**Resumen Proyecto y del Plan de Formación**

**Heme trafficking and metabolism at the host-parasite intersection: exploiting *Leishmania* heme auxotrophy (*LeisHeMed*)**

Leishmaniasis, one of the most devastating tropical diseases, is produced by different species of the protozoan parasite *Leishmania*. This disease is currently considered to be both emergent and neglected by the World Health Organization (WHO). Its control is based on chemotherapy, however, the drugs in use are inadequate, toxic, not very effective, difficult to administer, and/or threatened by resistance, thus highlighting the urgent need to find new drugs to treat this disease. One attractive strategy for identifying new drug targets involves exploiting the biochemical differences between this pathogen and its human host. Heme metabolism constitutes one of these differences since heme is a critical cofactor for many essential metabolic pathways and *Leishmania* cannot synthesize it. Thus, *Leishmania* must scavenge heme from the host to allow its intracellular replication in the parasitophorous vacuole of infected macrophages. The main objective of the Project, which is a direct continuation of the previous one financed by the National Plan, is to exploit this *Leishmania* heme dependency through the functional characterization of essential proteins involved in the use of host heme by *Leishmania*, and the identification of inhibitors of some of these proteins capable of killing the parasite. In the previous project, we identified, from a transcriptomic analysis, a series of heme-regulated genes confirming in animal models that some are attractive therapeutic targets. Here, we will carry out the functional characterization of the two most promising candidates. For that, in addition to different biochemical studies, we will perform complementation assays in mutant yeasts. In addition to the functional information they provide, this complemented yeast will be used for target-directed high throughput screening in a cellular context to find inhibitors that kill the parasite. On the other hand, we will study the essentiality and functionality of an HRG-9 (Heme Regulated Gene 9) homolog, a recently described protein involved in metazoan porphyrin trafficking, which we also identified in the parasite in the previous project. Its expression level in the parasite will be modulated (overexpression, Crispr-Cas9-mediated KO generation,…) and its role in heme trafficking/metabolism analyzed. The viability of modified parasites and their ability to infect and multiply in macrophages and cause disease in animal models will be determined. Using animal models, we will also study the leishmanicidal potential of a compound, identified in the previous project, which kills *Leishmania* by inhibiting an essential heme transporter, which could also bind to heme-interacting G quadruplex sequences of the parasite's mitochondrial DNA. These studies should make a major contribution towards our understanding of *Leishmania* biology and will open new avenues for pharmacological intervention in these and other trypanosomatid parasites responsible for devastating yet neglected diseases, as demonstrated by the support of the WHO and the interest of the international companies Ilender, and Rakta Therapeutics Inc., and the Spanish companies DOMCA, Hipra, and Fundación MEDINA.

***Training program planned***

The Predoctoral Investigator Personnel (PIP) will be integrated into the "Doctoral Program in Biochemistry and Molecular Biology" offered by the Ph.D. School of Health Sciences at the University of Granada (UGR). This program offers a broad spectrum of specialized courses for doctoral students (detailed at <https://doctorados.ugr.es/bioquimicaybiologiamolecular/pages/formacion/actividades_formativas>), including the course on Experimental Animal Handling, the Workshop on Strategies for Effective Research Publication, the course on Intellectual Property and Patentability, Databases and Bibliography Management, the Discovery Process and the Development of Therapeutic Agents, and Translational Research and Therapeutic Innovation, etc. In addition, the Doctoral Program promotes short stays at other research centers through grants awarded by the UGR International Graduate School via the Erasmus+ international mobility programme. In addition, several of our group's working practices are also designed to help the student obtain and develop complementary scientific skills. These include weekly group meetings where each group member can discuss their results and any problems they may have encountered. This process is designed to promote team building and improve presentational and oral skills. Furthermore, in the final two years of their training, the PIP is expected to supervise and train new undergraduate students recruited by the group, thereby ensuring that they develop research management and leadership skills. On the other hand, the PIP will join a group at the IPLN-CSIC that is very well-equipped and highly experienced in this type of research. This would, therefore, be an excellent opportunity for them to acquire both technical experience and scientific skills under the guidance of the project’s PI. Furthermore, as the proposed project is very ambitious, the PIP will receive extensive experimental training in different scientific disciplines, such as Parasitology, Cell Biology, Biochemistry, Pharmacology, Animal models, etc. Specifically, although the training objectives of the PIP are expected to cover numerous fields, they will include: i) Culture and molecular manipulation of the protozoan parasites *Leishmania* and *T. brucei*, macrophages, yeast, and bacteria; ii) Biochemical characterization of fluorescent substrate transport, gaining expertise in the use of spectrofluorimetry and flow cytometry; iii) Omics techniques including transcriptomic; iv) Molecular biology techniques, including gene cloning and CRISPR-mediated gene deletion, qRT-PCR, etc.; v) Cell biology techniques, including flow cytometry and microscopy (confocal microscopy, live cell fluorescent microscopy, image analysis, electron microscopy, etc.); vii) Animal models (including in vivo imagine system); and viii) HTS screening of drugs. Finally, part of the proposed project will be carried out in collaboration with the prestigious international groups. The PIP will be expected to spend some time in these laboratories and will, therefore, acquire experience working in a different scientific environment and benefit from this interaction with some of the world's top research groups in this field.