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Traditional Diagnostics versus Disruptive Technology: The role of the pathologist in the era of liquid biopsy

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Abstract

Precision medicine in cancer care is predicated on access to several fundamental pieces of data: (1) a precise tumor diagnosis, (2) accurate stage classification, and (3) protein or molecular biomarkers that predict efficacy of targeted therapies. For all cancer patients, these data points are generated by obtaining a tumor sample and subjecting it to analysis by a pathologist and, when appropriate, a molecular pathologist. While tumor diagnosis and pathologic staging (gross and microscopic examination of the primary tumor and draining lymph nodes) require the infrastructure and expertise of an anatomic pathology program, the advent of "liquid biopsy" has driven a shift in molecular biomarker testing away from local pathology labs and into high-throughput, centralized (and often for-profit) laboratories. What does this mean for patient care? How is the role of the pathologist affected? What are the implications for integration of diagnostic information and ultimately for appropriate therapy selection? This perspective will consider the current testing landscape, address current challenges in the use of liquid biopsy in clinical practice, and consider ways the pathologist should be involved in interpreting liquid biopsy data in the context of the patient's cancer diagnosis and stage.

Here we focus on cancer patients with solid tumors arising outside of the central nervous system. Liquid biopsy, in this context, is referring to detection of tumor-derived circulating DNA (ctDNA) within the patient's plasma. Tumors shed variable amounts of DNA into the circulation; the degree of shed depends in part on the tumor type (e.g. advanced colon carcinoma and small cell lung carcinoma appear to shed more than other solid tumors) and on the burden of disease (in lung cancer patients, distant metastatic spread correlates with greater ctDNA shed than disease confined to the chest). This ctDNA represents only a fraction of total cell free DNA (cfDNA) within the circulation; in most patients the majority of cfDNA represents genomic DNA generated from turnover of normal myeloid and lymphoid cells. For most patients with metastatic cancer, ctDNA represents 1% or less of total cfDNA; this poses a significant challenge for detection of tumor-derived variants and

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has only been made possible by significant advances in PCR and sequencing techniques that can accurately detect DNA changes at this level and below. Targeted next generation sequencing (NGS) assays optimized for sensitive and specific somatic variant detection can have broad applications in biomarker detection in cancer patients; hybrid capture NGS approaches in particular can detect a full array of genomic alterations including single nucleotide variants, insertion-deletions, copy number changes, and rearrangements, and can be optimized for variant detection from a liquid biopsy.

For patients with advanced non-small cell lung cancer (NSCLC) where genotyping is essential, it is estimated that about a third of patients cannot get appropriate testing when relying solely on tissue specimens.[1] Failure to obtain essential biomarker data- a particularly challenging issue for patients with non-small cell lung cancer- is a function of tumor tissue inadequacy or inaccessibility. The published literature indicates that a liquid biopsy can provide actionable biomarker data for at least a subset of patients who cannot otherwise undergo tissue molecular profiling[1] and that liquid biopsy is thus serving a much-needed role in clinical practice. Given the current excellent specificity of liquid biopsy testing, identification of certain genomic alterations in a well-defined disease context can permit the treating physician to make a confident decision about biomarker-driven targeted therapy. Examples include detection of *EGFR* p.L858R mutation in a patient with a diagnosis of NSCLC leading to selection of EGFR tyrosine kinase inhibitor therapy, or *NRAS* p.Q61K mutation in a patient with metastatic melanoma leading a clinician to forego use of combined RAF inhibitor therapy. In the context of relapsed disease, particularly for targeted therapies, the summative nature of a liquid biopsy may be ideally equipped to deliver information about potentially heterogeneous mechanisms of therapeutic resistance arising across sites of disease, without requiring that the patient undergo multiple invasive procedures. The value of this approach in detecting both on-target and bypass mechanisms of resistance has been demonstrated in NSCLC patients receiving tyrosine kinase inhibitors for targets including EGFR, ALK, and RET, as well as in detection of reversion mutations patients with BRCA1/2 mutated tumors receiving PARP inhibitor therapy.

