42. Drug target deconvolution studies in L*eishmania donovani*

Luciana Paradela[1], Sandra Carvalho[1], Julio Martin[2], Ian Gilbert[1] & Susan Wyllie[1]
1. The Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK; 2. Global Health R&D, GlaxoSmithKline, Tres Cantos, Spain.

Visceral leishmaniasis (VL) is caused by protozoan parasites from the *Leishmania* genus and is potentially fatal if left untreated. Despite its significant health impact, there are limited anti-leishmanial drugs available, with the lack of validated drug targets a serious impediment to the development of effective treatments for these diseases. Whole-cell phenotypic high-throughput screening of a set of 1.8 million compounds against *L. donovani* was performed by GlaxoSmithKline and resulted in the generation of a Leish-box containing approximately 200 active compounds. Since there is no information regarding the molecular targets and/or the mode of action (MoA) of these phenotypically-active compounds, comprehensive studies to determine their molecular targets are required. With this in mind, seven promising Leish-box compounds were selected in order to carry out drug target deconvolution studies. Genomic approaches have been applied and the new results are reported. Two promising hits, lanosterol synthase (LS) and a hypothetical protein (HP), from studies with compound C5 were identified from two unbiased genomic approaches – resistance generation followed by whole genomic sequencing and screen against a genome overexpression library. To validate LS as the target of C5, parasites overexpressing LS were generated and showed a marked gain of resistance to C5 and to an established LS specific inhibitor. These findings confirm LS as the first target validated in these studies. Further investigation will be employed to better understand the impact of LS as a target in *L. donovani* parasites.

43. Therapeutic efficacy of butenafine nanomedicines in experimental cutaneous leishmaniasis

Luiz Felipe D.Passero[1], A.B. Sousa[2], J.A.Jesus[2], Katerina Lalatsa[3], M.D. Laurenti[2]
1. São Paulo State University (Brazil), São Paulo University (Brazil); 2. Medical School of São Paulo University; 3. University of Portsmouth (United Kingdom).

The production of ergosterol lipid, important for the *Leishmania* membrane homeostasis, involves different enzymes. This pathway can be blocked to azoles and allylamines drugs, such as the antifungal butenafine chloride. This drug was active (*in vitro*) against *L. (L.) amazonensis* and *L. (V.) braziliensis*, the etiological agents of anergic diffuse and mucocutaneous leishmaniasis, respectively. Based on the leishmanicidal activity of butenafine and considering the absence of reports about the therapeutic potential of this drug in cutaneous leishmaniasis, the present work aimed at analyzing the efficacy of butenafine chloride formulated in two different topical delivery systems, such as self-nanoemulsifying drug delivery systems (SNEDD) and in a SNEDD-based nanogel as well as in the free form in murine cutaneous leishmaniasis. *L. (L.) amazonensis* infected BALB/c mice topically treated with SNEDD, nanogel or free butenafine during 15 days presented lesser lesion size and parasitism when compared with the control. Furthermore, animals treated with nanogel and free butenafine showed increased levels of IFN-γcytokine; histologically the skin of animals treated with nanogel and Glucantime were in healing process. In addition, the therapeutic potential of nanogel was similar with the potential of Glucantime. Based on these data, the antifungal drug butenafine chloride can be considered an interesting repurposed drug for the treatment of cutaneous leishmaniasis.

44. Lysyl-tRNA synthetase as a drug target in malaria and cryptosporidiosis

João P. Pisco[1], Sarah Niven[1], Beatriz Baragaña[1], Leah Torrie[1], Barbara Forte[1], Neil Norcross[1], Chimed Jansen[1], Caroline Peet[1], David Robinson[1], Irene Hallyburton[1], John Post[1], David W. Gray[1], Kevin D. Read[1], Christopher D. Huston[2], Stephen Nakazawa Hewitt[3,4], Ryan Choi[3,4], Chris Walpole[5], Wesley C. Van Voorhis[3,4], Ian H. Gilbert[1]
1. Drug Discovery Unit, Wellcome Centre for Anti-Infectives Research, Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee, DD1 5EH, UK; 2. University of Vermont, Stafford Hall, 95 Carrigan Drive, Burlington, Vermont 05405, USA; 3. University of Washington (UW), Center for Emerging and Reemerging Infectious Disease (CERID); 4. Seattle Structural Genomics Center for Infectious Disease (SSGCID); 5. Structure-guided Drug Discovery Coalition (SDDC), Structural Genomic Consortium.

Aminoacyl tRNA synthetases have previously been identified as suitable drug targets against different parasites, including *Plasmodium falciparum* (*Pf*), one of the parasites that causes malaria, and *Cryptosporidium parvum* (*Cp*), which causes cryptosporidiosis. This family of enzymes catalyses the attachment of an amino acid to its cognate tRNA molecule in a two-step reaction. First the amino acid is activated by ATP resulting in AMP-activated amino acid with loss of pyrophosphate, and secondly the amino acid is transferred onto the tRNA. Taking advantage of structural information, in this work we optimized a series of selective inhibitors of *Pf* and *Cp* lysyl t-RNA synthethase (KRS). We identified a drug-like selective inhibitor of both *Pf* and *Cp* KRS capable of clearing parasites from mouse models of malaria and cryptosporidiosis infection. Using a combination of crystallography, surface plasmon resonance (SPR) and steady-state kinetics experiments we characterized the binding and mode of inhibition of the compound. Our results demonstrated that the compound inhibits *Pf* and *Cp* KRS by binding to the ATP binding site, in the active site of KRS.