Hematopoiesis has been for many years seen as a straightforward process based on sequential restriction of cell fate potential leading to production of mature blood cells. In the last decade, however, several works documented an unexpected plasticity of hematopoietic cells with expanded potential of myeloid development from lymphoid progenitors and vice versa. Under physiologic conditions hematopoiesis is tightly controlled and the definite cell fate is denominated by multiple factors that all lead to changes in regulatory networks that include transcription factors, epigenetic changes and post-transcriptional modulations. Any disruption of this strict regulation, caused by mutations or other events, affects the proliferation and lineage fidelity of hematopoietic precursors. This may lead to clonal growth of variable significance or leukemogenesis and may possibly affect the treatment sensitivity of the hematological malignancies.

For better understanding of hematopoietic regulation we described gene expression changes during physiological development of lymphoid and myeloid lineages and in leukemic specimens using our own simplified real-time PCR based platform. We investigated expression of 95 genes connected with lymphoid and myeloid differentiation or with leukemogenesis in sorted hematopoietic progenitors and their malignant counterparts. We also investigated the expression changes in separate subpopulations of the newly described subset of childhood ALL - lineage switching leukemia and confronted them with observations in nonmalignant populations. Further, using flow cytometry, Ig/TCR rearrangements detection, cytogenetics and mutation studies and methylation status of *CEPBA* promoter we described this specific subset within its molecular context including phenotypic and genotypic peculiarities or epigenetic changes. Finally, using massively parallel sequencing we evaluated mutations in another unit from the spectrum of hematological malignancies - high risk MDS. Using sequential sampling before and during hypomethylation treatment with azacitidine we were able to evaluate mutations dynamics within the disease course.

We identified several lineage associated genes being upregulated during particular population development. *PAX5*, *FOXO1*, *TCF3*, *BCL11A* and others were upregulated during B-lymphocytes evolution whereas *BCL11B*, *TCF7*, *HOXB4*, *NOTCH3* are examples of T-cell fate regulators; besides others CEBP family, *ID2*, *KLF4* or *MNDA* were detected during myeloid maturation. Several significant differences were also observed between healthy and malignant populations. Besides the expected HOX family or *FLT3*, the most striking contrast was detected in expression levels of *CCDC26* and *PAWR*, which both correlate with therapy response in patients with ALL. We further described a rare phenomenon of lineage switch in ALL that mostly occurs within days 1 to 33 of induction regimen. All switching samples were

$CD2^{pos}$ at time of diagnosis, which was not true for non-switching leukemias. After the switch, the formed monocytoid population presented with the same phenotype and mRNA expression profile as normal monocytes from healthy controls. However, leukemia specific immunoglobulin rearrangements were detected in this population proving its leukemic provenience. Our expression platform depicted CEBPA as a possible key regulator of the lineage switch, which was further supported by the finding of hypomethylation of *CEBPA* promoter. No other common molecular or cytogenetic markers were identified in our cohort. Using the above mentioned mRNA expression data from healthy and leukemic specimens we prepared publicly available LeukoStage.org database. Finally, we described mutation architecture dynamics in MDS patients during hypomethylation treatment and identified several dynamics patterns correlating with the disease course. We were able to depict mutations that preclude resistance to the demethylation therapy or those selected by the treatment. We were also able to predict ongoing clinical relapse.