Pathologists at the Leading Edge of Optimizing the Tumor Tissue Journey for Diagnostic Accuracy and Molecular Testing

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ABSTRACT

Objectives: *Tumor biomarker analyses accompanying immuno-oncology therapies are coupled with a tumor tissue journey aiming to guide tissue procurement and allow for accurate diagnosis and delivery of test results. The engagement of pathologists in the tumor tissue journey is essential because they are able to link the preanalytic requirements of this process with pathologic evaluation and clinical information, ultimately influencing treatment decisions for patients with cancer. The aim of this review is to provide suggestions on how cancer diagnosis and the delivery of molecular test results may be optimized, based on the needs and available resources of institutions, by placing the tumor tissue journey under the leadership of pathologists.*

Methods: *Literature searches on PubMed and personal experience provided the necessary material to satisfy the objectives of this review.*

Results: *Pathologists are usually involved across many steps of the tumor tissue journey and have the requisite knowledge to ensure its efficiency.*

Conclusions: *The expansion of oncology diagnostic testing emphasizes the need for pathologists to acquire a leadership role in the multidisciplinary effort to optimize the accuracy, completeness, and delivery of diagnoses guiding personalized treatments.*

Key Points

- The expanded role of biomarker testing in oncology has led to a dynamic tumor tissue journey, with multiple considerations around tissue sampling and processing affecting diagnostic accuracy.
- Pathologists could be heavily involved in every step of the tumor tissue journey and have the requisite skills to optimize the processes leading to the delivery of diagnostic interpretations.
- Pathologists should be given the opportunity to assume a leading role within multidisciplinary medical teams, coordinating the delivery of cancer diagnosis and personalized treatment strategies.

The field of oncology is experiencing an expansion in cancer therapies that has paved the way toward precision medicine. As an example, the development of monoclonal antibodies has expanded treatment options, with their selection guided by accompanying diagnostic tests, thereby improving clinical outcomes in patients with cancer.[1,2] Therapeutic decisions are now guided by comprehensive diagnostic reports delivered by pathologists, who analyze not only the tumor histomorphology but also the molecular profile of each patient's tumor biology.[3]

As the indications for therapeutic agents in the oncology setting evolve and expand to include different tumor types, the need for accurate cancer diagnoses and reliable downstream molecular testing is constantly growing. Diagnostic assays interrogating DNA or RNA (such as next-generation sequencing [NGS]) and proteins (such as immunohistochemistry [IHC]) decipher the tumor biomarker profile and are already routinely implemented in the clinic.[4,5] In addition, development of NGS technologies has expanded the variety of possible genomic and transcriptomic assays. DNA tests, for example, can range from detecting single, clinically relevant

genetic mutations to genomic signatures.[6,7] Efforts to integrate the analysis of multiple biomarkers in a single test are ongoing to maximize the information obtained from limited specimens while avoiding sequential testing and delays in acquiring results.[7,8]

Biomarker testing comes with a number of specific requirements for tumor tissue sampling, handling, and processing, which should be seamlessly incorporated into the clinical workflow. From deciding on biopsy indication and procedure type to receipt of the clinical testing report, a specimen goes through a sequence of preanalytic and analytic steps involving various key personnel and technical resources, thereby creating a tumor tissue journey. The exact tissue journey steps and turnaround time for molecular testing are determined by the processing requirements of each test as well as the administrative needs of each institution pertaining to transport of tumor tissue during the preanalytic stages and manual generation of the clinical report.[9-11]

Traditionally, the role of pathologists in the context of cancer diagnosis has been behind the scenes, restricted to performing morphologic assessments and delivering a diagnosis.[12,13] Tumor morphologic assessment remains critically important, as highlighted by the prognostic value of scoring systems such as the Nottingham histologic grading system for breast cancer,[14] the histologic architectural patterns of lung adenocarcinoma,[15] and the French Federation of Cancer Centers Sarcoma Group grading for sarcoma.[16] However, the expansion of diagnostic assays routinely implemented in the clinic means that results from molecular testing contribute to the final diagnostic interpretation and are integrated into the pathology report.[12,13] For example, non-Hodgkin lymphomas are diagnosed by evaluating the clinical presentation, histomorphology and immune repertoire of the tumor, and molecular test results.[17] The routine use of molecular testing in the clinical oncology setting has also contributed to the complexity of managing the tumor tissue journey, given the additional considerations for sample adequacy and suitable processing.[10] Therefore, the role of pathologists has evolved to include new responsibilities for evaluating the tumor molecular profile while considering the histomorphology of each patient's tumor.[18]

The additional responsibilities in the era of molecular testing have led to the adoption of novel tools in the pathology department, such as digital image analysis and artificial intelligence, enabling an automated approach to pathologic assessments.[19] Being familiar with these technological advances, pathologists are uniquely equipped medical professionals able to interpret genomic, transcriptomic, and proteomic data in the context of tumor tissue morphology, and their expertise may be leveraged in every step of the tumor tissue journey.[13] Pathologists may also engage with industry by contributing to the development and validation of diagnostic assays[13] and by acting as caretakers of archival tissue biopsy specimens in clinical trials.[20]

This review aims to showcase the dynamic events occurring during the tumor tissue journey and to highlight the crucial role of pathologists in merging clinical, pathologic, and laboratory perspectives to improve the tumor tissue journey workflow. Suggestions will be offered on how the tumor tissue journey workflow could be optimized by any pathologist acquiring a leadership role, managing molecular testing, and appropriately informing therapeutic decisions.

