# Detection of recurrent somatic variants in cell-free DNA as a tool for disease monitoring in Hodgkin lymphoma

Jan Grohmann, Alexandra Kredatusova, Jana Navratilova, Veronika Hanackova, Helena Urbankova, Vit Prochazka, Tomas Papajik

Department of Hemato-oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital Olomouc, Olomouc, Czech Republic

**Background:** A significant challenge in the healthcare management of HL is finding the optimal balance between treatment efficacy and risk of toxicity. Nowadays, there is no reliable and precise tool for the evaluation of treatment response and MRD monitoring due to the scarce presence and difficult availability of neoplastic cells. The cell-free DNA (cfDNA) in HL reflects its mutational profile and could be a source for genotyping assays.

**Methods:** Our cohort consisted of 58 pts: 26 females/32 males; the median age at diagnosis was 39.5 years. A specific NGS panel covering coding sequences of 13 selected genes was designed. For library preparation, we used SureSelect XT HS2 technology (Agilent Technologies). Sequencing was performed on a NovaSeq6000 (Illumina). Data were analysed with the SureCall software (Agilent Technologies) with a sensitivity of 1,0 % VAF. The detected variants were annotated using COSMIC, dbSNP, Ensembl, and ClinVar. Selected variants were further monitored by dPCR (QIAcuity Digital PCR System; Qiagen) with a sensitivity of 0,1 % VAF.

**Results:** Mutations were detected in 33/58 (56,89 %) pts. The most frequently mutated genes were STAT6 (15/58 pts), TNFAIP3 (12/58 pts), XPO1 (8/58 pts), and SOCS1 (12/36 pts). Frameshift deletions prevailed in TNFAIP3 and SOCS1 genes. Most mutations in the STAT6 (p.N417Y/D) and XPO1 (p.E571K) genes were hotspots. We monitored levels of these variants by dPCR during the course of the disease and correlated results with clinical and PET-CT data.

**Conclusion:** Fast, sensitive, and non-invasive detection of mutations means an essential improvement in diagnostics, prognostics, and monitoring of HL. The NGS/dPCR approach would fundamentally refine the evaluation of treatment response. The correlation of mutational load with continuous PET examination would reduce the amount of false-positive results and enable us to use more precise and safe therapy de-escalation. DPCR proved to be a sensitive, fast, and affordable technology for MRD testing.

**Supported:** IGA_LF_2024_001, MZCZ-DRO(FNOL,00098892), Ministry of Health grant NU22-03-00182