An enduring goal of personalized medicine in cancer is the ability to identify patients who are likely to respond to specific therapies. Our growing understanding of the biology and molecular signatures of individual tumor types has facilitated the identification of predictive biomarkers and has led to an increasing number of diagnostic tests to be performed, often as serial and distinct assays on limited tumor specimens. The biomarker diagnostics field has been revolutionized by next-generation sequencing (NGS), which provides a comprehensive overview of the genomic profile of a tumor. Many preanalytic variables can influence the accuracy and reliability of NGS results. Standardization of preanalytic variables is, however, complicated by the plethora of specimen acquisition and processing methods. Variables across the tissue journey, including specimen acquisition, specimen fixation, and sectioning, as well as postfixation processing, such as nucleic acid extraction, library preparation, and choice of sequencing methods, are critical for the reliability of NGS analysis; thus, standardization would be beneficial. In this article, each step in the tissue journey is outlined, with specific focus on preanalytic variables that can influence NGS results. Practical considerations for standardization of these variables are provided to facilitate accurate, reliable, and reproducible NGS-based molecular characterization of tumors, ultimately informing diagnosis and guiding treatment.
quantity and quality of the tumor specimen. Preanalytic variables across the tissue journey, encompassing every stage from specimen acquisition to analysis, can influence the results obtained by biomarker diagnostic tools, ultimately affecting treatment decisions and, in turn, clinical outcomes. Among the increasingly available tests validated for clinical use, NGS permits simultaneous assessment of multiple genomic targets, thereby enabling the collection of complex biomarker data from a single specimen.\(^3\)\(^-\)\(^5\) NGS-based assays, however, rely on complex molecular methods compared with those used to interrogate single targets, and preanalytic variables, such as tissue availability, handling and processing, nucleic acid yield, and quality, may considerably influence the results.\(^6\) There is an urgent need for recommendations to optimize preanalytic procedures and specimen management for the purpose of ensuring accuracy and reliability, given the increasing relevance that NGS assays have in current clinical practice. This review highlights preanalytic considerations during biopsy procurement and throughout the tissue journey and provides guidance for tissue stewardship and standardization of NGS assays. Adherence to these procedures has the potential to enhance the reliability of NGS assays and at the same time benefit other biomarker diagnostic approaches.

**Figure 1** Key stages for biomarker determination. Biomarker diagnostics involve genomic, transcriptomic, and protein assays. Each diagnostic has specific requirements in specimen preparation, molecular isolation, management, and material yield, as well as analytical normalization. CNV, copy number variant; dMMR, mismatch repair deficiency; IHC, immunohistochemistry; indels, insertions/deletions; MSI, microsatellite instability; NGS, next-generation sequencing; PD-L1, programmed death ligand 1; SNV, single nucleotide variant; SV, structural variant; TMB, tumor mutational burden.

**Emerging Biomarker Diagnostic Tools in Oncology Clinical Practice**

Since the approval of trastuzumab as human epidermal growth factor receptor 2 targeted breast cancer therapy and the publication of recommendations for use of its companion IHC assay, biomarker diagnostics have diversified and are increasingly used in clinical oncology practice.\(^7\) Their use can be as a companion diagnostic, where testing is required as part of the regulatory approval of a therapy, or as a complementary diagnostic, where testing is optional but can nonetheless inform therapeutic decision making.\(^1\) Indeed, the recent approval of pembrolizumab for patients with microsatellite instability—high solid tumors marked the first approval of a tissue-agnostic cancer treatment based on a biomarker.\(^8\)

The linkage of precision medicine with the expanding availability of diagnostic technologies has increased the demand for sufficient quantity and high-quality biospecimens. Tumor assessment has evolved from traditional baseline morphologic assessment to include biomarker diagnostics inclusive of DNA, RNA, and protein, with tissue specimen prerequisites increasing beyond traditional basic requirements and reflecting the complexities linked to the...
different modalities of downstream analysis (Figure 1). With proper tissue stewardship, protein- and nucleic acid–based diagnostics are now frequently performed on samples from the same specimen. For example, IHC-based programmed death ligand 1 analysis can be used as a companion or complementary diagnostic on tissue biopsy specimens to determine patient eligibility for treatment with programmed death 1/programmed death 1 immune checkpoint inhibitors. Tissue from the same specimen, or blood from the same patient, may also be submitted for concurrent molecular analysis of actionable mutations to inform treatment decisions for non–small cell lung cancer.  

