Project title: Myeloid neoplasms: clinical and biological implications of genetic drivers, disease modelling and drug discovery
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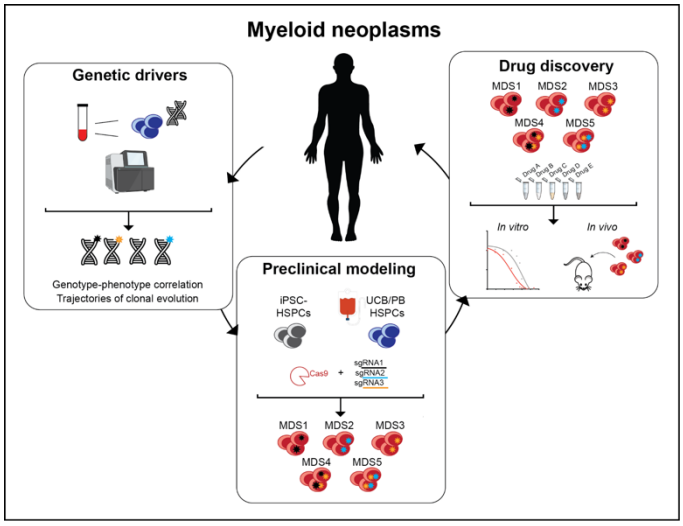
Background



Myeloid neoplasms are clonal hematopoietic stem cells (HSCs) disorders. Myelodysplastic syndromes/neoplasms (MDS) comprise a spectrum of myeloid malignancies characterized by ineffective hematopoiesis, morphologic dysplasia, and risk of progression to acute myeloid leukemia (AML). The development and evolution of myeloid neoplasms is caused by the acquisition of cooperating driver mutations leading to hematopoietic stem and progenitor cells (HSPCs) expansion and aberrant differentiation. The presence of underlying germline variants can predispose to the development of myeloid malignancies and shape the trajectories of clonal evolution. MDS are genetically heterogeneous with combinations of more than 50 distinct genetic lesions in epigenetic regulators, splicing and transcription factors, signal transduction regulators and DNA damage response pathways. Moreover, MDS is characterized by remarkable cellular heterogeneity in the HSPC hierarchy and architecture, known to modulate response to treatments. This heterogeneity has been recognized to be biologically and clinically significant. However, it is unclear how precise co-mutations interact to promote clonal expansion and perturb the hematopoietic hierarchy during disease evolution, due to the lack of human cellular models recapitulating MDS complexity. As such, targeted therapies for MDS remain limited. Our research work is aimed at addressing this unmet clinical need by (i) defining clinical and biological significance of driver mutations in myeloid neoplasms, (ii) developing preclinical models and (iii) identifying novel genotype-specific therapeutic vulnerabilities.

Aims

<u>Aim 1. Defining clinical and biological significance of genetic drivers in myeloid neoplasms</u>
To define the clinical implications of genetic drivers, patients diagnosed and treated according to evidence-based recommendations will undergo a comprehensive genomic profiling for somatic and germline mutations, transcriptomic and splicing analysis, at the time of diagnosis and during the disease course, as well as before and after treatment with disease-modifying agents. The resulting data will be correlated with phenotype variables, relevant clinical outcome measures and response to treatment, with the aim to recognize genetically-defined disease entities and precursor states, to validate non- or minimally-invasive approaches for early disease detection, to develop prognostic/predictive models and develop robust genetically informed criteria to guide therapeutic decision and measure response to treatment.

<u>Aim 2. Developing preclinical models of myeloid neoplasms</u>
To model genetic drivers of MDS development and evolution, we will introduce precise co-mutations in iPSC-derived and primary human HSPCs by CRISPR/Cas9 editing. Functional and molecular changes in gene-edited HSPCs will be assessed through differentiation and self-renewal assays and transcriptomic analysis. Relevant dysregulated genes in the selected genotypes will be functionally tested through genetic rescue experiments and will inform investigation of therapeutic vulnerabilities. In parallel, to identify extracellular context-dependent factors of MDS pathogenesis, we will conduct cytokine profiling and transcriptomic analysis of bone marrow niche cells in genetically-defined MDS

subtypes. Candidate pathways will be functionally tested in gene edited HSPCs and co-culture systems.

Aim 3. Identifying novel genotype-specific therapeutic vulnerabilities

To identify novel genotype-specific vulnerabilities, we will integrate unbiased drug screenings of approved and investigational compounds with targeted drug design. First, a panel of compounds will be tested in hematopoietic cell lines in a dose-response format to identify the half-maximal inhibitory concentration (IC50). Compounds selected from this initial screening will be further investigated in iPSC-derived and primary HSPCs, quantifying their impact on proliferation, self-renewal and differentiation *in vitro*. Finally, leading compounds will be validated *in vivo* using xenograft models of MDS/AML.

Techniques

Next generation sequencing (library preparation and data analysis), hematopoietic cell culture, CRISPR/Cas9 editing and lentiviral transduction, flow cytometry and cell sorting, molecular biology, computational approaches including unsupervised clustering analysis using Hierarchical Dirichlet Processes and machine learning-based approaches.