DRUG RESEARCH & DEVELOPMENT

SWISS START-UPs

Fighting Tuberculosis by Restoring Ethionamide's Antibiotic Power

Researchers from Lille in France, GlaxoSmithKline in Tres Cantos, Spain, and BioVersys AG, a biopharmaceutical company based in Basel, are cooperating in developing a preclinical candidate able to restore ethionamide's antibiotic power against Mycobacterium tuberculosis. BioVersys is also pursuing adjuvant strategies for potentiation of other antibiotics. The objective is to overcome antimicrobial resistance.

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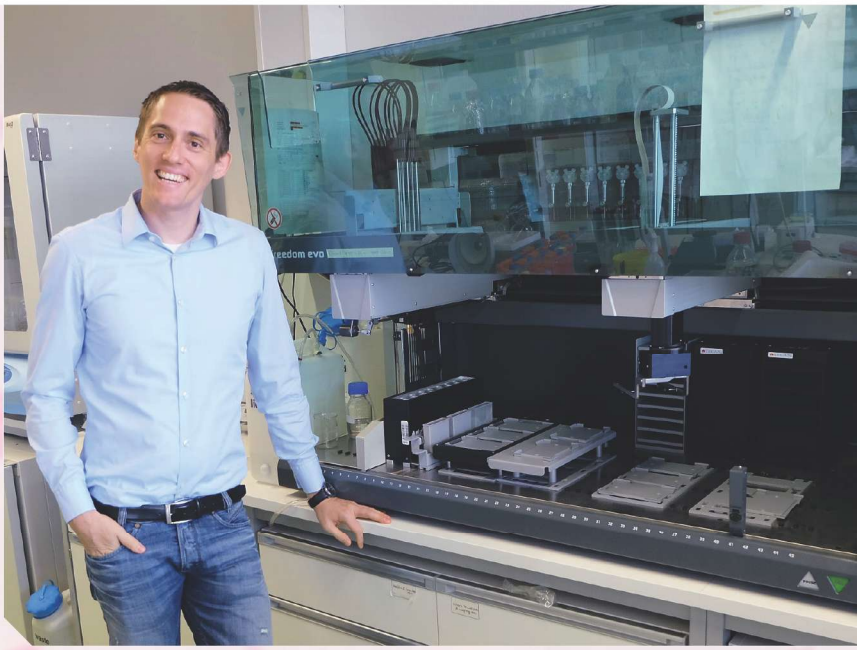
Synthetic biology is about combining genetic information of different origins to new functioning biologic cycle systems. Self-developed or purchasable nucleic acids sequences, so-called BioBricks, are assembled in a multi-species way and transferred to host cells. The extensive possibilities of use and application in this young field of research have given birth to several new business ideas and start-up companies. One of them is BioVersys AG, a biopharmaceutical company spin-off from the "Department of Biosystems Science and Engineering" (D-BSSE) of the "Swiss Federal Institute of Technology" in Zurich (ETHZ). BioVersys was founded in September 2008 and is based in the "Technologie Park Basel," where it rents offices and laboratory space. "We focus on transcriptional regulators, which we target with small molecules in order to inhibit resistance mechanisms that are switched-on in the presence of specific antibiotics," summarizes BioVersys' CEO and co-founder Marc Gitzinger. Suitable targets may be regulatory pathways controlling antibiotic induced thickening of the cell wall or biofilm production.

The company's researchers gain important insight into such kind of resistance mechanisms by doing knockout experiments on laboratory strains and studying patients' bacterial isolates of which BioVersys holds a large collection.

Understanding Resistance Mechanisms in Tuberculosis

Currently, work is carried out on three projects, addressing resistance in Gram-negative as well as Gram-positive bacteria; the most advanced one is in the field of tuberculosis (TB). Several antibiotics used in TB treatment are so called pro-drugs. These substances need to be bioactivated by enzymes of the pathogen before exerting antimicrobial activity. One such antitubercular pro-drug is ethionamide (ETH). This important second-line drug has been developed in the late 1950s and is still essential when treating patients suffering from an infection with multi-drug-resistant (MDR) tuberculosis pathogen Mycobacterium tuberculosis. MDR-TB treatment extends about two years and shows severe side effects due to the limited efficiency of ETH balanced by high dosages. The team of microbiologist Alain Baulard, Research Director at the Center of Infection and Immunity of the French Pasteur Institute in Lille, with expertise and specializing in mechanisms of action and metabolic activation of antibacterial pro-drugs, elucidated the transcriptional mechanism by which *M. tuberculosis* controls its own sensitivity to ethionamide (ETH).

