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Mutation and drug resistance

Novel leads and drugs and their mechanism of action in the field of vector borne parasitic disease

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One Health drugs against parasitic vector borne diseases in Europe and beyond

OneHealth*drugs*

The workshop "Novel leads and drugs and their mechanism of action in the field of vector borne parasitic disease" is jointly organized by WG1 "Compound libraries coordination and integration of compound design" and WG2 "Integration of early phase studies and low environmental impact actions" of the COST Action CA21111 OneHealthDrugs.
The focus of the workshop is to bring together experts to report and discuss common definitions of novel leads
and drugs and their mechanisms of action in view also of their impact on OneHealth needs. The workshop will give an overview about the state of art in the field and will help to plan a roadmap to achieve basic objectives of the COST Action. This workshop is connected with WG1/WG2 activity based on the handling of chemical compound information and the usage of compound databases/repositories and WG3 activity on the profile of leads and drug candidate.

BOOK OF ABSTRACTS

Quiescence in Leishmania, a target for a new generation of chemotherapeutic innovations

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The leishmaniases are a spectrum of neglected tropical diseases, causing lethal visceral organ damage or disfiguring skin lesions, that afflict millions of people and yet are difficult to cure with current drugs. Frequently, clinical cure is followed by recrudescence months to years after treatment, not associated with parasite drug resistance. This may be explained by parasites that enter a reversible quiescent state, refractory to drug treatment, thus resembling recalcitrant bacterial infections caused by so-called "persister forms". Historically, research into new drugs, vaccines and diagnostic tests for leishmaniasis screened replicative forms, creating a leishmaniasis intervention tool kit that neglects the impact of quiescent forms. Major stakeholders in drug discovery and development recognize the need for drugs that eliminate quiescent persister-like forms.

Research on quiescence in Leishmania is still in its infancy. My group is one of the few ones who published (see refs below) on that topic and we have developed a leading expertise in experimental research on quiescence in different Leishmania species, including the viscerotropic L. donovani. We developed 4 different in vitro models of quiescence and deeply characterized several of them, at cellular and molecular point of views (bulk and single cell technologies). Our most recent work highlighted a series of potential drivers/regulators of quiescence, which we are currently validating.

The knowledge generated through our work might guide R&D for new anti-leishmania drugs. On one hand, our in vitro models could be used for untargeted screening of compound libraries. On the other hand, the most relevant drivers/regulators of quiescence could be targeted by specific inhibitors.

Benznidazole uptake by *Trypanosoma cruzi* is a determinant of variable drug efficacy and treatment failure

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Benznidazole (BZ) is the front-line treatment for Chagas disease. However, there is extensive variation in susceptibility within natural populations of the causative agent *T. cruzi*, and treatment failures are widely reported. The underlying reasons for this diverse efficacy are unknown. We used a range of genetic, cell biology and biochemical approaches to dissect the mechanisms of BZ resistance in *T. cruzi*. In combination with high resolution imaging and *in vivo* studies, this allowed us to identify BZ uptake as a major determinant of parasite susceptibility.

We show that BZ uptake by *T. cruzi* is mediated by endocytosis and that stage-specific and strain-specific differences in this process have important roles in drug efficacy. There is also considerable heterogeneity in drug accumulation by amastigotes, the replicative intracellular form of the parasite, even within the same infected host cell. Following uptake, BZ rapidly transits to the mitochondrial network, the site where it undergoes reductive activation. In the infectious, non-replicative trypomastigote life-cycle stage, low-level drug uptake is associated with reduced susceptibility. In addition, naturally resistant parasites have a reduced drug uptake capacity, a phenotype associated with treatment failure in experimental infections. To add further complexity, BZ uptake by mammalian cells, which is also endocytosis-mediated, varies between different host cell types. Our results therefore demonstrate that differences in BZ uptake, acting at several levels, provide a mechanism to explain the wide divergence in sensitivity within the *T. cruzi* population and highlight why sterile cure with this drug can be difficult to achieve.

