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NEWSLETTER

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What's new in molecular genetic pathology 2021: solid tumors and NGS panel selection

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Abstract

The linchpin of precision medicine is molecular genetic and genomic testing. Molecular biomarkers are important for establishing precise diagnoses and for predicting therapeutic responses that enable cancer patients to receive personalized and targeted treatment. Below are highlights of the current considerations in next generation sequencing (NGS) panel selection, and in molecular testing of solid tumors of the lung, digestive system, thyroid and soft tissue.

NEXT GENERATION SEQUENCING PANEL SELECTION

- The use of targeted NGS panels (comprising up to several hundred genes) is more common than whole exome sequencing (WES) in clinical practice. Pros and cons of WES or large targeted panels compared to small hotspot panels include the following:
  - Comprehensive tumor profiling maximizes the chances of uncovering clinically relevant alterations.
  - They require more tumor tissue, cost more for data analysis and storage, and entail more complex interpretation.
- FDA-approved liquid biopsy using circulating cell-free DNA is an excellent tool when tumor tissue is inaccessible or limited [1]. While liquid biopsy has a faster turnaround time, it has a higher false-negative rate than standard biopsy NGS.

NON-SMALL CELL LUNG CARCINOMA (NSCLC)

- Molecular profiling is used to predict therapeutic efficacies in NSCLCs and typically includes *EGFR, KRAS, ROS1, ALK, MET, BRAF, RET, ERBB2 (HER2)*, and *NTRK* [2]. Also useful is PD-L1 (programmed cell death ligand-1) by immunohistochemistry (IHC) to predict response to immune checkpoint inhibitors.
- *EGFR* mutations involve 10%-35% of lung adenocarcinoma in Western populations (and as high as 50% in Asian populations) and 3% of lung squamous cell carcinoma (SqCC). Mutations can predict therapeutic response to EGFR-specific tyrosine kinase inhibitors (TKIs).
  - Acquired resistance inevitably develops, mostly commonly due to *EGFR* T790M missense mutation, accounting for ~50% of acquired resistance mutations. FDA-approved EGFR-inhibiting drugs that specifically target T790M or exon 20 insertion mutation are available.
  - *MET* amplification (by FISH or NGS) is responsible for 5%-20% of anti-EGFR resistance.
- *KRAS* mutations are detectable in 25%-35% of lung adenocarcinoma, and 5% of SqCC. Activating mutations in codons 12 and 13, and, less commonly, codon 61, predict unfavorable prognosis and EGFR-TKI resistance. A KRAS inhibitor drug that specifically targets the G12C missense mutation (seen in ~13% of NSCLCs) is available.
- *ALK* activating rearrangements occur in ~5% of lung adenocarcinoma, particularly in young non-smokers. The most common fusion gene product is *EML4-ALK*, which predicts response to ALK inhibitors. FISH is the gold standard for testing, but IHC, RT-PCR and NGS can also be used.
- *ROS1* gene fusion (1%-2% of NSCLCs) signifies response to certain ALK inhibitors due to homology between the rearranged *ROS1* and *ALK* genes.
- *MET* exon 14 skipping mutations, *BRAF* V600E mutation, *RET* gene fusion and *NTRK* gene fusion can predict therapeutic response to their respective inhibitors.

COLORECTAL CARCINOMA (CRC) AND CHOLANGIOCARCINOMA

- EGFR targeted treatment is effective in the absence of *KRAS* and *NRAS* mutations. Mutational analyses of *KRAS* and *NRAS* genes should include codons 12, 13, 59, 61, 117 and 146. Other drug resistance biomarkers include *BRAF* mutations and *PIK3CA/PTEN* deregulation.
- Despite its low prevalence in CRC (2%–3%), *ERBB2 (HER2)* amplification is emerging as a potential therapeutic target [3]. Furthermore, activation of *ERBB2 (HER2)* signaling causes resistance to anti-EGFR therapy in a subset of patients with metastatic CRC.
- *NTRK* fusions can be evaluated in CRC due to the availability of targeted therapies.
- *FGFR* gene fusion is detected in ~10% of intrahepatic cholangiocarcinoma for which two FGFR-targeted tyrosine kinase inhibitors have been approved - pemigatinib and infigratinib.