NGS has become an established methodology in the clinical laboratory for screening and diagnosis of germline (inherited) and somatic (acquired) genomic mutations and is most commonly used for genomic and transcriptomic analyses. NGS-based assays for genomic and transcriptomic biomarkers include whole-genome sequencing, whole-exome sequencing, RNA sequencing (RNA-seq), and genomic or gene expression panels (Table 1). Of these techniques, whole-genome sequencing has the highest coverage, generating information from the entire genome, including introns, whereas whole-exome sequencing is restricted to the exome. RNA-seq, typically focused on the transcriptome, can provide flexibility, sensitivity, and accuracy to gene expression measurements and gene fusion analysis, and although not yet widely adopted in the clinical laboratory practice, it is increasingly being used for the detection of translocations analysis in hematologic and solid malignancies. Because NGS assays range from targeted hotspot panels to comprehensive genome- or transcriptome-scale platforms, they can be used to detect specific genomic mutations and genomic signatures, such as microsatellite instability and tumor mutational burden or proxy or surrogate estimates for tumor mutational burden. Recent developments in NGS technology have provided highly accurate and reliable systems that facilitate the discovery of such genomic signatures. At the same time, the increasing association of these genomic signatures with clinical benefit from certain therapies, including immunotherapy, is accelerating the adoption of NGS in clinical practice. 

Biomarker diagnostics may benefit further from combining a variety of approaches. For example, combining genomic profiling via NGS with mismatch repair deficiency by IHC is being assessed for its accuracy in determining mutator phenotypes in a range of tumor types. Although single-biomarker diagnostics contribute to a more individualized approach to cancer treatment, the comprehensive diagnostic potential of NGS is actively revolutionizing clinical practice, providing comprehensive information on the genomic profile of a tumor to thereby inform clinical care. It is therefore clinically relevant to assess the importance of all factors that allow for reliable NGS outputs, including preanalytic considerations. 

Preanalytic Considerations Affecting NGS

Key steps of the tissue journey include specimen acquisition, processing and preservation, nucleic acid extraction, and NGS library preparation (Figure 2). Each of these steps may be considered as an individual opportunity for standardization in an effort to maintain suitable specimen quality, which can in turn optimize consistent and successful performance of downstream diagnostic biomarker analysis. Meanwhile, the development, validation, and implementation of downstream assays must also consider the reality of existing standard upstream practices. Molecular pathologists are among the key personnel in this process and are in a unique position to facilitate the implementation of informed protocols throughout the journey. A thorough consideration of preanalytic variables that may affect NGS-based biomarker diagnostics is warranted because this informs guidance for standardized approaches in the early stages of tissue management. 

Specimen Requirements and Tissue Acquisition

Fundamentally, microscopic imaging techniques are needed for morphologic analysis of a tissue specimen, which in turn, is necessary for the accurate and complete diagnosis and staging of most cancer types. Therefore, the primary use of biopsy specimens has traditionally been allocated toward diagnostic purposes. A number of different biopsy methods exist, depending on the site and size of the tumor, yet the least invasive method of biopsy should be undertaken. Surgical biopsies (including tissue biopsy, resection, and excision) are invasive but often necessary procedures. Core needle biopsies and cytology specimens obtained via fine-needle aspiration or as exfoliative cytology specimens are usually less invasive to obtain. A standard operating procedure should be established for the transportation of specimens to avoid errors and delays in tissue processing. Indeed, it is strongly recommended that the direct preservation of biopsy materials ideally follows a controlled and defined method, such as formalin fixation and embedding in paraffin beginning within 1 hour of removal. A short cold ischemia window is especially important for RNA analysis because RNA gets easily degraded. Specimens must also be placed into an adequate volume of buffered formalin because fixation time (approximately 16 hours) is also dependent on the specimen volume. Formalin-fixed, paraffin-embedded (FFPE) blocks, inclusive of cytology cell blocks, should then be sectioned to permit adequate morphologic assessment by hematoxylin and eosin staining. Extra sections may be cut at this stage to avoid tissue waste, especially if the amount of available diagnostic tissue is low. Standardization of procedures is critical not only to support reliable and reproducible hematoxylin and eosin assessment but also to mitigate tissue waste by facilitating efficient sectioning.
Procedures at this stage of the journey are best informed by the collaboration of surgeons, interventional radiologists, surgical and molecular pathologists, cytopathologists, and clinical oncologists. Multidisciplinary communication is therefore necessary to optimize specimen acquisition and processing.