The Role of the Pathologist During the Tumor Tissue Journey

Patient care benefits from the active engagement of multidisciplinary teams comprising physicians of various expertise.[21] Until recently, the role of pathologists in driving forward personalized medicine has been restricted to performing specific tissue sample analyses upon request.[13] However, the new paradigm of molecular diagnosis, biomarkers, and precision medicine has been a call to arms for all pathologists to assume a new and indispensable role in the process of cancer diagnosis and to establish a leading presence in the multidisciplinary effort to determine the optimal diagnostic and treatment strategy for patients with cancer. Indeed, similar to individual patient care, the same level of personalized attention should be given to tumor samples. Given the central role of the tumor tissue sample in this new era of precision medicine, we suggest that all anatomic pathologists (cytopathologists and surgical pathologists) be given the opportunity to provide oversight of the entire tumor tissue journey and deliver tumor diagnostic interpretations by considering the patient's clinical history and optimizing the use of resources and tumor tissue sampling and processing in a personalized diagnostic workflow. The tumor tissue journey spans multiple interconnected steps, as outlined in **Figure 1**, starting with decisions on the optimal procedure to obtain a tumor sample and defining the role of pathologists in the delivery of diagnosis on a case-by-case scenario. Subsequent events involve optimizing and ensuring sample adequacy, performing onsite microscopic evaluation during the course of the sampling procedure, and integrating and streamlining downstream testing, leading to the final interpretation and reporting of diagnostic results (Figure 1 and **Table 1**).

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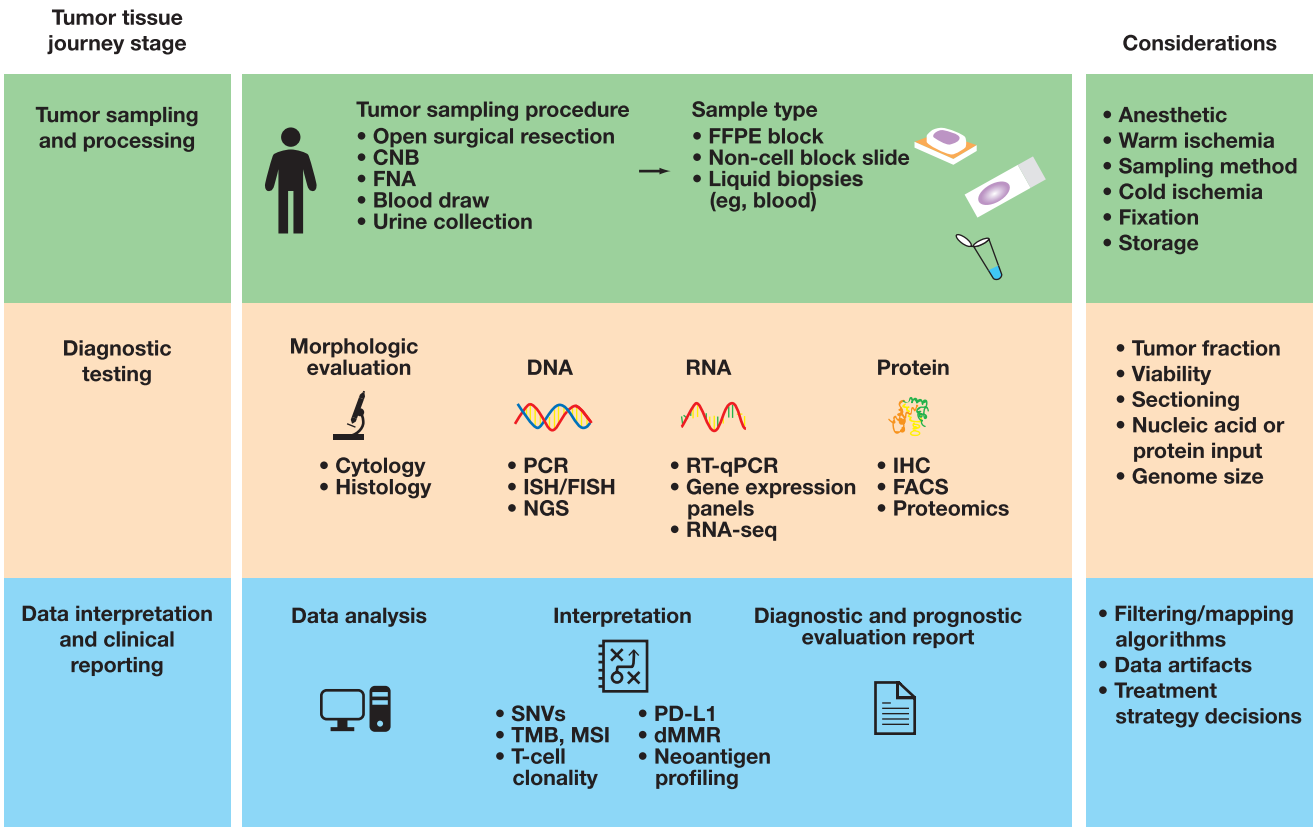

