Fellow ESR 15 (BRFAA), Project Title: The role of STAT5 target gene networks in leukemia. WP3 aims to investigate aspects of the cross-talk between intrinsic and extrinsic signaling regulating HSCs proliferation/differentiation and to identify novel molecular pathways amenable to therapeutic intervention. In this context ESR15 will investigate STAT5 signaling in AML and the role of STAT5 target gene networks in leukemia progression. Myelodysplastic syndromes (MDS) constitute a varied group of hematopoietic neoplasms, with an increased risk of evolution to acute myeloid leukemia (AML), a progression which occurs in about 30% of patients with MDS. Abnormal Signal Transducer and Activator of Transcription 3/5 (STAT3/5) signaling has been implicated in MDS and AML pathobiology, but the role of STAT5 in MDS, AML and MDS to AML progression is not well understood (Figure). Characterization of critical protein-DNA interactions and targets would thus provide multiple interfaces for potential targeted therapies that would impair not only aberrant growth and proliferation, but also self-renewal of leukemic cells.

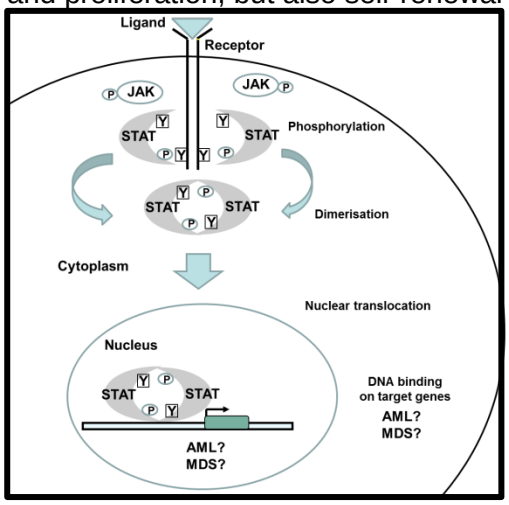

Figure. STAT5 signaling: Abnormal STAT5 signaling has been implicated in MDS and AML. Characterization of STAT5 target genes networks would provide interfaces for targeted therapies and biomarkers for stratification.

The project aims to understand STAT5 mechanisms of action in regulating targets and their functional role in MDS and AML, and to investigate if the characterised targets can be used as biomarkers/therapy targets. The **methodology** includes:

1. ChIP-seq and data analysis: MDS and AML cells will be used in Chromatin IPs (ChIPs) with antibodies against STAT5a and STAT5b. ChIP DNA will be used for library preparation and sequencing on an Illumina Next-Seq 500 platform. Thus, binding sites for STAT5a, STAT5b will be mapped across the entire genome.

2. RNA-seq and data analysis: STAT5a and STAT5b knock-down (KD) cells will be generated and used for RNA-seq on an Illumina Next-Seq 500 platform. RNA-seq data will be analyzed using Tophat2 to align short sequence-reads to the human genome. Transcriptome assembly and gene quantitation/differential expression analysis will be performed with HTSeq-count and DEseq2.

3. RNA-seq and ChIP-seq integration: RNA-seq data in combination with ChIP-seq data will then provide the means of cataloguing up- and down-regulated STAT5 target genes. The pathways linked to the function of the novel targets will be analysed and bioinformatics approaches will combine ChIP-seq, RNA-seq to answer questions on STAT5 target genes cross-talk in MDS/AML. Data will be used for data mining, simulations and will be modelled to create networks. This work will identify the best candidates to be tested as biomarkers.

4. Functional studies: The effects of KD or overexpression of STAT5 and/or selected targets will be investigated in proliferation, differentiation and apoptosis of MDS/AML cells.

4. Expression profiles of selected STAT5 targets: Depending on the findings we will test in MDS/AML samples whether STAT5 targets can be used to molecularly identify and categorize distinct types-stages of MDS/AML and to be used as biomarkers for therapeutic management and monitoring of disease progression.

The ESR15 will be enrolled in the PhD Program in Translational and Molecular Medicine - DIMET at the University of Milano-Bicocca http://www.dimet.org/