'Real-time' genome evolution in natural populations of *Leishmania donovani* across two successive drug eras

Jean-Claude Dujardin [1], Hideo Imamura[1], Tim Downing[2], An Mannaert[1], Manu Vanaerschot[1]; Maya Berg[1]; James Cotton[3], Shyam Sundar[4], Suman Rijal[5], Matt Berriman[3]

[1]Institute of Tropical Medicine, Antwerpen, Belgium; [2]College of Science, National University of Ireland, Galway, Ireland; [3]Wellcome Trust Sanger Institute, Hinxton, UK; [4]Banaras Hindu University, Varanasi, India; [5]B.P. Koirala Institute of Health Sciences, Dharan, Nepal.

Leishmania donovani causes up to 300,000 new cases of visceral leishmaniasis (VL) each year in the Indian subcontinent (ISC), with an estimated case fatality rate above 10%. The parasite population in the ISC underwent a major evolutionary bottleneck in the 60's during the DDT spraying campaigns and caused a massive epidemic after their interruption. During this period, this antroponotic parasite was submitted to drug pressure, (i) initially with antimonials (SSG), up to their replacement a decade ago because of resistance and toxicity and (ii) nowadays with miltefosine (MIL) in the frame of a regional control program. *L.donovani* from the ISC thus constitutes a unique model for studies of 'real-time' evolution of parasites and its clinical implications: such studies are now possible at an unprecedented depth, thanks to the undergoing 'omic and bio-informatic revolutions. In the frame of two collaborative projects (see kaladrug and Gemini in www.leishrisk.net/leishrisk), we followed cohorts of VL patients from the SSG and MIL eras, both in India and Nepal, collected and phenotyped 196 strains and sequenced their complete nuclear genome (mean 51x coverage). SNP analysis revealed a main and relatively homogeneous group within our sample (in average 173 SNPs/strain in 94 % of the strains), consistent with a post-DDT bottleneck clonal expansion. A few strains originating from Himalayan valleys of Nepal (hence called 'Yeti' strains) were shown to be highly divergent (about 55,000 SNPs away from core group) from the main population and could represent survivors of the pre-DDT bottleneck diversity. The scattering of parasites with a lower susceptibility to SSG or MIL across the SNP-based phylogenetic trees suggests multiple and independent events of drug resistance emergence, likely involving different adaptive mechanisms. In contrast with the SNP homogeneity observed in the main ISC *L.donovani* population, a high diversity was observed at genome structure level, essentially in the form of massive aneuploidy. Interestingly, the degree of aneuploidy was higher among strains of the SSG era than in most recent ones which date from the MIL era. Episomal gene amplification was present in all strains analysed, but amplified genes differed between the main population and Yeti ones and the quantity of amplicons could be related with treatment outcome. Gene dosage appears to be a major and fast adaptive strategy in natural populations of *Leishmania*; functional implications as well as challenges for further genomic studies will be discussed.