Figure 1 Representative stages of the tumor tissue journey. The tumor tissue journey starts with collecting the biopsy specimen, which then undergoes a series of diagnostic assessments to obtain the final clinical report incorporating diagnostic and prognostic data that will ensure a personalized approach in treating patients with cancer. Every stage comes with specific considerations that should be taken into account to ensure diagnostic accuracy. Considerations surrounding tumor sampling should dictate the sampling procedure employed on a case-by-case basis. Morphologic assessment is coupled with ancillary studies to ensure requirements for tissue adequacy and nucleic acid yield are met. Finally, treatment decisions are informed by careful interpretation of molecular testing after considering the analytic parameters affecting the final result. CNB, core needle biopsy; dMMR, mismatch repair deficiency; FACS, fluorescence-activated cell sorting; FFPE, formalin-fixed, paraffin-embedded; FISH, fluorescence in situ hybridization; FNA, fine-needle aspiration; IHC, immunohistochemistry; ISH, in situ hybridization; MSI, microsatellite instability; NGS, next-generation sequencing; PCR, polymerase chain reaction; PD-L1, programmed death ligand 1; RNA-seq, RNA sequencing; RT-qPCR, quantitative reverse transcription polymerase chain reaction; SNV, single nucleotide variant; TMB, tumor mutational burden.

Pathologists could be pivotally positioned in the multidisciplinary medical team to lead important changes and coordinate the proactive, undisrupted, and seamless communication between personnel involved in obtaining, handling, and processing biopsy specimens **Figure 2**.[9,12] Significantly, key processes with the potential to affect test results should be recognized, evaluated, customized to the needs and resources of each institution, and effectively implemented for the best possible care for patients with cancer. Table 1 provides an overview of preanalytic and analytic considerations for pathologists, as well as a series of evidence-based recommendations relating to key processes throughout the tumor tissue journey.

Tumor Tissue Sampling Procedures

At the beginning of the tumor tissue journey, decisions surrounding tumor sampling procedures might affect diagnosis as well as the execution and accuracy of downstream testing.[12,43,44] The interaction between the interventional physician performing the tissue biopsy (surgeon, oncologist, radiologist, pulmonologist, gastroenterologist, etc) and the pathologist, who has the necessary knowledge of tumor sampling requirements, is crucial to obtain optimal diagnostic material, meet downstream testing needs, and deliver a personalized cancer diagnosis.[12,44,45] Typically, a combination of imaging and sampling procedures is necessary to establish a diagnosis.[17,44] Molecular testing is ideally planned by clinicians before the biopsy procedure, although

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Table 1
Preanalytic and Analytic Considerations for Pathologists

Tumor Tissue Journey Stage	Considerations	Evidence-Based Recommendations
Tumor sampling	The anatomic pathologist and interventional radiologist should agree on the selection of the most suitable procedure to obtain sufficient tissue to fit assay requirements, facilitating both morphologic diagnosis and downstream biomarker testing, whether it is from a primary or metastatic site.	• Tumor type is an important consideration to determine if a sample from both primary tumor and metastases is required or if a sample from either primary tumor or metastatic site is sufficient.[22,23] • The least invasive method of biopsy should be undertaken to obtain sufficient tissue for the analyses required.[24] • In some situations, dual procedures, such as FNA followed by CNB, may be performed.[25] • Rapid onsite evaluation of cytologic material should be performed by a pathologist, cytopathology fellow, or trained cytotechnologist at the time of sampling to provide real-time feedback on sample quantity, quality, and suitability for molecular testing.[26-28] Telecytopathology, whereby a cytotechnologist uses a smart device and a microscope to live-stream slides to a remote pathologist, may alternatively be conducted.[29]
Tissue processing	Different tumor sample types are handled, fixed, and processed differently, which may influence their suitability for downstream diagnostic assays and other ancillary testing. Pathologists should consider the following parameters for the processing of biopsied tissue: • Warm ischemia • Cold ischemia • Anesthetic • Gross examination • Embedding • Fixation • Sectioning • Tumor fraction • Storage	• Total time between tumor harvesting and fixation, including warm and cold ischemia, should be kept to <1 hour and adequately recorded for samples processed for IHC. For example, the expression of estrogen or progesterone receptors decreases significantly 1 to 2 hours after sampling.[30] • Cold ischemia should ideally be <30 minutes in samples undergoing RNA or proteomic analysis.[30-32] • Anesthesia can induce biopsy tissue anoxia, leading to increased gene transcription; to minimize these changes, a representative portion of tissue should be snap-frozen in the operating room if NGS analysis is required.[33] • Direct preservation of biopsy materials should follow a controlled and defined method according to the downstream assessments needed, such as ultra-low-temperature freezing (for NGS assessments) or FFPE at room temperature (for morphologic and other pathologic assessments).[34] • FFPE blocks, including cytology cell blocks, should be processed to permit adequate morphologic assessment; multiple sections could be cut from tumor samples distributed or divided in more than one cassette to avoid tissue waste, especially if the amount of available diagnostic tissue is low.[35] • Low tumor fraction (the proportion of tumor cells in a specimen) may affect the reliability of molecular diagnostics; a tumor fraction more than 10% to 20% should be maintained during microdissection, although requirements vary across assays.[24] • A standard operating procedure should be established for the transportation and storage of specimens to avoid errors and delays in tissue processing.[34]
Diagnostic testing	Pathologists should play a key role in the integration and streamlining of downstream testing (eg, DNA/RNA/protein analyses) with initial morphologic evaluations (cytology/histology). Considerations for biopsied tissue include: • Sectioning • Tumor viability • Nucleic acid/protein input and genome size	• Tumor viability should be evaluated ahead of diagnostic testing; large cellular lesions are compatible with most NGS platforms, while necrotic regions may be incompatible with PCR-based sequencing.[36] • NGS assays have different requirements for nucleic acid input depending on the platform, gene panel size, and target enrichment method; input requirements vary from 10 to 300 ng.[36]
Data interpretation and clinical reporting	Pathologists should be responsible for the final interpretation and timely reporting of diagnostic results. Considerations for data analysis, interpretation, and reporting include: • Filtering/mapping algorithms • Diagnostic accuracy • Turnaround times • Influence in management decisions	• For IHC slides processed digitally, the quality of the tissue, histology slide, and scan should be confirmed to ensure that standards are met to collect meaningful and reproducible data. A consistently reproducible quality stain is critical to achieve the optimal value of image analysis.[37] • Analytic variables in NGS (eg, library preparation method, algorithms used for coverage depth, variant filtering, and mapping) can influence diagnostic accuracy and assay turnaround times[38-41]; such data interpretation algorithms should be standardized to ensure reliability of NGS results.[24] • Relevant pathology personnel should be trained in the use of computational analysis tools to manage large data sets such as those in the NGS.[40,42]