Typically, tumor specimens acquired by fine-needle aspiration have been preferentially processed as FFPE cell blocks because this specimen type most closely resembles surgical FFPE. However, cell blocks are not always available and may contain an insufficient number of cancer cells for molecular analysis. There is increasing evidence that the quality of the DNA yield from non—cell block (air-dried or alcohol-fixed) cytology specimens is adequate, with some studies reporting no significant differences in NGS results between cell-block and non—cell-block

<table>
<thead>
<tr>
<th>Table 1</th>
<th>NGS-Based Biomarker Assays</th>
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<tbody>
<tr>
<td><strong>NGS assay</strong></td>
<td>WGS</td>
</tr>
<tr>
<td>Total DNA and RNA requirement (ng)</td>
<td>250—1000</td>
</tr>
<tr>
<td>Tumor specimen requirement</td>
<td>FF tissue from at least one punch of 5 mm or FFPE tissue from two 1-mm-diameter core punches</td>
</tr>
<tr>
<td>Select examples of tests available</td>
<td>SNVs, CNAs, promoter and regulatory mutations, epigenetic modifications</td>
</tr>
<tr>
<td>Availability in clinical practice</td>
<td>Not widely adopted in clinical practice, usually limited to clinical research</td>
</tr>
<tr>
<td>Advantages</td>
<td>Allows for full complement of genomic analyses (intronic, exonic, intergenic regions)</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>High DNA input required; longer turnaround time vs genomic panels; requires paired blood for germline variation subtraction</td>
</tr>
</tbody>
</table>

*NGS assays are not directly comparable.

1Input requirements vary based on quality of DNA; 10 ng of DNA is equivalent to approximately 1500 to 3000 tumor cells.

HTG Molecular Diagnostics, Inc. (Tucson, AZ).

3In some cases, a matching normal specimen is also needed with its own specimen requirements.

*Tumor content requirements vary across different assays; cytology specimens may be applicable in some cases.


CNA, copy number alteration; FF, fresh frozen; FFPE, formalin-fixed, paraffin-embedded; indels, insertions/deletions; MSI, microsatellite instability; NGS, next-generation sequencing; RNA-seq, RNA sequencing; SNV, single nucleotide variant; TMB, tumor mutational burden; WES, whole-exome sequencing; WGS, whole-genome sequencing.
preparations. In addition, various cytology specimens, including archival stained smears, liquid-based cytology slides, archival unstained Cytospin preparations, FTA (Flinders Technology Associates) cards, and cryopreserved cells, can be used for molecular analysis. The technical requirements and features of each preparation type differ, but the quality and quantity of extracted DNA rely on adequate preanalytic assessment. Cytopathologists should therefore be familiar with different specimen types, associated technical procedures, and variables that can affect test results to correctly handle the material provided. It is recommended that the validation of NGS-based testing incorporates non–cell-block cytology specimens and that a cytopathologist is consulted as an expert to inform validation and liaison between the molecular laboratory and biopsy team.

A key preanalytic requirement for reliable biomarker analysis is that tissue specimens are of sufficient quantity and quality to permit morphologic diagnosis as well as ancillary biomarker profiling. Insufficient tissue specimens, for example because of inaccessible tumor location or late presentation with metastases at diagnosis, limit the available diagnostic testing options. For example, patients with lung cancer, particularly small cell lung cancer, are typically diagnosed through cytologic material only. However, large cell carcinomas of the lung cannot be identified with small biopsy or cytology specimens, and their diagnosis requires an invasive resection specimen. In addition, although biopsy methods of choice are used primarily based on patient safety, optimal procedures in the diagnostic setting should strive to obtain sufficient material, allowing morphologic diagnosis and downstream biomarker assays without delaying patient care and burdening a health care system with repeat biopsies.

