.3%) and 91 (12.1%) genes, using concentrations of 0.2 μg/ml and 0.4 μg/ml isoniazid. These findings suggest that cells that are grown in the presence of high concentrations of delamanid suffer more from respiratory poisoning than from inhibition of cell wall synthesis, although this last one is hypothesized to be one of the main modes of action of delamanid [4].

### 3.3. Delamanid impacts genes involved in aerobic respiration

Since delamanid is a bicyclic nitroimidazole which, like pretomanid, needs bio-activation by the nitroreductase Ddn, it is highly likely that reactive nitrogen species (e.g. NO) are produced during this process. One of the first effects of the release of NO is to block the type I dehydrogenase responsible for aerobic respiration, Ndh (Rv1854c). Stress induced by pretomanid, but also by KCN and NO, therefore induces a relatively fast up-regulation of transcription of this enzyme [7,14]. This was equally observed in our data of delamanid after 6 h of incubation and was maintained at 24 h. However, the reported up-regulation of the cytochrome bd oxidase operon (cydCDBA) and nitrate reductase narG-HHIJ that compensates for this respiratory poisoning [7,14] was not found for delamanid. On the other hand, a significant induction of two other cytochromes cyp135A1 (Rv0327c) and cyp 138 (Rv0136) could be noticed (Table S1).

Apart from these direct effects of respiratory poisoning, a large number of indirect expression patterns were observed (Fig. 2). Fe-S clusters are thought to act as regulatory proteins that sense iron deprivation but also reactive oxygen species and gases like O2 and NO [15]. In our dataset, we notice a significant up-regulation of Fe-S clusters are thought to act as regulatory proteins that sense iron deprivation but also reactive oxygen species and gases like O2 and NO. For example, we observe clear repression of elongation factor Tu (Rv0685), greE2 (Rv0440) and the ATP synthase (Rv1304-11), as well as the transcriptional machinery itself (rpoB-rpoC, Rv0067-Rv0668). A last clear observation is the down-regulation of almost all ribosomal proteins and many ESAT-6-like proteins (esxA-esxW) (Fig. 2). All these observations were shared with the response of MTB to pretomanid, but also to respiratory stress induced by KCN and/or NO [7,8,14]. Moreover, several less characterized genes and operons experienced comparable up and down regulation, for example Rv3011, Rv0874c-76c, Rv1195-96, Rv2825c-27c, Rv3188-89, Rv3360 and Rv3550-53 (Fig. 2).

### 3.4. Low impact on expression of cell wall biosynthesis genes

It has been found that delamanid works as an inhibitor for mycolic acid synthesis [4]. However, only a small number of genes involved in mycolic acid synthesis showed significant changes in expression upon DLM exposure (Fig. 2). Moreover, and in contrast to other cell wall inhibitors like isoniazid and ethionamide, these genes were mostly down-regulated. A prime example is operon Rv2243-Rv2247, for which the abundance increased 2-3 times in the presence of isoniazid [17,18], while DLM caused a 1-2 fold decrease. Only three genes characterized for their role in cell wall biosynthesis [19] were up-regulated in both tested strains: the mannosyltransferases Rv1635c and Rv2181 and fatty acid synthase fas (Rv2524c).

Beside the genes that are directly involved in cell wall biosynthesis, mycolic acid inhibitors are reported to influence the expression of multiple other genes with no direct relations to this process. In their findings, Manjunatha and co-workers highlighted a group of 30 genes that were co-regulated between pretomanid and fatty acid synthesis inhibitors, but not by KCN [8]. Fig. S3 shows that this co-regulation was mainly present at low concentrations of pretomanid, while this effect diminished with increasing concentrations of the drug. Our data demonstrate that for most of these genes, the response to delamanid is more related to the response to KCN and high concentration of pretomanid. This corresponds well to the fact that high concentrations of delamanid were used during this study. It is therefore reasonable to assume that at low concentrations, delamanid might influence cell wall synthesis.