CNB, core needle biopsy; FFPE, formalin-fixed, paraffin-embedded; FNA, fine-needle aspiration; IHC, immunohistochemistry; NGS, next-generation sequencing; PCR, polymerase chain reaction.

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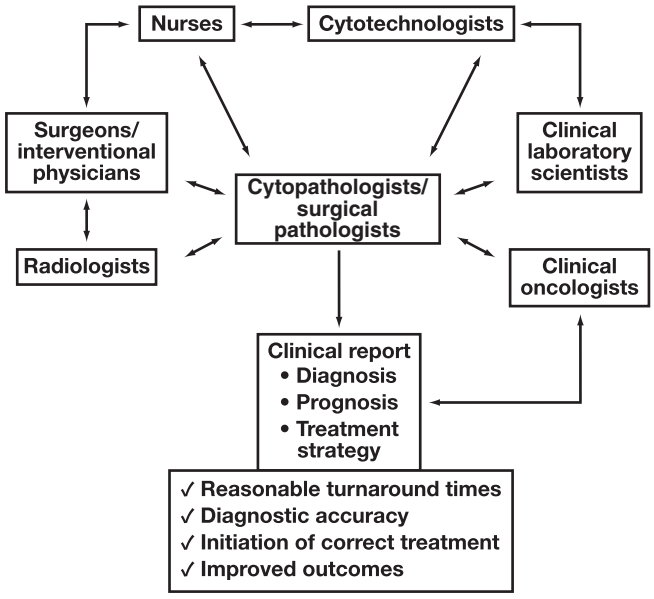

Figure 2 Suggested tumor tissue journey workflow for cancer diagnosis. Pathologists can lead a multidisciplinary team involved in cancer diagnosis by coordinating the interactions between the different personnel and ensuring that the right processes and methods are performed in a timely manner. We propose a diagnostic workflow whereby a pathologist is present throughout the tumor tissue journey and liaises with all the various personnel involved, ranging from interventional physicians at the time of tissue sampling to laboratory scientists during the interpretation of molecular testing. Pathologists can be engaged during every stage of the diagnostic workflow, advising on optimal tissue sampling, triage, and processing to ensure efficiency, diagnostic accuracy, and the delivery of the clinical report in a timely manner.

pathologic evaluation may expand or change the necessary panel of tests. The input of the pathologist on selecting a target lesion is crucial. For example, sampling distant metastases may be deemed appropriate for diagnosis and staging of non–small cell lung cancer (NSCLC), especially if it involves a more accessible location than the primary tumor, thus allowing a less invasive sampling procedure[44] (Table 1). The pathologist should therefore effectively flag such scenarios to the interventional physician so that sampling can be performed accordingly. In addition, the quality and amount of tumor biopsy sample obtained may differ depending on the tumor type, location, viability, and size.[46]

During tumor sampling, the physician performing the procedure aims to obtain an appropriate amount of cellular material for diagnosis and downstream testing while minimizing the risk of tumor spread, morbidity, and disruption of further procedures or treatment.[47] Open (excisional or incisional) biopsy has been reported to have a diagnostic accuracy of 94% to 99%.[48] However, such surgical options may be adding to the expense of obtaining a diagnostic tissue sample and carry an up to 16% risk of complications, including the development of hematoma, tumor spread, and wound-healing problems that may delay the initiation of adjuvant treatment.[48]