Molecular profiles can be affected by intratumoral and intertumoral heterogeneity as well as prior therapies. These factors should therefore be considered when NGS is performed on specimens derived from patients with metastatic disease and/or prior treatment. Clinical scenarios may exist that require distinct biopsies before and after treatment and from any resistant or progressive lesions after treatment to facilitate monitoring of differential gene expression patterns that may influence treatment.
decisions. Tumor fraction, also known as tumor purity, refers to the proportion of tumor cells in a specimen and is an important factor to consider. A low tumor fraction may affect the reliability of molecular diagnostics, leading potentially to false-negative results; however, this could be partially alleviated by using macrodissection. A molecular pathologist with anatomic and cytopathology training can be particularly useful at this stage of the tissue journey in optimizing tissue triage and guiding nucleic acid extraction for optimal yield in downstream processes. In the absence of a molecular pathologist with such training, the involvement of an anatomic pathologist may also be useful for optimizing downstream testing. Training of staff involved in biopsy acquisition, triage, and rapid onsite evaluation of tissue adequacy during minimally invasive biopsy is also important.

Tumor fraction requirements commonly vary across different NGS assays and depend on the technical sensitivity of the NGS platform used. The incidence of successful NGS is generally lower in small specimens (core needle biopsy and cytology specimens) compared with larger specimens (resections and excisions), which may not only yield more nucleic acid but also include more nontumor cells, such as stromal or inflammatory cells. However, some tumor types tend to have low cellularity (eg, sclerotic or cystic) or may contain necrotic regions that can affect the nucleic acid yield. NGS readouts are usually considered to be consistently reliable from specimens with a tumor fraction and surface area >10% to 20% and 5 mm², respectively. The tumor fraction directly affects the nucleic acid yield; at the same time, the NGS requirements for nucleic acid input depend on the platform, gene panel size, and target enrichment method (Table 1). Indeed, insufficient nucleic acid input as well as substandard quality may result in library preparation failure or a high degree of sequencing artifacts, rendering the specimen unsuitable for diagnostic assessment. The quality of the nucleic acid extracted is paramount, requiring appropriate tissue specimen preparation and handling to obtain reliable results. (Table 1). Some NGS assays may have specific requirements for matched control specimens that should be considered at the time of tissue sampling (Figure 1). However, obtaining matched normal tissue specimens can be challenging for many solid tumors because of local invasion of the tumor within the surrounding normal tissue or field effects in apparently normal tissue adjacent to tumor. Therefore, this approach is not widely adopted in clinical practice. For nonhematologic malignancies, blood specimens can be obtained without many of the challenges that exist for tissue specimens. Matched blood specimens are required for whole-exome sequencing and some targeted gene panels to filter out germline alterations and rare variants and can enable a definitive diagnosis of inherited cancer predisposition syndromes. However, this process can be expensive, logistically challenging, and time-consuming and may not align with current clinical practices. Some targeted gene panels use prediction algorithms or databases to filter out the most common germline alterations to yield tumor-derived mutations, negating the need for a matching control specimen. Such panels rely on algorithms to filter out potential germline variants according to the published databases of known germline polymorphisms.

Tissue Processing

Methodologic variability in both prefixation and tissue preservation can affect the quality of tissue processing because changes in the molecular profiles of cells can occur as specimens are obtained, stored, and processed. Therefore, variables in sample processing should be considered when assessing NGS results, especially when performing RNA-seq and potentially methylome NGS analyses. In cases in which biopsy specimens are taken during surgery, the type of anesthetic used might be considered. In addition, clamping off the blood supply during surgery induces local tissue anoxia and hypoxia, which, together with pH changes and environmental stress, can affect the molecular profile of the tissue specimen. Prolonged time between tissue sampling and fixation also contributes to tissue ischemia, which can induce changes in gene and protein expression, such as decreased estrogen receptor expression in breast cancer tissue. The surgeon and pathologist should implement standard operating procedures to support timely tissue collection to minimize time from tissue acquisition to fixation.