For some genome regions, we nevertheless observed similar patterns of differential expression induced by isoniazid and delamanid. For example, operon iniBAC, which is known to be up-regulated by cell wall inhibitors [20], was also induced in the presence of delamanid. However, this increase was also seen in data of KCN and NO pressure [7,14]. More generally, of the 127 genes that were found to be significant affected by both delamanid and isoniazid (0.2 μg/ml or 0.4 μg/ml) after 6 h of incubation, 51.2% were influenced by KCN as well (using 5 μg/ml and 20 μg/ml KCN) [7]). This suggests that these responses are probably global stress related responses rather than specific responses to cell wall biosynthesis inhibition.

### 3.5. Specific delamanid induced expression patterns arise

A specific response of MTB cells that might be expected in the

---

**Table 2: Comparison of the transcriptional trends induced by delamanid vs. pretomanid, KCN and isoniazid.**

<table>
<thead>
<tr>
<th>Delamanid** AND</th>
<th>Pretomanid**</th>
<th>KCN**</th>
<th>Isoniazid**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 μg/ml</td>
<td>0.4 μg/ml</td>
<td>2.0 μg/ml</td>
<td>5.0 μg/ml</td>
</tr>
<tr>
<td>6h*</td>
<td>Up-regulation</td>
<td>372 (100%)</td>
<td>162 (43.5%)</td>
</tr>
<tr>
<td></td>
<td>Up-regulation</td>
<td>378 (100%)</td>
<td>194 (51.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>750 (100%)</td>
<td>356 (47.4%)</td>
<td>502 (66.9%)</td>
</tr>
<tr>
<td>24h*</td>
<td>Up-regulation</td>
<td>482 (100%)</td>
<td>166 (34.4%)</td>
</tr>
<tr>
<td></td>
<td>Down-regulation</td>
<td>496 (100%)</td>
<td>214 (43.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>978 (100%)</td>
<td>380 (38.8%)</td>
<td>559 (57.1%)</td>
</tr>
</tbody>
</table>

The number of significantly induced or repressed genes that are shared between the RNA-seq data for delamanid in this study and microarray data for pretomanid, KCN and isoniazid [7]. Both data sets represent differentially expressed genes after 6 h of incubation with the respective drug.

* For both strains (H37Rv, and ITM-04-1195), | log2FC | > 1, P < 0.001.
** Microarray data of Boshoff et al., 2004, for H37Rv, | log2FC | > 0.5.
presence of delamanid, is a repression of the enzyme responsible for bio-activation. Indeed, in all tested samples, the expression of $ddn$ was significantly lower than in the control samples. For $fbiC$, known to be involved in resistance, a small induction was observed. In contrast, no changes in expression level were detected in other enzymes that are found to be involved in resistance, including $fbiA$, $fbiB$, and $fgd1$ [6] (Table S1). These expression patterns were equally observed for pretomanid [7].

Apart from the LexA regulon, seven other operons were strongly up-regulated at both time points (Fig. 2). A remarkable strong induction (log2FC up to 9) was observed for operon Rv2487-90c, composed of two PE-PGRS proteins, two hypothetical proteins and a LuxR-like transcription regulator. The exact function of these proteins is not known yet, although luxR-like regulators can respond to a wide range of environmental signals [21]. This massive gene induction, absent in pretomanid-treated cultures [7], extended into the neighboring operon containing the hypothetical proteins Rv2491-92. Two other operons that were only affected by delamanid are Rv1129c-31c and Rv3160c-61c. The first one encodes the methylcitrate synthase PrpC, methylcitrate dehydratase PrpD and a transcriptional regulator. Both enzymes are part of the methylcitrate cycle needed for respiration on odd-chain fatty acids like propionate, which is mainly important for virulence and survival in macrophages [22]. The second operon comprises a dioxygenase and a TetR transcription regulator. This operon is also induced in lipid-rich environments [23]. In addition, Rv3161c was also found to be induced by anti-tuberculous benzene-containing compounds such as thiabendazole and triclorobenzoquinone, however, without influencing resistance levels to these compounds [24]. The other three highly induced operons were found to be equally up-regulated by pretomanid pressure and respiratory stress. The operon containing protein kinase G PknG and glutamine-binding lipoprotein GlnH (Rv0410c-12c) that regulates glutamine transport across the membrane, monoxygenase EthA and its regulator EthR (Rv 3854c-55) known for its role in the bio-activation of the drug ethionamide and the operon Rv3287c-90c consisting of two uncharacterized proteins, anti-sigma B factor RsbW and $\varepsilon$-lysine-$\varepsilon$-aminotransferase Lat. The latest operon being highly induced in nutrient starvation models [25].

