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Global transcriptional analyses of Mycobacterium tuberculosis using old and new drugs

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Background: With 1.5 million deaths in 2014, the most recent report of the WHO ranks tuberculosis (TB) alongside HIV as a leading cause of death by infectious diseases. In combination, the spread of (multi-)drug resistant *Mycobacterium tuberculosis* is rising (480 000 new cases), impeding the treatment of infected patients.

Current TB drug resistance research is mainly focused on the genomic level, using WGS to search for resistance-causing mutations. However, transcriptional analyses can be a powerful tool as well. Analyses on susceptible strains provide information on the response to the stress a drug is causing, showing which mechanisms are activated to counter the effect of the drug. This might lead to new strategies for the development of new/complimentary drugs. On the other hand, this response gives an view on the working mechanism of the drug. Moreover, comparing the response of sensitive and resistant strains can yield additional information, like the activation of specific efflux pumps.

However, due to the high cost of RNAseq analyses, genome wide transcriptional analyses of drug influences on *M. tuberculosis* remain rather limited. Here we present a global study of the response of two pansensitive *M. tuberculosis* strains to eight TB-drugs. By using the approach called RNAtag-Seq, multiple samples were combined in one run, reducing time and cost.

Material/methods: For each drug (isoniazid, rifampicin, ethambutol, capreomycin, amikacin, linezolid, moxifloxacin and bedaquilin), twice the critical concentration was added to the cultures of two pansensitive *M. tuberculosis* strains. Samples were taken 0h, 2h, 6h and 24h after the administration of the drug. RNA was extracted combining bead-beating in TRIzol and the Direct-Zol purification kit. After quality control, the extracts were fragmented, barcoded and pooled, cDNA libraries were

constructed and sequenced using the HiSeq technology (Illumina®). Reads were mapped to the reference genome and normalized read counts were calculated per gene.

Results: For each drug a specific transcriptional response was mapped, revealing lists of up- and down-regulated genes. As an example and in accordance with previous studies, the highest induced genes in the presence of isoniazid belong to a cluster of genes that encodes components of the FAS-II (fatty acid synthase II) complex, which is targeted by isoniazid. On the other hand, a remarkable down-regulation of the NADH-dehydrogenase (ndh) cluster genes was noted. This can be assigned to the dependence of isoniazid target inhA on NADH. Lowering the ndh-activity is also seen in resistant strains.

Conclusions: By using RNAtag-seq, a global study of the transcriptional response of *M. tuberculosis* to several drugs could be made. These analyses can reveal the working mechanisms of existing and new drugs and provide new insights on the mechanism of resistance of strains.