For tissue preservation, FFPE tumor tissue and ultralow temperature freezing (−80°C to −190°C) as well as cytology samples are the preferred approaches to preserve the molecular profiles of cells. Ultralow temperature freezing is linked with superior preservation and quality of nucleic acid yield. At the same time, freezing disrupts the morphologic features and tumor fraction assessment, which, together with the additional cost, infrastructure, and process requirements associated with freezing procedures, renders the FFPE method the most common approach to preserve and store biopsy specimens in clinical practice. Cytology samples, such as fine-needle aspiration smears and Cytospin preparations, retain intact nuclei and thus can be valuable sources of quality tumor nucleic acid.

Alongside the preservation process, it is important to consider the type of fixative used and its mechanism of action. Formaldehyde, as a 10% neutral buffered formalin solution, is able to preserve the morphologic structure and architecture of a variety of tissues, hence its status as the most widely used fixative. FFPE specimens can provide a sufficient quantity and quality of DNA and RNA for analysis. However, concerns about the action of formalin on nucleic acids have been raised after the discovery of artificial mutations in formalin-fixed materials: formalin forms cross-links with DNA, which may result in sequence alterations during PCR amplification. Methanol fixation of tissue specimens is an alternative option, which has been
shown to yield higher-quality DNA with longer fragment size and more accurate copy number calling than FFPE specimens in whole-genome sequencing. The use of fixatives that incorporate picric acid, mercuric chloride, or tannic acid to enhance tissue penetration or fixatives that contain phenol or heavy-metal solutions to enhance protein precipitation has been validated in IHC studies, but data are lacking on their use as DNA-preserving agents. Methods used to preserve the morphologic structure of cytology samples vary considerably, thereby challenging the validation of these specimens in a clinical setting. Fixing with methanol, ethanol, isopropanol spray, or air drying, combined with different stains, are all commonly used methods that may also influence the quality of the nucleic acid yield.

Additional tissue preservation variables to consider include temperature, pH, and fixation time. Temperature can have an effect on the DNA yield because fixation at room temperature induces more DNA degradation than at lower temperatures. Immediate fixation at a low temperature is therefore recommended, although this may take more time than room temperature fixation and decrease the staining capacity of the specimen. pH is a critical parameter that affects the quality of nucleic acid yield; tissue fixed in formaldehyde at low pH is characterized by extensive DNA damage compared with tissue fixed in neutral pH. Finally, fixation time is a crucial consideration given that under-fixation can lead to nucleic acid and protein degradation or a change in gene expression within tissue regions that have not been permeated by the fixative solution, whereas over-fixation can result in extensive cross-linking and DNA fragmentation, which makes extraction of usable nucleic acids and proteins more difficult. Optimal fixation time is dependent on a number of other variables, including the tissue, fixative, and fixation temperature.

Molecular lability is an important factor to consider when specimens spend a long time in storage, especially when RNA analyses are to be conducted. Meanwhile, DNA and proteins can remain intact at −70°C for the long term. When storing material in a biorepository, appropriate specimen labeling and logging of all storage metadata are essential to assessing a specimen’s analytical properties.

Postfixation Variables

Postfixation variables that can affect the quality of FFPE tissue and non—cell block cytology sample processing, and hence the readout obtained, include histologic processing, cytopreparation and macrodissection or microdissection, staining, nucleic acid extraction and quality assessment, library preparation, sequencing methods, and bioinformatics. Triage of the tumor specimen can also affect the result because it is dependent on how well the pathologist directs to tumor-rich areas and viable tumor cells to allow for sufficient nucleic acid yield. Therefore, tissue processing for morphologic analysis should be informed by collaboration among the anatomic pathologist, molecular pathologist, and oncologist so that specimen preparation is efficiently conducted to minimize specimen waste and thereby optimize the tissue triage for ancillary testing. To maximize tissue availability from FFPE tissue, suitable sectioning and staining should be performed by experienced technicians using specialized microtomes that make use of the whole tissue specimen. Specific diagnostics have different requirements for thickness and staining of FFPE specimen sections (Table 1). Thin 2- to 5-μm sections are usually prepared for hematoxylin and eosin staining and ancillary studies, such as IHC staining, in situ hybridization, and molecular testing. It is also common practice to cut multiple (approximately 20) unstained sections to store for ancillary testing to minimize unnecessary tissue loss through recutting or refacing of FFPE blocks.