THYROID CARCINOMA

- Papillary thyroid carcinoma may harbor *BRAF* mutations (especially V600E), *TERT* promoter mutations, and, less commonly, *RET/PTC1/2/3* rearrangements and *RAS* mutations.
- *PAX8-PPARγ* fusion, *RAS* mutations, and *TERT* mutations are enriched in follicular thyroid carcinoma, while *BRAF* V600E is unusual. *RAS* mutations are also seen in a minority of benign follicular adenomas.

- More than 60% of medullary thyroid carcinomas show somatic *RET* mutations.
- Anaplastic thyroid carcinomas can harbor *TERT, TP53* and/or *BRAF* mutations.
- Preoperative molecular testing is valuable in thyroid nodules with indeterminate FNA cytology. The gene panel typically includes *TERT, BRAF, PAX8/PPARγ, RAS, RET/PTC* and *TP53*. Below are three commonly used commercial platforms:
  - Thyroseq™ involves next-generation DNA and RNA sequencing of 112 genes to stratify thyroid nodules as likely benign or likely malignant.
  - Afirma® Gene Sequencing Classifier is an RNA-based test. Similar to ThyroSeq™, it is mostly a rule-out assay with acceptable rule-in capability.
  - ThyGeNEXT®/ThyraMIR® uses a combination of two tests. If no mutation is found in the first panel by DNA and RNA sequencing, another test is performed using microRNA expression.

BONE AND SOFT TISSUE TUMORS

- Molecular testing has led to the discovery of new mesenchymal tumor entities. For example, *CIC-DUX4* sarcoma is a recently described small round blue cell tumor associated with more aggressive disease than Ewing sarcoma with characteristic *EWSR1-FLI1* fusion. Immunohistochemical detection of ETV4, a transcriptional target of *CIC-DUX4*, is a useful diagnostic tool.
- Amplification of the *MDM2* gene detectable by IHC/FISH/NGS is helpful for confirming a diagnosis of atypical lipomatous tumor/well-differentiated liposarcoma (~93%) or dedifferentiated liposarcoma (~97%). The oncogenicity of *MDM2* is related to *TP53* inactivation (Fig. 1).
- Gene fusion testing by FISH or NGS is helpful for classifying rhabdomyosarcomas [4].
  - Recurrent gene fusions are detected in ~80% of alveolar rhabdomyosarcoma (ARMS), and involve mostly the *FOXO1* gene fusing with either *PAX3* or *PAX7*. Fusion positive ARMS is associated with a worse prognosis than fusion negative ARMS.
  - Embryonal rhabdomyosarcoma (ERMS) characteristically lacks gene fusions, but shows chromosomal losses/gains and gene mutations. ERMS is similar to fusion negative ARMS in clinical behavior.
  - Most congenital/infantile spindle cell and sclerosing rhabdomyosarcomas show *NCOA2* in addition to *VGLL2* gene fusions. This entity is associated with a favorable prognosis. However, patients who harbor *MYOD1* mutations tend to have aggressive disease.