Targeting epigenetics to combat vector borne parasitic diseases

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Our research focus is `Chemical Epigenetics´ and deals with the development and application of chemical and biochemical tools to dissect biological pathways, to validate therapeutic targets and to discover and optimize potential drugs addressing a wide range of epigenetic targets. This includes bifunctional Proteolysis targeting chimeras (PROTACs) for chemically induces protein degradation. Here we present work to target the epigenetic machinery of eukaryotic parasites (*Schistosoma*, *Trypanosoma*) as well as targeting host cell epigenetics to combat human macrophage subversion by *Leishmania*.

From drug discovery to drug development: The role of intermolecular interactions in drug solid forms

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All available strategies for drug discovery, both the searching the known receptor/enzyme structures and testing the metabolic paths for the unique molecular structures of the new synthetized or isolated compounds are relied on intermolecular recognition on the binding sites. Crystal engineering, on the other hand, utilizes the inherent structural moieties "synthons" formed by spatial interactions that nature and geometry depend on the type of the functional groups and/or molecular structures leading to their reproducibility during the molecules/ions pack each other during the macroscopic crystal growth. Therefore, analysis of around 138 000 small-molecule structures deposited in the Cambridge Structural Database (CSD) offer opportunities for statistical assessment of the propensity of occurring different type of synthons in different classes of chemical compounds which molecular structures are packed in crystallographic classes of monocomponent or multicomponent single phases of crystals. In order to screen the specific binding compatibility with the protein targets, extrapolation of the synthons in the CSD, that describes the shape and interaction propensities of molecules in their crystal structures, enables to optimize the high-throughput screening new leading compounds for their favorable drug–protein crystal structures. In addition, electron-density-based intermolecular boundary surfaces in small-molecule crystal structures and in target-protein binding sites are tool to identify potential ligand molecules from the CSD based on 3D shape and intermolecular interaction matching [1]. Crystal engineering concept becomes crosscut stage where overlapping drug discovery and drug development enable tailoring the desirable properties through screening the solid phases and selection the appropriate one. The nature of non-covalent interactions among molecular/ionic counterparts in stoichiometric ratio influence either crystallization of the single component solids (polymorphs) or multicomponent solids (solvates/hydrates, salts, cocrystals or inclusion complexes etc.) to occur beside the amorphous solid phases [2].
The presented case studies for searched crystal structures for several compound classes of antiparasitic drugs which are deposited in Cambridge Structural Database (https://www.ccdc.cam.ac.uk/) reveal the opportunities for studying the frequency, prevalence and hierarchy of appearing the non-covalent interactions in their molecular crystals and to perceive the opportunities for designing new solid phases for multicomponent crystals of either the combinations of antiparasitic drugs with different molecular structures or antiparasitic drug cocrystalized with reliable conformer that improve the drug properties.

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Preclinical development of a novel therapeutic candidate for the treatment of animal trypanosomiasis

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Animal trypanosomiasis (AT) is a widespread disease caused by *Trypanosoma spp.* and has a devastating effect on animal husbandry all over the world due to the scarcity of efficient drugs and development of drug resistance, hence emphasizing the need for novel treatment options. Following previous identification of 3’-deoxytubercidin as a highly potent trypanocide with curative activity in mouse models of both stage-1 and stage-2 Human African Trypanosomiasis (HAT), we now present a comprehensive preclinical evaluation of new 6-amino substituted tubercidin analogues with promising activity against a broad range of AT species. Potent hits were identified *in vitro* across all important AT species, *i.e. T. b. brucei*, sensitive and isometamidium (ISM)-resistant *T. congolense*, *T. vivax*, *T. evansi* (type A and B) and *T. equiperdum*. Selected ‘hits’ were further tested for *in vitro* metabolic stability (using bovine, horse and piglet liver microsomes), *in vivo* mouse models for each AT species, genotoxicity assays and mode-of-action studies (*i.e.* genome-wide RNA interference library screening, metabolomics). Analogue **3** was highly active in *T. vivax*, *T. congolense*, *T. equiperdum*, *T. evansi* and *T. brucei* curative mouse models. Furthermore, there was no indication of *in vitro* genotoxicity as confirmed by Vitotox®, the micronucleus and the comet assays. Mode-of-action studies for **3** revealed that the P1 nucleoside transporter and adenosine kinase are involved in drug uptake and activation, respectively. Given the preferred target product profile for a broad-spectrum drug against AT, analogue **3** represents a promising ‘lead’ candidate for treatment of animal trypanosomiasis, regardless of the causative species.