The type of ancillary diagnostic assay should also advise the method of nucleic acid extraction. Several manual and automated methods for DNA and/or RNA extraction from FFPE cancer cell line samples are available, including dual DNA and RNA extraction, specialized single extraction, manual silica column—based extraction, and automated magnetic bead—based extraction, each with different yield quantities and qualities. Various factors at this stage of the tissue journey may affect the extraction. For example, qualitative comparison of different extraction kits indicates that the quality of nucleic acid yields is influenced by the amount of processed tissue and the elution volume. The presence of salts is also essential for enhancing DNA precipitation from the alcohol, maximizing the resulting DNA yield. Therefore, the quality and quantity of the material required should be considered when choosing an extraction method. For cytology specimens, for example, seemingly small details, such as the type of glass slide used for direct smears, can affect DNA yield. Reverse transcription is an additional step needed for gene expression profiling and fusion analysis on RNA specimens isolated from fresh frozen or FFPE specimens. Prior protein extraction reduces nucleic acid yield from the same tissue specimen and vice versa. This means that if different diagnostic assays are planned, a suitable amount of specimen should be procured.

Before introducing any new laboratory-developed molecular assay into clinical practice, its performance characteristics must be validated. Several publications provide guidance on the process of preparing a new molecular assay for clinical use to assist clinical laboratories with the validation and ongoing monitoring of NGS testing to ensure high-quality sequencing results.

Analytic Considerations That Affect NGS Performance

Focusing on the analysis for genomic signatures, several variables that fall into the analytical phase may still
Table 2  Considerations for Reliable NGS Clinical Analysis

Stage: Tissue acquisition

<table>
<thead>
<tr>
<th>Considerations</th>
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<tbody>
<tr>
<td>• Use rapid onsite assessment of minimally invasive biopsies if applicable</td>
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<tr>
<td>• The performance parameters and specimen requirements, as informed by clinical validation of an NGS assay, are key educational points for personnel before genomic analysis. Assay development and validation should reflect the needs and capacity of local biopsy and anatomical pathologic practices</td>
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</table>

Stage: Tissue management

<table>
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<tr>
<th>Considerations</th>
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<tbody>
<tr>
<td>• Standardized tissue management processes should ideally be informed by the requirements of downstream analyses</td>
</tr>
<tr>
<td>• Appropriate specimen management to mitigate tissue waste and ensure suitable nucleic acid yield</td>
</tr>
<tr>
<td>• For small biopsy specimens, cores can be divided among multiple blocks rather than being limited to a single block</td>
</tr>
<tr>
<td>• FFPE tissue has traditionally been the preferred specimen type for molecular analysis. However, non—cell block cytology specimens remain an underused source of relatively well-preserved tumor. In the era of genomic analysis, this should be more formally acknowledged and incorporated into clinical practice and power comprehensive interinstitutional validation work</td>
</tr>
<tr>
<td>• Specimen labeling, storage, and shipping should be standardized and continuously monitored</td>
</tr>
<tr>
<td>• External quality assessment for accuracy, quality, and reproducibility of NGS assays</td>
</tr>
<tr>
<td>• Ancillary testing methods should be performed simultaneously as part of a comprehensive analysis of a single-input specimen</td>
</tr>
<tr>
<td>• Routine clinical testing in the oncology setting should move toward the use of comprehensive genomic testing and the identification of multiple diagnostic variables. Correlations between quantitative traits (eg, TMB, MSI) and qualitative traits (eg, alterations in DNA mismatch repair genes) have been identified</td>
</tr>
<tr>
<td>• Harmonization efforts across different laboratories, assays, and platforms, comparing data from the same pathology specimen, can be used to validate existing assays for use with a wider spectrum of specimen types</td>
</tr>
<tr>
<td>• Biomarker assessment should be routinely incorporated into clinical trials, with patients stratified to generate supportive data linking molecular biomarkers to clinical outcomes. A joint international effort has been proposed to help maximize data generation and support clinical trial designs</td>
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</table>

