INTENDED CLINICAL USE

AURASEQ-FUSIONS

METHODOLOGY

Next Generation Sequencing

CLINICAL UTILITY

The concept of precision medicine consists in the accomplishment of therapy individualized to each tumor by exploiting these alterations as predictive biomarkers as well as targets of therapy, such as tyrosine kinase inhibitors (TKIs) (1). The utilization of next generation sequencing (NGS) based testing in clinical practice is adding new dimensions to molecular testing for cancer patients. The expanding use of NGS now allows us to detect gene fusions, such as those driven by ALK, ROS1 and NTRK in lung cancer and other tumor types. Such fusions, include gene partners that often encode protein kinases, which are the molecular targets of TKIs. In addition to gene fusions, other genomic alterations such as single nucleotide variants (SNVs), small insertions and deletions (Indels), and copy number variants (CNVs) have been identified as molecular targets of TKIs as well. Effective targeted therapeutics have been FDA-approved for these variants and many more are currently in clinical trials. Thus, the AuraSeq-Fusions panel, a highly multiplexed assay based on an NGS platform, allows the simultaneous identification of drug targets caused by different genomic abnormalities, assisting physicians to select personalized therapies (2).

INTENDED USE

The intended use of this assay is to detect somatic SNVs, small Indels, CNVs and fusions across 52 genes using NGS technology in metastatic or advanced lung adenocarcinoma (NSCLC) (3), prostate cancer, bladder cancer, thyroid tumors (4), soft tissue sarcomas and other advanced carcinomas who had failed to respond to conventional chemotherapy or are about to start a TKI regime. The AuraSeq-Fusions panel can be used on formalin-fixed, paraffin embedded (FFPE) tissue sections on an organ basis, as follows:

Lung adenocarcinoma: Following NCCN, CAP, IASLC, and AMP guidelines (5), the AuraSeq-Fusions test is indicated for the management of all patients with nonsquamous NSCLC, and it may be considered in cases of squamous histological findings with unique clinical phenotypes (e.g., in never-smokers or in patients with mixed adenosquamous subtypes), as it surveys hotspot regions of 35 cancer-related genes, including EGFR (including T790M), KRAS, NRAS and BRAF. In addition, the AuraSeq-Fusions test can detect the presence of 23 different gene drivers in 270 gene fusions relevant to NSCLC, such as those involving ALK, ROS1, MET (including MET exon 14 skipping), RET, NTRK1, NTRK2, NTRK3, etc…

Prostate cancer: Three fusion genes have been characterized in prostate cancer, occurring in 50% to 70% of patients (6). These fusions join the androgen-regulated promoter for the TMPRSS2 gene with the ERG, ETV1 and ETV4 genes, which lead to the overexpression of these oncogenic transcription factors in an androgen-regulated manner. Similarly fusions containing another androgen-regulated promoter gene (SLC45A3) and the ERG, ETV1 and ETV4 genes are also present in prostate cancer cases. Recently, a fusion of the SLC45A3 gene and the tyrosine kinase encoding FGFR2 gene has been reported to be targetable by TKIs. Thus, the AuraSeq-Fusions panel can detect all major fusions expressed in this cancer type.

Bladder cancer: Bladder cancer is associated with activating mutations in the FGFR3 gene, including the FGFR3-TACC3 fusion, which occurs in about 10% of patients (7). This fusion was found to promote MAPK signaling in bladder cancer cell lines, as well as increased cell proliferation and transformation. In addition, the FGFR3-BAIAP2L1 gene fusion has been described to be expressed in the bladder, liver, testes, heart, and lung. Thus, the AuraSeq-Fusions panel can detect all major fusions expressed in this cancer type.

Thyroid cancer: Following the American Thyroid Association's (ATA) guidelines, nodule FNA cytology results showing atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), or Bethesda category III with an associated risks of cancer of 10% to 30% (8), are recommended to undergo mutational testing to resolve the uncertainty of this category. A comprehensive genetic characterization of papillary thyroid carcinoma (PTC) identified genetic alterations in ~97% of tumors. Specifically, BRAFV600E mutations have been described in classic papillary, whereas RAS mutations were predominant in encapsulated
follicular variant PTC. In addition, NTRK1 rearrangements have been described in PTC (9), as well as a rare chromosomal rearrangement in sporadic thyroid cancer, but more frequent in radiation-related tumors, the ETV6-NTRK3 gene fusion. As opposed to PTC, in follicular thyroid carcinoma the PAX8-PPARG fusion has been identified in 60% of such cases. Thus, the AuraSeq-Fusions panel can detect all major genomic abnormalities present in thyroid cancers.

**Glioblastoma:** The WHO has recently recommended an integrated diagnosis to classify glioblastoma (GBM) as IDH1/IDH2 mutant, or IDH-wildtype which corresponds to a resistant-to-treatment disease with a dismal prognosis and standard of care that includes concomitant radio-chemotherapy. Some histological variants of GBM IDH-wildtype have been found to carry targetable oncogenic drivers such as EGFR amplification (its active mutant EGFRvIII), BRAF V600E mutation and the FGFR3-TACC3 gene fusion, a potentially targetable biomarker. The FGFR3-TACC3 fusion is an oncogenic driver mutually exclusive with IDH mutations and EGFR amplification (10). Gene fusions occur in near 30 - 50% of GBM cases, and NTRK1 fusions have been described among them. Thus, the AuraSeq-Fusions panel can detect all major genomic alterations described in GBM.

**Sarcomas and other uncommon tumors:** Recent studies have shown that 38% of NTRK fusion-positive tumor types corresponded to a diagnosis of sarcoma including soft tissue, infantile fibrosarcoma and GIST. Overall, the estimated prevalence rate of NTRK fusions in sarcomas ranges from 1% in adults to 92% in patients with congenital fibrosarcoma, being the ETV6-NTRK3 fusion he most common. Secretory breast cancer and mammary analog secretory carcinoma (MASC) of the salivary gland are rare tumors with distinct clinical and pathological features. However, they also harbor the ETV6-NTRK3 fusion in 92% and 100% of secretory breast cancer and MASC cases, respectively (11). Thus, the AuraSeq-Fusions panel can detect all major gene fusions described in these rare cancers.

**REFERENCES**