00230 Detection of BCOR Exon 15 Internal Tandem Duplication in Formalin-fixed Paraffin- Embedded Sections of Clear Cell Sarcoma of Kidney by Direct PCR and Gel Electrophoresis

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Aims: Clear cell sarcoma of kidney (CCSK) has an exon 15 internal tandem duplication (ITD) in a majority of cases. Various duplication breakpoints (DB) have been reported in a hotspot region of the last 60 coding nucleotides of exon 15 of BCOR. As formalin-fixed paraffin-embedded (FFPE) tissue contains degraded and shorter fragments of genomic material, it can be challenging to cover all DB with only a single set of primers. In this report, we describe a direct PCR method utilizing two sets of primers. This technique can detect all described DB in the hotspot region of BCOR.

Methodology: We extracted DNA from FFPE material of 15 CCSK cases with known BCOR ITD, five neuroblastomas, and five Wilms tumours. We performed PCR amplification utilizing two sets of primers flanking the DB, generating 98 bp and 112 bp amplicons. The amplicons were resolved by electrophoresis using a 3% agarose gel. The presence of a second amplicon of doubled size that is identified along with the expected 98 bp or 112 bp amplicons indicates the presence of a BCOR ITD event.

Result: All 15 CCSK cases demonstrated second amplicons of doubled size together with the 98 bp and 112 bp amplicons. The BCOR ITDs were confirmed by Sanger sequencing. None of the neuroblastomas and Wilms tumours had second amplicons. The turnaround time was one day.

Conclusion: We successfully detected all ITD events in BCOR exon 15 ITD-positive CCSK in FFPE specimen by the use of two sets of primers. This direct PCR method is quick, and sensitivity and specificity are both 100%. This assay can be set up in a hospital pathology department with PCR capabilities to enhance diagnostic accuracy of CCSK in the clinical setting.