Validation and clinical application of a targeted next-generation sequencing gene panel for solid and hematologic malignancies

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ABSTRACT

Background. Next-generation sequencing (NGS) is a high-throughput technology that has become widely integrated in molecular diagnostics laboratories. Among the large diversity of NGS-based panels, the Trusight Tumor 26 (TsT26) enables the detection of low-frequency variants across 26 genes using the MiSeq platform.

Methods. We describe the inter-laboratory validation and subsequent clinical application of the panel in 399 patients presenting a range of tumor types, including gastrointestinal (GI, 29%), hematologic (18%), lung (13%), gynecological and breast (8% each), among others.

Results. The panel is highly accurate with a test sensitivity of 92%, and demonstrated high specificity and positive predictive values (95% and 96%, respectively). Sequencing testing was successful in two-thirds of patients, while the remaining third failed due to unsuccessful quality-control filtering. Most detected variants were observed in the *TP53* (28%), *KRAS* (16%), *APC* (10%) and *PIK3CA* (8%) genes. Overall, 372 variants were identified, primarily distributed as missense (81%), stop gain (9%) and frameshift (7%) altered sequences and mostly reported as pathogenic (78%) and variants of uncertain significance (19%). Only 14% of patients received targeted treatment based on the variant determined by the panel. The variants most frequently observed in GI and lung tumors were: *KRAS* c.35G > A (p.G12D), c.35G > T (p.G12V) and c.34G > T (p.G12C).

Conclusions. Prior panel validation allowed its use in the laboratory daily practice by providing several relevant and potentially targetable variants across multiple tumors. However, this study is limited by high sample inadequacy rate, raising doubts as to continuity in the clinical setting.

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