Bone marrow assembloids – an ultimate three-dimensional model to study hemato-oncologic dissorders

Janstová L.[1,2], Janoušková K.[3], Švubová V.[1,2], Brodská B. [1], Starková, J.[3], Frič J.[1,4,5]

[1] *Institute of Hematology and Blood Transfusion, Prague, Czechia*
[2]*Faculty of Science, Charles University, Prague, Czechia*
[3]*CLIP – Childhood Leukaemia Investigation Prague, Prague, Czechia; Dept. of Pediatric Hematology and Oncology, Second Faculty of Medicine, Charles University, Prague, Czechia*
[4]*International Clinical Research Center of St. Anne's University Hospital in Brno (FNUSA-ICRC), Brno, Czechia*
[5]*Faculty of Medicine, Masaryk University, Brno, Czechia*

Leukemia cells can interact with different cell types within the bone marrow (BM) microenvironment, including mesenchymal stromal cells, endothelial cells, and immune cells. These interactions can promote leukemia cell survival, proliferation, and resistance to chemotherapy. Furthermore, the BM environment can also influence leukemic cells, affecting their metabolism and drug response. In order to study this interplay and address specific questions, we are developing a unique three-dimensional *in vitro* model of BM assembloids, mimicking the leukemic niche. This model allows us to explore leukemic cell behaviour, drug sensitivity, and interactions with immune cells like NK cells.

In our pilot experiments, we created BM assembloids using immortalized mesenchymal stromal cell line hTERT MSCs, endothelial cells HUVECs, and two types of leukemic cell lines (RS411, NALM-6 for ALL, THP-1 for AML). We individually labeled all cell lines with different CellTrace (CT; Invitrogen) dyes before co-culturing. MSCs and HUVECs were mixed in a 1:1 ratio in Aggrewell 400 (STEMCELL). After 3 days, some assembloids were analyzed using confocal microscopy. Next, leukemic cells were introduced into the remaining assembloids at a 1:1:1 ratio (MSCs:HUVECs:leukemic cells), and co-culture continued for 4 more days. Afterward, we used confocal microscopy and flow cytometry to examine the assembloids, particularly assessing the migration of leukemic cells toward the BM assembloids.

The initial experiments yielded promising results, demonstrating that MSCs and HUVECs are capable of creating cohesive, three-dimensional structures. Notably, in a pilot study leukemic cells exhibited a migration

response towards these assembloids and maintained viability over a 4-day period. This extended viability period offers a valuable opportunity to investigate various aspects, including the metabolism of leukemic cells, their sensitivity to drugs, and their interactions with NK cells.
This robust methodology also paves the way for the development of personalized models using patient samples. This advancement holds potential for enhancing our understanding of hematological disorders and their treatment strategies, marking a significant step forward in the field of hematology research and therapy.

Supported by MH CZ – DRO (Institute of Hematology and Blood Transfusion – ÚHKT, 00023736).