Cytology sampling techniques were developed as less invasive alternatives to open biopsies and have led to the expansion of the cytopathology field. Fine-needle aspiration (FNA) is a cost-effective and repeatable tissue sampling technique using a needle no bigger than 22 gauge and can be performed as an outpatient procedure requiring little equipment without raising any significant safety concerns related to discomfort, complications, wound healing, or tumor spread.[47-49] When coupled with onsite evaluation, FNA allows the fast assessment of morphologic results even for inoperable tumors, reducing the need for invasive exploratory procedures and the use of frozen tissue sections.[47,48] Nonetheless, the diagnostic accuracy of FNA is dependent on the location and size of the tumor, the expertise of the physician performing the procedure, and the quality of the sample preparation. In addition, the diagnostic interpretation skills of the pathologist evaluating the cytology sample, developed with appropriate training and dedication, are also crucial for the accurate assessment of an FNA cytology sample.[47-50] Furthermore, FNA cytology may not provide enough material to allow for ancillary studies and lacks information on the architectural features of the tissue sampled.[47] This observation has deterred some institutions from effectively adopting FNA in their diagnostic workflow.[47]

Core needle biopsy (CNB) was developed as an alternative to FNA, using a 10- to 16-gauge needle to provide a cylindrical core of tissue, thereby allowing for the evaluation of tissue architectural features.[47,48] Both CNB and FNA are easy techniques for the interventional physicians to learn and perform, and they offer a satisfactory safety profile, fast sample preparation, and fast turnaround of preliminary evaluation compared with open biopsies.[47,48] However, CNB provides larger tumor tissue samples but comes with added risks of complications compared with FNA, such as bleeding, added costs, and longer turnaround time.[25] Similar to FNA, the use and reliability of CNB are also dependent on the accessibility of the tumor. Overall, CNB has been reported to provide higher diagnostic accuracy, specificity, and sensitivity than FNA.[48,50]

In some institutions, interventional radiologists will perform FNA, CNB, or both, depending on tumor accessibility and risk of complications. FNA may be performed first to document accessibility of the lesion, followed by one or more CNBs.[25,47,48] This dual procedure can be advantageous in some circumstances since, by using FNA to confirm an appropriate biopsy location, CNBs can

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be placed directly in fixative without any further manipulation that may compromise the tissue sample. In such cases, the role of the pathologist is pivotal, regardless of who performs the procedures. It is therefore important that interventional physicians and pathologists remain familiar with both techniques. However, some physicians remain unconvinced by the diagnostic utility of FNA because of the small amount of material obtained. As a result, the collection and processing of cytology material lack standardization across different medical centers, with some institutions relying on other diagnostic workflows such as open biopsies.[47] Nonetheless, FNA has evolved into an effective diagnostic tool, widely recognized for its feasibility, its contribution to the delivery of reliable diagnoses, and lack of significant risks associated with more invasive procedures.[47]

Tumor Tissue Sampling Optimization and Real-Time Microscopic Evaluation

Considerable advances in cytopathology and sampling procedures have expedited delivery of diagnoses and improved diagnostic precision, leading to the establishment of rapid onsite evaluation (ROSE) of cytologic material as a widely adopted practice.[26,47] The presence of cytopathologists or trained cytotechnologists at the time of sampling is important because they can provide the interventional physician with real-time feedback in terms of the quantity and suitability of the specimen for morphologic assessment, tumor tissue procurement, and ancillary testing, whether it is an intraoperative frozen section or a cytology sample.[26-28] Crucial factors such as tumor viability, necrosis, and percentage of tumor cellularity are evaluated by pathologists when the specimen is scheduled to undergo NGS-based diagnostic testing.[36] Assessing these factors at an early stage can decrease the incidence of insufficient tumor samples, mitigate the need for resampling, and, in turn, reduce delays in test results and initiation of appropriate treatment.[51-53]

In addition, pathologists can effectively identify and supervise challenging situations where the physician performing the sampling procedure may not adequately determine sample quality. For example, pathologists who have assessed the available clinical and radiologic findings are the ideal candidates to decide during real-time microscopic evaluation whether a lymph node FNA sample is representative and adequate or whether it has been compromised by tumor contamination during sampling. This is particularly important not only to avoid false-positive results but also to prevent the need for additional sampling procedures.[28,47,51] Furthermore, the inclusion of the onsite cytopathologic assessment in the final pathology report may provide clarifying comments and further insight to the referring clinician.[49]

Ultimately, ROSE of small specimens has been shown to improve specimen adequacy, diagnostic accuracy, and turnaround times, as well as support optimal triage across several tumor types.[51,52] Diagnostic results obtained through ROSE have shown high agreement with final cytologic evaluations.[26] Therefore, ROSE could expedite reliable therapeutic decisions.