Apart from the clear down-regulation of the transcription of the ribosomal proteins, the genes of the dosR regulon were largely repressed in the presence of delamanid. This regulon, that contains about 50 genes, is involved in dormancy and survival in the non-replicating state. Contradictory to the parallels observed, this regulon was induced in conditions of respiratory poisoning by hypoxia, NO or CO [26]. In the data of pretomanid only a few of these genes were mildly influenced (induction and repression), however, for most of these genes no significant changes were observed. These observations suggest that, although the general overlap with aerobic poisoning, cells are not induced by bicyclic nitroimidazoles to adapt to a non-replicating state.
4. Conclusions

For the first time, global transcriptional analyses were performed on MTB cells under drug pressure of the bicyclic nitrimidazolide delamanid. The obtained RNA sequencing data were compared with published work to detect similarities and differences in the response of M. tuberculosis towards other drugs and compounds. Although the technical parameters and limitations of both techniques are different, clear parallels could be detected. A key observation is that delamanid induces a response that shows many similarities with the response to the antituberculous drug from the same class pretomanid.

A confirmation on its proposed major mode of action, being the inhibition of mycacidic acid synthesis, could not directly be elucidated from these transcriptional data. Genes that are known to be involved in cell wall synthesis were only mildly affected under the applied experimental conditions, and only few parallels with other cell wall inhibiting drugs were found. In contrast, the data revealed that aerobic respiratio was affected in the treated cells. Many of the identified responses were equally observed in transcriptomic data of respiratory inhibitors. As for pretomanid, this demonstrates that respiratory poisoning has a crucial role in the bactericidal effect of delamanid. However, due to the re-oxidizing effect of oxygen in aerobic environments, this poisoning is probably mainly important for the bactericidal effect of delamanid in anaerobic conditions and might therefore contribute to the activity of delamanid on dormant, non-replicating cells.

The direct target of delamanid remains to be uncovered. For pretomanid, the effect on cell wall biosynthesis was more pronounced using low, suboptimal drug concentrations. Therefore, it can be suggested that in our dataset, the massive impact of high concentrations of delamanid in anaerobic conditions and might therefore contribute to the activity of delamanid on dormant, non-replicating cells. The direct target of delamanid remains to be uncovered. For pretomanid, the effect on cell wall biosynthesis was more pronounced using low, suboptimal drug concentrations. Therefore, it can be suggested that in our dataset, the massive impact of high concentrations of delamanid in anaerobic conditions and might therefore contribute to the activity of delamanid on dormant, non-replicating cells.

Our results point to at least a dual mode of action for delamanid, in which the biochemically observed effect on cell wall biosynthesis is accompanied by or replaced by respiratory poisoning depending on the environmental conditions. The direct target(s) of the drug are still unknown, but first clues to the fully uncovering of the pathways affected by delamanid might be found in the uncharacterized genes that were found to be highly induced by M. tuberculosis cells in the presence of delamanid.

Declarations of interests

None.

Acknowledgements

We thank Otsuka Novel Products GmbH (Munich, Germany) for providing us with pure delamanid powder and the Belgian Co-ordinated Collections of Micro-organisms as co-ordinator (BCCM) for the distribution of the ITM-04-1195 strain. The Transcriptome and Epigenome Platform is a member of the France Génomique consortium (ANR10-NBS-09-08). This research was supported by an International Network of Institute Pasteur: ACIP grant N°03–2016 from the Institute Pasteur in Paris.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2019.05.002.

References