At the same time, the field is struggling to define an “informative” liquid biopsy result in the absence of a disease-specific alteration,[2] a challenge driven largely by our inability to anticipate the load of ctDNA in any given patient plasma specimen. This is unique to liquid biopsy; in tumor tissue biopsies, the pathologist always assesses the tissue sample for tumor content and adequacy for the validated molecular test. The somatic variant allele fractions can therefore be anticipated based on the input material and results can be interpreted in a sample-specific context. If the tumor content is estimated to be low based on microscopic examination, absence of an oncogenic driver or characteristic tumor suppressor gene variant profile may point to a falsely negative result and drive testing of an alternative specimen. Conversely, if molecular testing of a clearly adequate sample is unrevealing, further testing may be indicated (e.g. negative DNA sequencing in a never smoker with NSCLC triggering use of RNA sequencing for fusion detection). In a liquid biopsy, a negative result may indicate either that the tumor is truly negative for the tested variants or that the amount of ctDNA in the specimen is below the limit of detection (LOD) of the molecular assay. When the overall number of mutant gene copies in a sample is near the LOD, tests become more imprecise.[3] The challenge of distinguishing true mutant signal from background noise likely

explains a substantial number of the reported discrepancies when samples are tested by different commercial or in-house assays. Limited sensitivity and precision can be mitigated only in part by higher depth of sequencing—if the target of interest is extremely rare, larger sample volumes (possibly beyond the limits of clinical feasibility) may be required to get an informative result. Further complicating the situation, identification of mutations in a tumor suppressor gene like *TP53* point to the presence of a neoplastic clone but lack specificity for any particular site of origin. *TP53* in particular is highly mutated in myeloid neoplasia, including in "clonal hematopoiesis of indeterminate potential"; recent high-depth sequencing studies of patients with lung, breast, and prostate cancers have shown that mutations derived from clonal hematopoiesis in the white blood cells comprise a greater fraction of variants found in cfDNA than do variants derived from the solid tumor of interest.[4] Thus, in the absence of *a priori* knowledge of the patient's tumor genotype and/or parallel sequencing and subtraction of clonal variants derived from the white blood cells, individual tumor suppressor gene variants cannot always be assumed to be reflective of their cancer. Variants derived from clonal hematopoiesis can also be detected in tumor sequencing, however these are typically present at a much lower allele fraction than the tumor variants and can be subtracted by performing paired tumor-normal sequencing.[5]

Even oncogenic alterations must be interpreted with caution, if not highly specific to a particular disease. While *EGFR* kinase domain mutations are almost exclusively detected in NSCLC, other common alterations such as *KRAS* hotspot mutations or *BRAF* p.V600E are common across a large number of solid tumors as well as some hematologic malignancies. This creates an absolute necessity for accurate tumor diagnosis; at the present time, a tumor diagnosis requires a biopsy or other tissue specimen for morphologic and possibly immunohistochemical characterization by a pathologist. KRAS is not considered targetable (with some rare exceptions in the clinical trial context) thus the principle driver of therapeutic decision making is the pathologic diagnosis and clinical stage informed by appropriate radiographic studies. For *BRAF* p.V600E, knowledge of the diagnostic context is essential to therapeutic choice: in melanoma, combined RAF and MEK inhibitor may be indicated with immunotherapy offering a compelling alternative; in NSCLC, front line combined dabrafenib and trametinib is indicated; in colon cancer, combined RAF and EGFR inhibitors are approved in the second line. In the worst case scenario, identification of an apparent oncogenic "driver" alteration may lead to the conclusion that the tumor in question has undergone appropriate biomarker profiling, whereas in reality such changes may simply reflect another unrecognized or subclinical clonal process, and the real driver – such as a fusion alteration that may not be readily detected with available liquid biopsy technologies--goes undetected.

