IBEC-VHIR INTERNATIONAL PhD PROGRAMME

Position

1. Project Title/ Job Position title:
   YAT2150: Elucidation of its Targets and their Validation in *Leishmania*

2. Research project/ Research Group description

The problems associated with the drugs currently used to treat leishmaniasis, including resistance, toxicity, and the high cost of some formulations, call for the urgent identification of new therapeutic agents with novel modes of action. Treatment of *Leishmania infantum* cultures with protein aggregation inhibitors showed a significant *in vitro* antileishmanial effect. In particular, the aggregated protein dye YAT2150 had a better *in vitro* activity against *L. infantum* (IC_{50} of ca. 0.5 µM for both amastigote and promastigote forms) than most drugs currently used for the clinical treatment of leishmaniasis, such as miltefosine, pentamidine and paromomycin. The presumed antiparasitic mode of action of YAT2150 is the inhibition of functional protein aggregation in the pathogen, a mechanism that would target multiple proteins, which might in turn significantly reduce the emergence of resistance in *Leishmania* to this compound. Encapsulation in liposomes of YAT2150 significantly improved the *in vitro* antileishmanial activity relative to the free drug and reduced its unspecific cytotoxicity. The synthesis of several YAT2150 derivatives provided compounds which maintained a high activity against *L. infantum* but with a much lower *in vivo* toxicity in the *Caenorhabditis elegans* model. These last two approaches led to selectivity indexes >100 and therefore to good perspectives regarding the entry in the preclinical pipeline of YAT2150 or some of its derivatives. In this PhD, to investigate the mechanism through which this family of compounds kills *Leishmania*, we will do (i) whole genome sequencing of *L. infantum* lines resistant to YAT2150, (ii) flow cytometry-based sorting and (iii) cellular thermal shift assays (CETSA) to identify the proteins binding YAT2150, and (iv) transcriptomics (RNAseq) analysis of drug-treated and non-treated samples to identify differentially displayed transcripts. This robust analysis performed with four complementary techniques will be completed by validation of the candidate target proteins through the generation of knock-out parasite lines. The gained knowledge will be essential to advance YAT2150 and its derivatives towards entering the preclinical pipeline, and also for the development of new antileishmanial drugs targeting protein aggregation, a potential Achilles' heel of the pathogen.

3. Job position description

We look for an enthusiastic, organized, and autonomous PhD candidate with a degree in Microbiology or similar areas. The thesis will be divided in 4 phases. During the first semester the candidate will become familiar with all necessary methods and will start the generation of *L. infantum* lines resistant to YAT2150. In Phase 2, the candidate will perform flow cytometry-based sorting, CETSA and RNAseq assays. In Phase 3, the fellow will characterize the proteins eventually identified in Phases 1 and 2 and will generate knock-out (KO) lines for them. Phases 2 and 3 will overlap and complement each other until the first semester of year 3. Finally, during the last semester of year 3 and the first semester of year 4, the characterization of the target proteins and their corresponding KO lines will be performed, together with completion of all analyses, statistics and interpretation of results. The writing of the PhD Thesis and its defense will be done during the second semester of year 4. The results of the PhD will be published in peer-reviewed journals within the first quartile of their respective fields and disseminated in international conferences. During the 4 years of the fellowship, the PhD fellow will acquire a strong knowledge of the cellular biology of *L. infantum* through lab work, regular meetings with the PhD co-supervisors, bi-weekly journal clubs and weekly group meetings, and through daily readings of literature related to the field.

Specific requirements: Training in cell cultures, fluorescence microscopy, genomic analysis, RNAseq and cellular and molecular biology techniques, experience with *in vitro* cultures of *L. infantum* will be a plus.

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Group Leader at IBEC

1. Title: Prof.
2. Full name: Xavier Fernàndez Busquets
3. Email: xfernandez@ibecbarcelona.eu
4. Research Group: Nanomalaria

Collaborator at VHIR

1. Title: Prof.
2. Full name: Israel Molina Romero
3. Email: israel.molina@vallhebron.cat
4. Institute: Vall d'Hebron Institut de Recerca (VHIR)
5. Research group: Unitat de Salut Internacional Vall d'Hebron-Drassanes. Servei de Malalties Infeccioses