"The reason for ETH's limited efficiency is that the transcriptional repressor EthR negatively regulates the expression of the gene coding for the enzyme EthA, a Flavin-containing bacterial mono-oxygenase, which catalyzes



Dr. Marc Gitzinger, CEO of BioVersys (Picture: Peiseler)

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DR. MARC GITZINGER. CEO BioVersys

The next milestone will be the nomination of a substance candidate for development from out of the five different substance classes, which the consortium holds in hands thanks to joining their forces.

the required activation of ETH," explains Gitzinger. Transcriptional repressors as well as activators mediate transcription-inhibiting or -activating signals via specific gene sequences called operators. EthR suppresses the biosynthesis of EthA via the operator OethR. As a result, EthA is produced in insufficient amounts: an intrinsic resistance mechanism, protecting *M. tuberculosis* from ETH's toxic effect.

Based on this know-how, Gitzinger, together with former colleagues from the research group of Martin Fussenegger, Professor at D-BSSE and co-founder of BioVersys, assembled a synthetic network, which is displaying the mechanism of ETH resistance. It was linked to a quantitative reporter gene expression readout and transferred to mammalian cells. The artificial cellular system was used to screen for compounds preventing EthR from binding to OethR. It turned out that the nontoxic flavor 2-phenylethyl-butyrate abolishes EthR's repressor function inside human cells, in mice, and within *M. tuberculosis*, where it triggers derepression of ethA and increases the pathogen's sensitivity to ETII. The findings were published in PNAS in July 2008. The efficient screening system, developed during Gitzinger's doctoral studies, set the starting point for Bioversys' TRIC- technology (transcriptional regulator inhibitory compound) platform and was decisive for company foundation. It enabled the discovery of transcriptional regulator inhibitory compounds differing from the structure of 2-phenylethyl-butyrate and largely superior in activity. "Our screening and medicinal chemistry activities have yielded new compounds that fully reactivate ETH sensitivity of all tested clinical MDR and extensively drug-resistant (XDR) TB isolates *in vitro* at very low concentrations," says Gitzinger.

Working in the Same Direction

At the same time, Benoîit Déprez and colleagues from the 2007-founded "Pole of Interdisciplinary Research on Drugs" (PRIM: Pôle de Recherche Inter-disciplinaire sur le Médicament) in Lille started searching for EthR inhibitors that would boost antituberculous activity of ETH. The French researchers developed a chemical screening system, indicating compounds that disrupt the interaction of EthR with DNA. Co-crystallization of such molecules with EthR brought additional information about the complex's three-dimensional structure and led to the synthesis and optimization of improved analogs. As well as the team from BioVersys, the crew from PRIM was able to develop active substances, which increase the potency of ethionamide by a multiple: results that were published in Nature Medicine in 2009. ›

Knocking out Bacterial Resistance Mechanisms

In January 2015, Nature Magazine published a paper on the discovery of teixobactin, a natural compound isolated from the soil bacterium Eleftheria terrae, showing activity against Gram-positive bacteria such as Staphylococcus aureus and Mycobacterium tuberculosis by binding to important cell wall forming precursor molecules. "It looks like a promising compound, but knowing the drug development risks, we need more early stage projects like this", comments BioVersys CEO Marc Gitzinger Nature's rating of teixobactin as an "irresistible newcomer." Gitzinger recalls that nature is a large pool for resistance genes. At least the organism, from which a natural compound has been isolated, is in possession of an intrinsic resistance mechanism. Besides acquiring antibiotic resistance through spontaneous mutations, bacteria can pick up resistance genes from other bacteria by horizontal gene transfer when undergoing conjugation or by intervention of viruses (bacteriophages). Bacteria can also acquire naked DNA from their environment. Resistance genes can be accumulated in bacterial genomes over time or at one fell swoop, leading to resistance against many different families of antibiotics. It seems to be just a matter of time, until an antibiotic loses its power given against resistant bacterial strains. This is the reason why BioVersys wants to explore alternative ways. The Swiss company is closely looking on resistance mechanisms, which could be inhibited by non-antibiotic compounds, following the example of Augmentin, a medicine that combines the β-lactamase inhibitor clavulanic acid with penicillin-group antibiotics. Clavulanic acid its able to fight antibiotic resistance in bacteria that secrete β-lactamase, which otherwise inactivates most penicillins.