At the same time, pathologists stand to gain from ready access to liquid biopsy data. Because liquid biopsy testing is a straightforward process requiring no more than a blood draw and transportation to the local laboratory or central testing center, the turnaround time is almost inevitably shorter than that for tumor tissue genotyping. Tumor tissue handling is an operationally complex process requiring infrastructure for the sample acquisition and histologic processing that often adds a week or more to the molecular test turnaround time. In some cases, liquid biopsy results can return while a specimen is still undergoing diagnostic workup or can inform an established nonspecific diagnosis. Undifferentiated

carcinomas or carcinomas of unknown primary by definition lack morphologic and immunohistochemical features specific to their site of origin; occasionally, however, these tumors can harbor alterations that point the way, including *EGFR* kinase domain mutations for lung cancers, *IDH1/2* or *FGFR* family activating mutations in cholangiocarcinomas, or mutational signatures like tobacco or ultraviolet light, pointing to pulmonary or cutaneous origin, respectively.[6]

It is possible and indeed likely that advances in bioinformatics and approaches to cfDNA testing beyond mutational analysis will lead to improvements in clinical utility. The unique tumor-specific fragmentation and methylation patterns of ctDNA may enable more sensitive detection of ctDNA and inform site of origin.[7] Fragment size selection may also be used to enrich the ctDNA fraction to enhance sensitivity of molecular diagnostics techniques.[8] Rigorous filtering of variants derived from clonal hematopoiesis could simplify the interpretation of results from broad panel sequencing of cfDNA and hone in on those alterations truly relevant to the solid tumor, a particularly relevant challenge when interpreting tumor mutational burden or mutational signatures derived from blood-based analyses. Optimization of preanalytic handling, including techniques to isolate exosomal contents, may lead to enhanced detection of alterations in both the tumor genome and transcriptome. It is unlikely, however, that these solutions will provide the type of clinicopathologic correlation required for optimal management of any individual patient. The local pathologist, especially one with molecular expertise, can guide the clinician on the pros and cons of available commercial tests, be they tissue or liquid based, including the likelihood that a given commercial offering is likely to add benefit to any local testing. Used responsibly, these external resources can provide information not available in the local laboratory. Excessive reliance on external testing drives up costs for the hospital and patient; access to experts that can vet the quality and necessity of this testing can serve as a resource to treating clinicians who may lack the time to investigate the commercial offerings and who may be swayed by aggressive sales tactics or by patient requests informed by direct-to-patient marketing rather than evidence-based medicine. Many hospitals already rely on pathologists to serve as a gateway to esoteric and/or expensive clinical testing. These experts can examine comparable commercial or reference laboratory offerings in the context of their value and cost and make a recommendation to the treating clinician. Leveraging the pathologist to manage tumor genotyping, or at a minimum to serve as a conduit for return of results, also allows the pathologist to incorporate the molecular information into an integrated diagnosis and ensure that these results are properly documented in the medical record.

Molecular pathologists are uniquely trained to interpret tumor molecular data in the context of the known advantages and pitfalls of the particular PCR or sequencing assay. The molecular genetics of tumors is not generally a major component of clinical oncology training and many oncologists are less than comfortable interpreting tumor molecular results.[9] Consultation with trained molecular pathologists during the process of clinical decision-making can reduce the risk of overinterpretation of irrelevant variants or oversight of potentially important and targetable alterations in the liquid biopsy data. Molecular diagnostics experts, including those trained in pathology, play a crucial role in the quality control and interpretation of data within any genomics laboratory. However, at the local

level, pathologists with and without molecular expertise supply critical diagnostic insights and can inform decisions in the molecular tumor board setting. All centers providing cancer care are encouraged to support routine multidisciplinary meetings where genomic data can be interpreted in the context of the diagnosis and clinical status of a given patient.[10] Cancer care is, ultimately, a team effort and a combination of clinical, diagnostic, and genomic expertise is likely to provide the greatest value to the patient.

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Conflict of interests

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