Developing in vitro and in vivo models to reveal the molecular and cellular mechanisms of testicular infiltration in pediatric ALL

Tessa Skroblyn[1], **Madlen Pfau**[2], **Uta Höpken**[3], **Cornelia Eckert**[4]
[1]***Max-Delbrück-Centrum für Molekulare Medizin , Charité Pädiatrie Mit Schwerpunkt Onkologie und Hämatologie, Berlin, Germany***
[2]***Pädiatrie Mit Schwerpunkt Onkologie und Hämatologie, Berlin, Germany***
[3]***Max-Delbrück-Centrum für Molekulare Medizin, Tumor- und Immungenetik, Berlin, Germany***
[4]***CC 17: Frauen-, Kinder- & Jugendmedizin Mit Perinatalzentrum, Klinik für Pädiatrie Mit Schwerpunkt Onkologie und Hämato, Berlin, Germany***

Introduction: One critical source of acute lymphoblastic leukemia (ALL) relapse in boys is the testis. To prevent testicular relapse, the molecular factors responsible for the infiltration need to be discovered. Here, we present two models which can support further research.
Method: Stroma cell cultures, derived from human bone marrow (BM) and/or testis from patients with ALL relapse, were characterized by flow cytometry for mesenchymal stem cell (MSC) markers. The ability of the cultured stroma cells to support migration and proliferation was tested by migration and co-culture assays using NALM6 cells. Conditioned medium was analyzed by a multiplex protein assay covering 10 different cytokines or growth factors.
Additionally, primary lymphoblastic leukemia cells obtained from relapse patients were adoptively transferred into NOD/SCID Gamma/J (NSG) mice. Differences in tumor cell load and surface markers derived from BM, spleen and testis were analyzed by flow cytometry. Additionally the testes infiltration was quantified by immunohistochemistry.
Results: Testis-derived stroma cells show a moderate downregulation of MSC markers compared to BMMSC. However, NALM6 proliferation is significant higher, when cultured on testicular stroma. Conditioned medium of both groups induces migration of NALM6 cells, which can be inhibited by blocking the CXCR4 receptor. An association between the level of SDF-1 secretion by the stroma and the migratory capacity of the tumor cells was observed.
NSG mice developed severe ALL within five to seven weeks after xenograft transplantation. At the time of sacrifice, BM and spleen are highly infiltrated with leukemia cells, whereas the tumor load in testes is low. Particularly older mice show no testicular involvement. In young animals before puberty leukemia cells are more frequently detected in this extramedullary location.
Conclusion: While BMMSCs are a widely studied in leukemia research, testis derived stroma cells can complement relapse studies. Their ability to support proliferation and migration defines them as an important component in the testicular niche in pediatric ALL. Since *in vitro* models are limited in revealing complex molecular mechanisms an *in vivo* mouse model for testicular involvement is warranted.