Animal trypanosomiasis | drug discovery | nucleoside analogues | RNAi

Deciphering the mechanism of action of VP343, a drug candidate for the treatment of visceral leishmaniasis

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The work that I would like to present will focus on the mechanism of action of a drug candidate, VP343, showing promising activities on L. infantum, both in vitro on infected macrophages and in vivo in a mouse model of visceral leishmaniasis. By using host-cell trafficking markers in confocal microscopy to analyze the action of VP343 at the cellular level and a proteomic approach to identify the molecular pathways targeted the compound, our data showed that VP343 interferes in the interaction between the parasitophorous vacuole and host-cell endolysosomal compartments, with direct consequences on parasite proliferation, leading to their elimination from the host cell.

Comparative *in silico* and *in vitro* search for dual inhibitors of the *Trypanosoma brucei* and *Leishmania major* pteridine reductase 1 and dihydrofolate reductase

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The protozoan parasites *Trypanosoma brucei* (*Tb*) and *Leishmania major* (*Lm*) are responsible for the insect-borne tropical diseases sleeping sickness, Nagana and cutaneous leishmaniasis. Every year, millions of humans as well as animals living in tropical to subtropical climates fall victim to the illnesses' broad-ranging negative medical and economical effects. Both mentioned parasites are extremely prone to drug resistances, threatening the efficacy of the current treatment options, and hard to control due to widely spread insect and animal reservoirs [1].
The pteridine-auxotrophy of the family Trypanosomatidae caused *Tb* and *Lm* to develop a corresponding enzyme system consisting of the dihydrofolate reductase-thymidylate synthase (DHFR-TS) and pteridine reductase 1 (PTR1), to ensure cell survival [2]. A comparative study of the *T. brucei* (*Tb*DHFR, *Tb*PTR1) and *L. major* (*Lm*DHFR, *Lm*PTR1) enzymes was employed, to identify lead structures with dual inhibitory effect against these promising targets. *In silico* experiments were performed to preselect possible inhibitors towards the respective parasite enzymes, encompassing a pharmacophore-based virtual screening of four natural product databases (ca. 5000 compounds). Building on these *in silico* results, the inhibitory potential of promising compounds was determined *in vitro* using spectrophotometric enzyme assays against recombinant DHFR and PTR1. Out of 97 tested natural products, thirteen compounds were identified as dual inhibitors against the *Tb* enzymes (0.2µM<IC50<85.1µM). Furthermore, nine natural products inhibited both of the respective *Lm* enzymes (0.58µM<IC50<84.5µM). These results encourage the future utilization of the trypanosomatid pteridine metabolism as a drug target.
Acknowledgement: We thank Prof. Dr. M.P. Costi and colleagues (University of Modena and Reggio Emilia, Modena, Italy) for providing the plasmids for the investigated PTR1 enzymes.