"The inhibition of enzymes like β-lactamase has been known for more than 20 years. We focus on more complex resistance mechanisms, which are tightly controlled by transcriptional regulators. Since their constitutive expression would mean an unnecessary energetic cost for the bacterium, such resistance mechanisms are up-regulated only in the presence of antibiotics, which they turn against," explains Gitzinger. Next to an inhibition of the EthR/OethR interplay in Mycobacterium tuberculosis in order to fight resistance to ethionamide (see: Fighting tuberculosis by restoring ethionamide's antibiotic powermain article), the expression of some efflux pumps, may be another appropriate target. Such transport proteins are found in both Gram-positive and -negative bacteria as well as in eukaryotic organisms. They are involved in the extrusion of toxic substrates like antibiotics from within cells into the external environment. Pumps may be specific for one substrate or may transport a range of structurally dissimilar compounds. The latter are associated with multiple drug resistance. BioVersys is investigating such resistance mechanisms. Together with the world-renowned antibiotics expert David Livermore, Professor of Medical Microbiology at the University of East Anglia and a member of BioVersys' scientific advisory board, the Swiss scientists are also looking on transcription-regulated pathways central crucial for cell wall thickening and biofilm production.

Extensively drug-resistant tuberculosis is a tuberculosis form that is resistant to at least four of the core anti-TB drugs.

"When the French scientists and we noticed that we were working in the same direction, contact was established with the doctor Alain Baulard, and the professors Nicolas Willand, and Benoît Déprez, all three leading scientists in TB research and drug discovery from different institutions in Lille," tells Gitzinger. The researchers agreed to work together and started cooperation in 2011. In 2012, the "Société d'Accélération du Transfert de Technologie" (SATT Nord), a center for technology transfer, took over all patents concerning the cooperation that were formerly held by different institutions: the Pasteur Institute of Lille, the University of Lille, the National Center of Scientific Research (CNRS), and the National Institute of Health and Medical Research (Inserm). "This smart move made collaboration much easier. We currently share all rights on intellectual property while commercial rights are completely with BioVersys. All IP generating research falls under one same contract," explains the CEO of BioVersys. The collaboration of the Swiss and French researchers yielded new promising results, and in 2014, UK drug giant GlaxoSmithKline (GSK) joined the team. The object is to develop a preclinical candidate against tuberculosis. According to Gitzinger, there are 14 people working full-time on the project. All work on absorption, distribution, metabolism, and excretion of active com-

pounds as well as some *in vivo* experiments are conducted at GSK. while *in vitro* and *in vivo* experiments with *M. tuberculosis*, requiring biological safety labs, and a main part of the medicinal chemistry activities are carried out in Lille. All other work is done at BioVersys in Basel. The Welcome Trust, who was already supporting GSK in order to push some early-stage projects focusing on finding treatments for diseases largely affecting the developing world, co-funds the project, which is based on milestones. This means that further funding follows demonstrated progress. The next milestone will be the nomination of a substance candidate for development from out of the five different substance classes, which the consortium holds in hands thanks to joining their forces. GSK is granted the possibility to negotiate an overall license with BioVersys.

Tuberculosis Remains a Life-Threatening Health Problem

Behind HIV infection, tuberculosis (TB) is on the second place of deadly infectious diseases, caused by a single infectious agent. Nine million new TB cases were reported in 2013. In the same year, 1.5 million people died from the contagious disease, which is transmitted by infectious aerosol droplets. TB is most prevalent in Africa, India, China and Eastern Europe; infections with multidrug-resistant (MDR) and even extensively drug-resistant (XDR) Mycobacterium tuberculosis are on the rise. MDR-TB is when *M. tuberculosis* fails to respond to a combination of two of the four first line anti-TB drugs (rifampicin and isoniazid). Patients usually acquire drug resistant TB either as a result of spread of a drug resistant strain from another TB sufferer or as a result of inappropriate or incomplete treatment. XDR-TB is a form of TB that is resistant to at least four of the core anti-TB drugs (rifampicin, isoniazid, fluoroquinolones and second-line injectable agents). In December 2012, the US Food and Drug Administration (FDA) approved bedaquiline, the first new anti-TB drug in 40 years. Bedaquiline affects the proton pump for ATP synthase and thereby thwarts the bacterial energy supply. Another new anti-TB drug, delamanid, received its first global approval at the end of 2013 from the European Medical Agency (EMA). Delamanid damages the pathogen's cell wall. Both, bedaquiline and delamanid, are exclusively reserved for the treatment of proven MDR- and XDR-TB. Anyway, despite these two new anti-TB drugs, the pipeline of high-quality treatments remains thin. "The existing price/volume business model for antibiotics is not working and is a key barrier to achieving more rapid progress on resistance," argues Kevin Outterson, Professor at the University of Boston, in a recently published report on the broken market for antibiotic innovation. The authors recommend the authorities to continue "building on recent promising steps by boosting funding for basic research and development, surveillance, and antibiotic stewardship; targeting these initiatives to prior unmet needs; and reforming reimbursement to support effective antibiotic access and use rather than volume."