**The use of a targeted RNA sequencing-based approach for the detection of clinically relevant fusion genes in pediatric cancer patients**

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Childhood cancers are among the rare diseases with an annual incidence of around 350-400 new cases in the Czech Republic and represent the second most common cause of death in this age group. With significant advances in molecular profiling techniques and their successful implementation within various precision oncology programs and pediatric pan-cancer profiling initiatives, different NGS-based methods are becoming more routinely used in a clinical setting. Fusion gene analysis is of great importance for diagnostic and prognostic stratification and therapeutic planning among these methods. Between September 2019 and May 2022, 243 pediatric cancer patients underwent fusion gene analysis using targeted RNA sequencing. In 210 cases, the analysis was carried out as a part of a complex tumor profiling within a precision oncology program. Sequencing libraries from both fresh-frozen and FFPE tissue were prepared using TruSight RNA Pan-Cancer Panel (Illumina) covering 1385 cancer-associated genes and sequenced on NextSeq 500 platform using NextSeq Mid Output Kit (150 cycles) (Illumina). Raw reads were quality checked with the FastQC package (version 0.11.9). Adapter sequences were identified and trimmed with the Trimmomatic tool. Trimmed reads were then mapped to reference genome hg38 using a STAR aligner with parameters set to allow fusion gene detection. Mapping quality was checked using QualiMap and Picard tools. Fusion genes were identified using Arriba and STARfusion. Visual verification of identified fusion genes was performed using IGV software. In 26 % of cases, a clinically relevant fusion gene was identified. The most commonly found fusions included known drivers of sarcoma tumorigenesis, such as *EWSR1*-*FLI1*, *PAX3*-*FOXO1*, and *SS18*-*SSX1*/*2*. The second largest group consisted of fusion genes associated with CNS tumors, mainly low-grade gliomas, e.g., *KIAA1549*-*BRAF* or other MAPK-activating fusions. Approximately one-third of identified fusion genes were therapeutically actionable. In one case, a novel fusion gene *DVL3*-*TFE3* was identified, relevant to the renal cell carcinoma diagnosis of the analyzed patient. Targeted RNA sequencing has proven to be a sensitive and feasible strategy contributing to patient stratification and treatment selection. Its ability to detect both clinically established and novel fusion genes is superior to PCR-based approaches that show only a limited throughput. Further use of this method will allow a better understanding of cancer biology.