Optimizing Clinically Actionable Biomarker Detection in Advanced Cancers by Next Generation Sequencing (NGS) Panel and Specimen Type

Husain, H[1], Troxel, S[2], Urban, B[2], Kutumbaka, K[2], Sathyan, P[3], Lococo, J[3], Tierno, MB[3], Weinroth, M[3], Moss, TJ[2], Eterovic, AK[2]

1. Moores Cancer Center at UC San Diego Health, La Jolla, CA 1.
2. Eurofins Viracor Biopharma, Lenexa, KS
3. Illumina Inc., San Diego, CA

Background:

There are many factors to consider when choosing the optimal NGS approach for successful biomarker testing. We present NGS data from 82 advanced solid tumor cases to illustrate how different NGS panels [small amplicon-based or comprehensive genomic profiling (CGP)] and specimen types [tumor tissue or circulating tumor DNA (ctDNA)] can influence detection of actionable variants for clinical decision-making.

Methods:

Matched ctDNA and tumor tissue specimens from 82 patients with advanced solid tumors were sequenced using the hybrid-capture TruSight™ Oncology 500 (TSO500) tissue and ctDNAv1 CGP (523 genes DNA, 55 genes RNA) assays and the amplicon-based oncoReveal™ Core LBx (104 genes) and multi-Cancer DNA (60 genes DNA) and RNA (18 genes) panels. Oncogenic variants were identified using the respective panels' bioinformatics pipelines.

Results:

Concordance between tissue and ctDNA variant detection with TSO500 CGP was influenced by tumor type and ctDNA fraction. 6,360 total COSMIC variants were identified: 1,998 in ctDNA only, 642 in tissue only, and 3,720 in both. 52% of cases had ctDNA fractions <1%, 30% had ctDNA fractions 1-10%, with 18% of cases >10%. Colorectal (CRC) and non-small cell lung cancers (NSCLC) had the highest number of cases with ctDNA fraction >1%. Higher ctDNA fraction correlated with greater concordance between tissue and ctDNA findings and increased sensitivity. Tissue-only variants were predominantly observed in cases with low ctDNA fractions, emphasizing the value of tissue testing in clinical scenarios with low estimated ctDNA fraction. ctDNA-only variants reflected tumor heterogeneity, indicating a maximized yield of oncogenic variant detection when testing both specimens.

Select clinical cases further illustrate how testing results are influenced by NGS strategies. An actionable *KRAS* G12C variant was detected by all NGS panel and specimen types in CRC and NSCLC cases. However, a *LANCL2-EGFR* fusion was detected in tissue and ctDNA specimens from a gastric cancer case exclusively by the hybrid capture-based TSO500 panel, due to greater gene coverage and detection of novel fusions. Clinical relevance was confirmed by 6,000-8,000-fold

higher *EGFR* expression levels, suggesting sensitivity to EGFR inhibitors. A *PIK3CA* E545K resistance mutation to EGFR inhibitors in a CRC case was found in ctDNA by both NGS panels but not in tissue, highlighting utility of ctDNA in capturing tumor heterogeneity.

Conclusions:

NGS panel and specimen selection must be tailored to the actionable variant classes specific to the individual's tumor type and specific clinical scenarios impacting ctDNA fraction and tumor content. Hybrid-capture CGP panels can outperform small amplicon-based panels in detecting fusions and gene signatures, while ctDNA testing identifies tumor heterogeneity in clinical scenarios with higher estimated ctDNA fraction.