Despite the reported advantages associated with ROSE, the necessity of an onsite pathologist communicating with the clinical personnel retrieving the specimen has been perceived by some investigators as a disadvantage due to concerns over the added cost and the additional need for human resource, especially in nonacademic centers where the availability of trained pathologists may be limited.[26,51] Nonetheless, it constitutes a timely opportunity for the pathologist to obtain pertinent clinical history, analyze tumor tissue adequacy, perform appropriate triage, and improve turnaround times.[51] In underresourced centers, telecytopathology, whereby a cytotechnologist uses a smart device and a conventional microscope to live-stream slides to a remote cytopathologist, may be employed.[29] Undisrupted communication and mutual trust between the remote pathologist and onsite personnel, as well as optimized specimen handling during sampling procedures, are of paramount importance for the implementation of this workflow. Alternatively, onsite adequacy assessment and tissue triaging can be performed by an available cytotechnologist, with final evaluation of the biopsied material to be performed subsequently off-site by a pathologist.[25] The implementation of this workflow is dependent on the competency of the cytotechnologist to accurately perform ROSE with appropriate specimen triage and correctly allocate specimens for ancillary testing. Implementation of a standardized protocol for tissue management during ROSE is therefore crucial to enhance tissue preservation and minimize wastage.[52,54]

It is important to note that ROSE is not restricted to FNA samples. Imprint cytology (or touch preparation) samples can be generated by gently pressing or rolling a CNB sample on a glass slide.[55] The resulting cytology sample can be readily assessed in terms of its adequacy and diagnostic utility, as well as be used to determine whether further sampling is required. Moreover, it can also be used for a morphologic assessment to provide initial diagnostic interpretations.[55,56] Intraoperative samples are assessed by generating frozen sections[27] or tumor microsampling, which involves taking 1- to 2-mm^3 samples from the lesion and squashing them between glass slides.[57]

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Even though onsite evaluation may be applied to different sampling scenarios, it is also important to note that different sample types, including FNA and CNB, are handled, fixed, and processed differently, which may influence their suitability for downstream diagnostic assays and other ancillary testing.[45] FNA material is usually smeared on slides, but other sample types (eg, small tissue biopsy specimens) may require different preparations, such as tissue squashing.[57,58] This further emphasizes the importance of placing pathologists at the leading edge of driving the tumor tissue journey workflow while leveraging their deep understanding of downstream variables that affect diagnostic testing.

Additional Factors Affecting Sample Adequacy and Diagnostic Accuracy

Additional variables encountered during the tumor tissue journey may influence tumor tissue sample adequacy and diagnostic reliability. For excisional biopsy sampling, the attending physicians should consider and predetermine surgical variables such as the administration of anesthesia. For example, the intralesional administration of local anesthetic could alter the phosphorylation state of signaling pathways and induce artifacts that would ultimately change gene expression and compromise tissue adequacy for downstream testing.[59,60]

The durations of warm and cold ischemia have the potential to affect the integrity and molecular repertoire of the tumor.[30,33,61] Therefore, the time of clamping-induced warm ischemia, as well as the time between excision and application of an appropriate fixative to stabilize the tissue, should be kept to a minimum and adequately recorded.[30] Short durations of both warm and cold ischemia are especially important for proteomic and RNA analysis.[30] Warm ischemia has been proven to affect RNA quality and level detection, while cold ischemia duration also decreases RNA integrity levels to a modest degree.[30] Cold ischemia interval should remain below 1 hour.[30,62] However, evidence exists that cold ischemia effects on gene expression in biopsy tissues may be significant in under 30 minutes. In proteomics, factors such as postexcision hypoxia and stress-response signals may alter expression levels of certain kinase proteins during cold ischemia delays of as little as 10 to 15 minutes.[30] In biopsy samples requiring proteomic analysis, it is recommended that the elapsed time between tissue extraction and stabilization should not exceed 20 minutes.[32]

Furthermore, disease incidence, as seen in NSCLC with nodal involvement, as well as the skill of the physician performing the sampling procedure, could also influence diagnostic accuracy.[63] This is highlighted by hospitals receiving a high volume of patients. Such hospitals regularly perform sampling procedures and achieve higher diagnostic accuracy than hospitals treating fewer patients, which may be attributed to differences in personnel experience and expertise.[64] Moreover, optimization of sampling procedures should be in line with the needs and available resources of each therapeutic center to achieve realistic improvement in the diagnostic workflow.[64]

Tissue Processing

Following tissue sample acquisition, a number of additional preanalytic variables should be considered, as they can potentially influence the outcomes of diagnostic assays. These include the type of preservation (fixation vs freezing), type of fixative, time of fixation, pH, temperature during fixation, section surface area, and molecular extraction.[33,34,36,44] Tissue-processing factors may affect pathologic assessments differently from molecular analyses. For example, bone lesion biopsy specimens require decalcification that necessitates the active engagement of the pathologist in dictating tissue processing and downstream testing. Acid decalcification can cause acid hydrolysis of DNA with prolonged treatment, negatively affecting downstream molecular analysis.[65] In contrast, the use of chelating agents such as EDTA for decalcification has been shown to circumvent the negative effects of acids on DNA quality and may be a more suitable choice for specimens in which molecular analysis is anticipated.[65] Alternatively, a limited decalcification protocol involving a weak acid that is compatible with both morphologic assessment and molecular testing without degrading DNA, such as formic acid, may be employed.[36] Thus, the panel of tests to be used should be agreed upon by the multidisciplinary team and guide the methods surrounding tissue processing.

