LEISHMANIA INFANTUM DYRK1 IN THE DEVELOPMENT OF INFECTIVE PROMASTIGOTES AND IN STAGE DIFFERENTIATION

Vinícius Pinto Costa Rocha[1,2], Mariko Dacher[3], Foteini Kolokousi[4], Antonia Efstathiou[4], Gerald F. Späth[3], Milena B. P. Soares[1,2], Despina Smirlis[3,4]

1. Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil & Centro de Biotecnologia e Terapia Celular do Hospital São Rafael, Salvador, Brazil, 3. Pasteur Institute & INSERM U1201, Parasitology and Signaling Unit, Paris, France 4. Hellenic Pasteur Institute, Molecular Parasitology Lab, Athens, Greece

DYRKs comprise a family of pro-survival eukaryotic protein kinases against stress and differentiation stimuli. For combating leishmaniasis, a serious disease caused by the protozoan parasite *Leishmania,* it is essential to increase the validated drug target repertoire. To this end we have selected a member of the *Leishmania* DYRK family, *L. infantum (Lin)* DYRK1, for probing its druggability potential. We employed a facilitated null mutant analysis in promastigotes that ectopically express DYRK1 from a plasmid that carries a negative selection marker, and a GFP reporter. Episomal persistence in logarithmic promastigotes after 15 passages under negative selection was used as a readout of essentiality. Our results showed that episome loss was tolerated in logarithmic parasites, suggesting that *Lin*DYRK1 deletion could be compensated. However, early stationary phase *Lin*DYRK1$^{-/-}$ promastigotes displayed a round morphology, mitotic arrest, and subsequent parasite death. Moreover, *Lin*DYRK1$^{-/-}$ parasites showed both reduced thermotolerance to heat-shock, a signal required for stage differentiation, and reduced intracellular survival. Over-expression of *Lin*DYRK1 resulted in G1 cell-cycle phase prolongation. Overall our results suggest that *Lin*DYRK1 is an important pro-survival kinase in stationary growth phase and parasite differentiation, and thus a potential drug target for the prophylaxis against leishmaniasis.