High-throughput sequencing technologies for genome methylation analysis and possible epigenetic intervention strategies in the model bacterium *Helicobacter pylori*

Lubna Patel[1,3], Florent Ailloud[1,3], Fabian Neukirchinger[1,3], Mark Brönstrup [2,4], Ursula Bilitewski[2,4], Sebastian Suerbaum[1,3], Christine Josenhans[1,3]
[1] Max von Pettenkofer-Institut, Chair for Medical Bacteriology and Hygiene, Ludwig Maximilians Universität München, Pettenkoferstr. 9a, 80336 München, Germany
[2] Department for Chemical Biology, Helmholtz Center for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany; [3] DZIF site Munich, [4] DZIF site Hannover-Braunschweig

Background and Questions. We have recently defined, in a compound screening approach, a novel small molecule family, which can act as an antibacterial "pathoblocker" against bacterial motility, in the model bacterium *Helicobacter pylori*. Active compounds were applied in a preclinical therapeutic mouse model, and one pathoblocker was able to remove *H. pylori* from the mouse stomach in a one-week, one-course, monotherapeutic antibacterial regimen, acting even more successfully than classical antibiotics [1]. On the quest for a compound target, we found that family members of the compound family act on bacterial epigenetics. Bacterial epigenetics is a recently expanding field of study, which has shown that numerous bacterial species express dedicated methyltransferases (Mtases) which methylate genomic DNA at specific nucleotide motifs. *H. pylori* is currently one of those bacterial species which possess the highest number and the most variably expressed set of DNA Mtases [2,3]. It is well established that DNA methylation is contributing to genomic DNA integrity in bacteria, and many recent studies expanded Mtase functions also to genome-wide gene regulation, similarly to human epigenetics. Using the compounds of our new pathoblocker family, we now need to better characterize their activity under various environmental conditions, developing and using novel quantitative measurements of global genome methylation.
Methods and Results. Pathoblocker compounds active against bacterial motility were successfully used to remove bacteria in a preclinical therapeutic mouse infection model. We have assessed resistance development in vitro and in vivo and found it to be negligible. We have also employed various methods, including biochemical methods, mutant analyses, and enzymatically aided sequencing-based detection, to quantitate effects on bacterial genome-wide methylation, even at single base resolution. Site-specific methylation patterns of the *H. pylori* genome were derived, also under the influence of bacterial epigenetic pathoblockers. Alternative compound targets and direct effects on bacterial and human methyltransferases have been tested. We have thereby identified basic conditions that modulate genome-wide nucleotide methylation in *H. pylori*. Furthermore, we have collected information on compound activities and bacterial whole genome methylation.
Conclusions. We have obtained active pathoblocker compounds against bacterial motility and identified bacterial epigenetics as one pathoblocker target in *H. pylori*. This not only increases our fundamental understanding but also makes bacterial epigenetic modulation in general more accessible for possible therapeutic approaches.

1. Suerbaum, S. et al., mBio 2021; doi: 10.1128/mbio.03755-21.
2. Krebes, J. et al., Nucleic Acids Research 2014; doi: 10.1093/nar/gkt1201.
3. Estibariz, I. et al., Nucleic Acids Research 2019; doi: 10.1093/nar/gky1307.