Considering that pathologists perform these pathologic assessments and contribute to decisions surrounding downstream testing, they are also strategically placed to determine the most appropriate panel of tests to be performed and can identify the suitable methods that will ensure diagnostic accuracy. For example, ultra-low-temperature freezing is associated with a higher-quality nucleic acid yield, whereas formalin-fixed, paraffin-embedded (FFPE) tissue at room temperature is best suited for morphologic and other pathologic assessments.[34] Findings from IHC validation studies have suggested that tissue-processing variables, such as the type of fixative and the staining platform used, can affect results.[2,66] For example, formalin is the recommended fixative

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for accurate assessment of programmed death ligand 1 expression using IHC.[66] Nonetheless, methanol fixation may be a more suitable alternative for downstream NGS testing because it is associated with improved nucleic acid yield and longer fragment size.[67] Notably, nucleic acid extraction can hinder protein separation. Hence, this should be considered if a specimen is scheduled to be evaluated using both NGS- and IHC-based assays.

In addition, storage and transport conditions may also influence the interpretation of downstream testing. Fresh samples may yield superior staining intensity compared with stored archival tissue.[66] Time in transport or storage may influence the quality of the specimen if conditions are not optimal and may also affect the turnaround time of test results. Indeed, pathologists are the most suitable physicians to customize, on a case-by-case basis, the variable methods for optimal tissue preservation and storage.

Pathologists are also in a position to perform microdissection and appropriate tissue sectioning protocols to minimize tissue waste while considering the section surface area requirements of downstream testing as well as the quality and inherent characteristics of the specimen.[20,36,44,68] When there is enough specimen, separating tissue into several cassette blocks at the time of initial gross assessment may mitigate tissue waste and be more accommodating for the different diagnostic assessments.[35] Once molecular testing is ordered, pathologists are tasked with determining the optimal tissue block for downstream processing depending on tissue availability and testing requirements. Tumor purity is a key determining factor for DNA yield and subsequent diagnostic testing success.[24] For example, fluorescence in situ hybridization analysis requires approximately 100 tumor cells per section while genomic sequencing and mutational analysis require at least 10% to 20% of tumor content.[24,35] Depending on their tumor content, sections should therefore be allocated to ancillary testing accordingly.[69] Tumor purity is also affected by the presence of inflammatory cells, stromal cells, and fibrotic or necrotic regions, as well as blood and mucin.[24,36] To ensure accuracy of NGS testing with high DNA input requirements, sections with high tumor purity and predicted DNA yield should be used.[36] Pathologists may, however, consider that DNA fragments caused by treatment-induced tumor apoptosis but not necrosis may still be compatible with NGS testing.[36] Section regions appearing morphologically dead may still be viable options for molecular testing when tissue is limited.[36] In addition, even though molecular testing is traditionally performed on FFPE samples, there is growing evidence that quality nucleic acid yield may be derived from cytology smears.[58,70]

Molecular yield is influenced by both the amount of processed sample and protocol parameters, such as the nucleic acid concentration recommended for NGS platforms.[36] In addition, nucleic acid input is dictated by the downstream ancillary diagnostic assay, illustrating the dynamic nature of the tumor tissue journey. Different NGS assays have different requirements of nucleic acid input (eg, targeted gene panels require less DNA than whole-exome sequencing).[36] By considering the amount of tissue retrieved as well as the inherent characteristics of the specimen, pathologists can strategically optimize tissue triage and liaise with the clinicians ordering diagnostic testing, the interventional physicians performing the tissue biopsy, and the laboratory scientists to determine sample requirements and the ideal panel of downstream tests. Pathologists are therefore the ideal medical professionals to assume tissue stewardship roles in the context of clinical trials and tissue banks.[71]

Tissue banking has an integral role in the development of diagnostic biomarkers.[71] Pathologists represent the managerial liaison between research and medical care, ensuring optimal tissue procurement, appropriate documentation, the establishment of evidence-based preservation protocols, and allocation to appropriate biomarker studies.[71] In addition, the marked increase in available clinical trials of precision oncology pharmaceuticals, many of which require 10 to 25 slides of diagnostic tissue for enrollment and subsequent correlative studies of biomarkers with response, has created additional responsibilities for pathologists.[20,71] When diagnostic tissue is limited, pathologists are tasked with managing the submission of tissue slides in order for patients to benefit from potentially life-extending therapy while ensuring that diagnostic tissue is not exhausted.[20] In such scenarios, pathologists are guided by their experience in minimizing tissue waste and also by ethical and legal considerations pertaining to patient informed consent and access to their tissue for subsequent diagnostic testing.[20] In fact, pathologists may act as patient advocates by raising awareness and striving for better management of the amount of tissue allocated for trial enrollment and retrospective correlative studies.[20]

Diagnostic Evaluation, Interpretation, and Integration of Downstream Testing

Despite the recent developments in diagnostic practices, traditional histologic and cytologic evaluations are often performed separately from molecular assessments, with little interdepartmental interaction and communication.[72] This compartmentalized approach to the comprehensive evaluation of tumor specimens may obstruct

