P1402 ALTERATIONS OF HUMAN BONE MARROW NICHE IN CLONAL HEMATOPOIESIS AND AML

**Topic:** Hematopoiesis, stem cells and microenvironment

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**Background:** Acute myeloid leukemia (AML) is a heterogeneous disease, predominantly caused by acquisition of mutations in the hematopoietic stem and progenitor cells. Some of these mutations are acquired in a preleukemic state termed clonal hematopoiesis of indeterminate potential (CHIP). Recent studies show that alterations in the bone marrow niche can support progression and therapy resistance of myeloid neoplasia. To date, it is unclear when these niche alterations occur and how they contribute to disease progression. In our study, we aim to elucidate alterations in the bone marrow niche, in particular in mesenchymal stem cells (MSC), during the evolution from healthy to CHIP and further to AML.

**Aims:** Primary goal of this project is to examine how hematopoietic and niche cells from different health status (healthy, CHIP and AML) interact with each other and to what extent the presence of clonal hematopoiesis and leukemia have an impact on function and structure of the bone marrow niche.

**Methods:** We collected primary human bone marrow (BM) and peripheral blood (pB) from hematologically healthy individuals who underwent hip replacement surgery at the LMU University Hospital. BM samples from AML patients are provided by the Laboratory for Leukemia Diagnostics, LMU University Hospital. Individuals are assigned to non-CHIP or CHIP cohorts based on targeted mutation analyses on pB samples. To study transcriptional changes in the bone marrow compartments, non-CHIP, CHIP and AML BM samples were FACS-sorted for 10 hematopoietic and non-hematopoietic cell types, and further processed for bulkRNA sequencing. MSC from donor BM samples were isolated via plastic adherence over 10-14 days of in vitro culture. MSC were characterized regarding their surface marker expression (FACS), differentiation (osteogenic, adipogenic, chondrogenic) potential and metabolic activity (Seahorse XF).

**Results:** So far, we have included 98 healthy individuals and 64 have undergone targeted sequencing, revealing 19 individuals (30%) with CHIP. While there was no difference between the gender of the non-CHIP and CHIP groups, individuals with CHIP tended to be of older age (median, 64 y non-CHIP vs. 73.5 y CHIP). Most common mutations were DNMT3A (10/19), TET2 (7/19), ASXL1 (2/19), with variant allele frequencies varying between 1.0% and 16.8%, and 1.42 mutations per individual on average. The amount of mutations per individual increased with age. Analysis of MSCs from non-CHIP (n=5) and CHIP (n=5) individuals revealed no difference in surface marker expression over 10 passages. Differentiation assays of MSCs (non-CHIP=4, CHIP= 2) showed no difference in adipogenic and chondrogenic differentiation after 21 days. For osteogenesis, we noticed a trend towards an increased differentiation potential in the MSCs of CHIP individuals. To gain deeper insights into the BM niche we sorted 4 non-hematopoietic and 6 hematopoietic cell populations from non-CHIP (n=3), CHIP (n=4) and AML (n=4) individuals and performed bulkRNA sequencing on each population. So far changes in the composition of the bone marrow niche were only detected in the AML samples.

**Summary/Conclusion:** Currently, we are analyzing the bulkRNA sequencing data and performing further experiments on cultured MSC to increase group sizes, as well as MSC and HSPC co-cultures in vitro to gain more insight into the bone marrow niche in non-CHIP, CHIP and AML. Additionally, we are continuously recruiting new individuals into...
our study. A comprehensive update on our cohort, sequencing data and MSC characterization will be shown at the conference.