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Targeting casein kinase 1 as a novel strategy for the design of antileishmanial agents

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According to a recent WHO report[1], leishmaniasis affects nearly 12 million people, with 350 million others at risk, and is responsible for nearly 40,000 deaths per year. In 2012, mainly due to global warming, visceral leishmaniasis (VL) was declared as a new emerging disease in Europe. Today, there is no effective vaccine, and the limited treatments are unfortunately too toxic and costly. In this context, there is a real emergency to develop new paradigms for antileishmanial therapy, which also limit the devastating impact of parasite resistance. While most of the current drugs as well as those in development target the parasite biology, we propose to target the exoproteome of *Leishmania*, and particularly excreted signalling kinases, to inhibit host-parasite interactions, which will restore the host cell ability to fight the parasite and limit the risk of resistance.
To this end, we selected and validated *Leishmania* casein Kinase I paralog 2 (L-CK1.2) as a drug target[2,3]. L-CK1.2 is essential for intracellular parasite survival and released in macrophages via extracellular vesicles[2]. Moreover, several evidence suggest that L-CK1.2 has been evolutionary selected to interact with and phosphorylate host proteins subverting the biological and immune functions of the macrophage[2]. Because of its dual role in the parasite and the host cell, targeting L-CK1.2 would kill the parasite while limiting the emergence of parasite resistance.
We previously reported the discovery of **CTN1122**[4,5], an imidazo[1,2-*a*]pyrazine derivative with promising antileishmanial properties that targets L-CK1.2 (Figure 1).

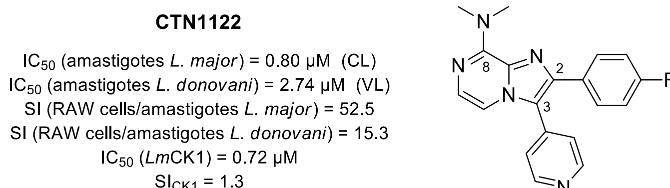

Figure 1. The lead compound **CTN1122**.

When tested *in vivo*, it reduces the parasite load in the liver and spleen of mice infected with *L. donovani*, as well as in the lesion of mice infected with *L. major* with a significant decrease in the size of the lesion. Here, we present the TEXLEISH consortium, which, through a research program dedicated to this chemical series and its target, focuses on three main objectives (1) the optimization of this lead compound by generating new pharmacomodulations of **CTN1122,** (2) the identification of potential off-target effects to limit toxicity and side-effects by using state-of-the-art deconvolution methods and (3) the better understanding of the role of L-CK1.2 in host-pathogen interactions by using system-levels analyses. The TEXLEISH consortium will provide the first evidence that targeting the exoproteome of parasite for drug treatment is an innovative way to discover potent new drugs against leishmaniasis limiting the risk of selecting for drug resistant parasites.

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Optimising leads for parasitic diseases: specificity by target or by targeting?

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When a drug target is known the optimisation strategy commonly involves the isolation and purification of this target (enzyme) and develop stronger inhibitors, often with the aid of structural information like X-ray crystallography, cryo-EM of modelling/docking software. After a Medicinal Chemistry SAR campaign the best inhibitor is then tested on whole cells to confirm the improved antiparasite activity. However, this strategy may fail if the new inhibitor cannot reach the target for which it was optimised. Drug uptake into cells is overwhelmingly mediated by various transport processes – mostly by transport proteins but also by binding to cell-surface proteins followed by endocytosis, for instance (receptor-mediated endocytosis). These are highly specific processes and in the process of optimising a phenotypic screening hit to a strong enzyme inhibitor the efficiency of uptake could be lost or severely diminished, thereby also reducing the antiparasite efficacy of course. Therefore, the inhibitor SAR must match the specificity of both target and transport process. In addition, the intracellular distribution must be taken into account. One example is Trypanosome Alternative Oxidase (TAO) an essential enzyme located in the inner mitochondrial membrane. Although good inhibitors have been known for a long time they did not accumulate in the mitochondria, resulting in low anti-parasite activity. Improving the mitochondrial targeting by adding a triphenylphosphonium group achieved up to 10,000-fold higher activity against *T. brucei*. Selectivity was excellent as mammalian cells do not have an equivalent Alternative Oxidase.

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