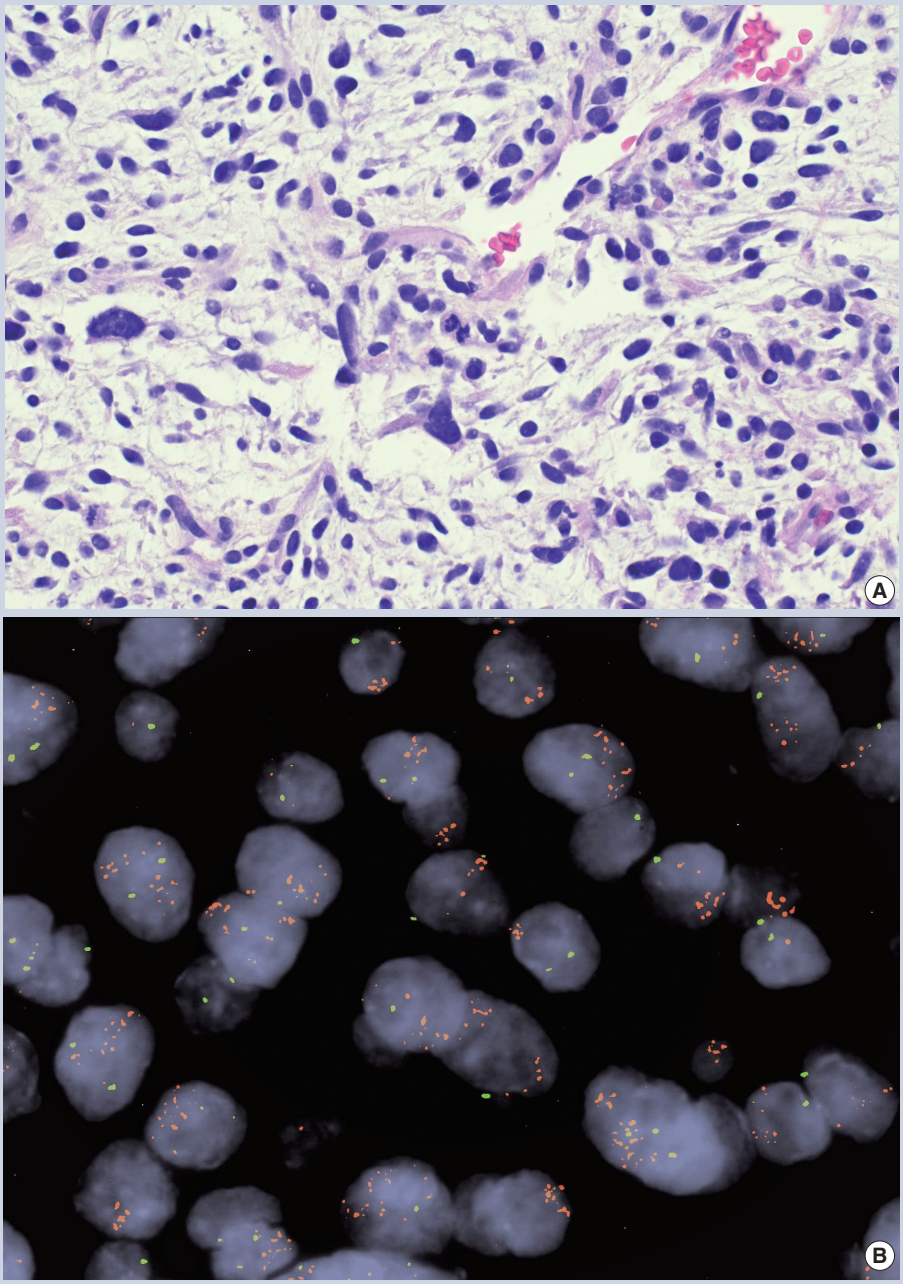

Fig. 1. Dedifferentiated liposarcoma with neoplastic spindle cells in perigastric mass. (A) H&E stain, 40 x. (B) FISH shows abnormally amplified *MDM2* gene (orange signals) relative to CEP12 control (green). *MDM2*/CEP12 ratio > 2.0:1 normal cut-off.

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Meet the Authors

Dr. Chen is an Associate and Attending pathologist at Geisinger Medical Center, where he participates in clinical, academic and teaching services in surgical pathology and molecular diagnostics. He has actively published and lectured on topics related to molecular pathology of solid tumors.

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