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POSTER 11: Impact of microenvironment changes on NK-cell immunosurveillance during leukemic progression of myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) are clonal disorders of the Bone Marrow (BM), characterized by an immune escape and an increasing excess of leukemic cells (blasts) that in 30% of cases evolve into secondary Acute Myeloid Leukemia (sAML). The Mesenchymal Stromal Cells (MSC) and the Natural Killer (NK) lymphocytes, both impaired in these pathologies, are known to take an active part in the disease evolution. In this study, we sought to modelize the MDS microenvironment, with a particular focus on NK-MSC-blasts cell interactions, to uncover the immunological and environmental changes leading to the disease progression by integrating *in vitro* and *ex vivo* multidimensional data.

To better reproduce cell interactions in the BM niche, we modelized the progressive blast invasion trough 3D *in vitro* cell culture system allowing to co-culture NK cells, MSC from different pathology stages together with increasing number of Molm13 (sAML cell line). Data obtained from multiparametric spectral cytometry, performed on each cell type, and multiplex ELISA were integrated in a statistical model recapitulating the reciprocal impacts of the different cell protagonists during the disease evolution. Single cell RNAseq was performed on *ex vivo* sorted NK, MSC and CD34+ cells on longitudinal samples from MDS patients in progression.

The phenotype and secretome data allowed us to observed major dysregulations of cell-cell adhesion (ex: VCAM1), NK cell functions (ex: DNAM1) and immune regulation (ex: S100A8, CCL2). Interestingly, integrating these data into a statistical model led to the conclusion that the stromal microenvironment, and not blast proliferation, is the major player in those changes. Longitudinal single cell RNAseq data, not only validated most of *in vitro* observations, but also confirmed the progressive loss of function of NK cells trough the evolution of MDS to sAML. *In silico*, we were thus able to reconstruct a three-cell interactome and observed the NK-cell immune defects induced by the microenvironment that could no longer prevent blast proliferation.

The extensive *in vitro* and *ex vivo* deep characterization of NK-MSC-blast interaction allowed us to uncover the main pathways through which microenvironmental changes could affect NK cell response during the disease progression. Altogether, this study proposes an integrative approach deciphering microenvironmental and immunological changes occurring during leukemic transformation of MDS.