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the collection of clinical information, leading to lost or insufficient information available to the multidisciplinary clinical team and delays in diagnosis. The presence and interaction of pathologists with laboratory scientists and bioinformaticians are also recommended in the analytic stages of the tumor tissue journey to identify potential factors distorting the interpretation of test results. It is also important for these stakeholders to agree on a desirable turnaround time that will in turn influence the analytic methods to be employed. According to the Association for Molecular Pathology and the College of American Pathologists, NGS assays feature a number of analytic variables that can influence diagnostic accuracy and assay turnaround times.[38] Parameters within the NGS workflow that may influence consistency in the results and reasonable turnaround times include the choice of library preparation method and the algorithms used for coverage depth, variant calling, germline variant filtering, and mapping.[39-42] The portion of the genome inspected may influence the reliability of the result when determining genomic signatures but can also significantly affect the duration of test run.[8,38] Ultimately, the interpretation of the diagnostic results needs to align with the morphologic context of the tissue sample, highlighting the importance of undisrupted and seamless communication between the personnel involved and the engagement of pathologists throughout the tumor tissue journey. For example, during the tissue journey of suspected lung cancer samples, when pathologists are present during sampling, they can determine lung cancer subtype and guide molecular testing based on the morphologic results, which will in turn guide treatment decisions.[44,68] Furthermore, on occasions where the test results conflict with the morphologic features of the specimen, both of these assessments should be questioned and revisited to resolve any discordant results. For example, lobular carcinoma of the breast is expected to express estrogen and progesterone receptors.[73] Absence of these markers in a suspected lobular breast cancer sample might be explained by either a specimen compromised by prolonged cold ischemic time or sampling or interpretation errors.

Envisioning the Role of Pathologists in the Biomarker Era

Pathologists perform indispensable roles and, with appropriate training, can become integral to the delivery of personalized medicine. Indeed, pathologists should oversee and manage the tumor tissue journey while communicating with the key personnel involved (including members of the multidisciplinary clinical team), consulting on diagnostic assay outputs, and interpreting results in the morphologic context for each case to inform and expedite therapeutic decisions (Figure 2).[68,74] It is therefore essential that all pathologists, especially those who undertake the responsibility of overseeing and managing the entire tumor tissue journey, stay up to date with molecular testing requirements to better triage specimens, serve as patient advocates, and advise clinicians about further testing possibilities.

Despite the advantages presented by pathologists being actively engaged in tumor boards and multidisciplinary medical teams, treating physicians are frequently unaware of the molecular pathology approach to cancer diagnosis. This provides pathologists with ethical obligations and opportunities to assume a role in the education of the physicians regarding the tumor tissue journey requirements for biomarker diagnostics in cancer care. To access the full benefits of diagnostic testing for their patients, physicians should be aware of the importance of the tumor tissue journey variables involved, taking into account the outcome, costs, and turnaround times associated with molecular testing.[4,75] The management of the tumor tissue journey should be seen as a dynamic process at every institution based on their needs, available physical resources, and workforce.

The administrative responsibility of pathologists to oversee the undisrupted flow of the tumor tissue journey should be aided by the establishment and utilization of standard operating procedures. These should focus on standardizing decisions regarding specimen handling, triaging, identification, and transport requirements.[10] The role of the pathologist may potentially be supported by the creation of a laboratory navigator system. Traditional navigator systems are run by nurses who support the continuity of health care and alleviate patient anxiety associated with disease.[76] A modified navigator system supporting the tumor tissue journey under the guidance of pathologists could involve designated laboratory personnel being assigned to monitor the completion of each step so that suitable and timely handling and processing are performed on tumor specimens.[77]

As the role of pathologists in the biomarker era evolves, residents as well as experienced professionals should be encouraged to assume leadership roles through appropriate training and awareness initiatives. Institutions can organize formal lectures and seminars focused on pathologist-led scenarios, such as ROSE, where the benefit of the intervention to patient care may be immediately evident. Mentorship and shadowing schemes may also be established so that pathologists in training can be exposed to the varied duties of pathologists across the tumor tissue journey. Appropriate morphomolecular training needs to

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be addressed in pathology curriculums to engage pathologists in the final stages of the tumor tissue journey. This could be approached by either incorporating molecular pathology modules in the residency curriculums or introducing postgraduate degrees focusing on molecular pathology.[72,78] Considering the vastness of molecular pathology and the wide range of molecular tests currently used in the clinic, personal portfolio forms could be used to record competency, supervision, and training needs.[78] The involvement and collaboration of specialist centers and national institutions focusing on pathology may also benefit from the establishment of such training modules.[72] Such training and level of engagement will provide pathologists with the necessary tools to create strong ties with other physicians while educating them during daily interpersonal interactions in the workplace, as well as at tumor boards and multidisciplinary team meetings. It will also allow pathologists to not only provide an expert opinion for the customization of assay procedures and interpretation of results but also to provide feedback and inform assay development institutions on the requirements, practicalities, and turnaround times associated with diagnostic procedures. The expert opinion of pathologists, with a deep understanding of the tumor tissue journey, may also improve the execution, applicability, and interpretation of biomarker studies in clinical trials.

The era of biomarker diagnostics in the oncology setting has brought new responsibilities to the role of pathologists. Aspiring pathologists should lead the management of the tumor tissue journey to provide prompt and reliable diagnoses, informing treatment decisions that could lead to improved patient outcomes.

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