Stage: Engagement of multidisciplinary personnel in the tissue journey

<table>
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<tr>
<th>Considerations</th>
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<tbody>
<tr>
<td>• The tissue journey would benefit from stewardship by a molecular pathologist with NGS expertise</td>
</tr>
<tr>
<td>• The steward should identify, engage, and educate all key personnel (involved with biopsy acquisition on the rapid onsite evaluation sometimes available during minimally invasive biopsies) about the assay requirements, optimizing the value of precision medicine (eg, oncologists, radiologists, and surgeons should be informed of the minimum tissue requirements for NGS assay)</td>
</tr>
<tr>
<td>• Information services personnel should also be involved, facilitating transdisciplinary communication throughout the tissue journey</td>
</tr>
<tr>
<td>• Key personnel in the development of therapeutics with companion diagnostics could inform assay development. This can potentially address reimbursement challenges for NGS-based testing laboratories, especially as the scope for TMB/MSI testing assays and their associated costs expand</td>
</tr>
<tr>
<td>• Payers should also be engaged early in both therapeutic and clinical assay development</td>
</tr>
</tbody>
</table>

FFPE, formalin-fixed, paraffin-embedded; MSI, microsatellite instability; NGS, next-generation sequencing; TMB, tumor mutational burden.

influence the final result and should be acknowledged. Library preparation processes for NGS can induce discrepancies in the assay readout, risking misinterpretation of NGS data. PCR amplification can be a particular source of bias in DNA sequencing protocols, with different polymerases performing better than others. Depending on the platform used, different amplification methods may be used, such as bridge amplification, emulsion PCR, and the PacBio
DNA library preparation method. PCR-free protocols have been developed, but these protocols require large amounts of input material. The choice of sequencing method is also crucial; targeted cancer gene panels use capture hybridization-based or amplicon-based sequencing, both bearing their own specific characteristics. The choice of capture kit can also influence NGS results, particularly for RNA-seq assays.

There are two main types of NGS platforms used in molecular oncology clinical laboratories: Illumina and Ion Torrent systems. Both systems have sequential quality control checkpoints, including preanalytic tissue qualification, DNA quantification, library preparation, clonal amplification, sequencing, and postanalytical data, to ensure that high-quality data are generated. However, the platforms differ in their chemistry, detection methods, and individual specifications. Consequently, the two platforms differ in their determination of differentially expressed genes, even though leading to the same biological conclusions. This difference could be attributable to technical differences in the library preparation and sequencing technologies adopted by each platform.

The size of the genome area inspected by different NGS assays can also affect the results obtained. The size and composition of targeted panels may vary among laboratories, and any given gene within a panel may be completely or only partially (hotspot regions) sequenced. In addition, differences in protocol specification used to conduct biomarker analyses may also introduce bias and skew the result. Such variables have yet to be standardized in clinical practice; therefore, assay harmonization is recommended.

As the field of molecular diagnostics shifts focus from single-gene variants to genomic profiling, the likelihood of detecting incidental findings unrelated to the targeted disease increases. Accurate interpretation of sequence variants is complex when applied to a large cancer gene panel, and establishing robust criteria for the classification of specific gene variants as pathogenic or benign is challenging. However, standardization of data sources and workflows used for medical interpretation is strongly recommended to ensure reliability of NGS results, including the standardization of algorithms applied for coverage depth and evenness, enrichment in targeted regions, performance in guanine cytosine-rich regions, variant calling, germline variant filtering, and sequencing artifact removal.

Conclusions

Current clinical practice of cancer diagnostics can benefit from standardization of various activities within the tissue journey. This standardization can facilitate tumor specimen management and thereby optimize consistently accurate and reliable biomarker testing to guide patient diagnosis and treatment. Throughout the tissue journey, several processes risk tumor specimen attrition and compromise NGS result reliability. Factors that range from the quality of the tumor biopsy, specimen preservation processing, and specimen handling and storage, through to nucleic acid extraction, as well as various analytic points within the NGS workflow, such as the choice of sequencing platform, may yield discrepancies that could influence the decision-making process for patient treatment. Considerations for the standardization of preanalytic methods for NGS assessment are hence outlined to minimize inconsistency and ultimately improve patient outcomes (Table 2).

In summary, tumor biopsy specimens are being increasingly subjected to an ever-expanding array of ancillary molecular assays. With careful consideration of the optimal preanalytic techniques required for such testing by a multidisciplinary team, specimen preparation and management can be effectively standardized to minimize preanalytic variability across the tissue journey and to generate accurate biomarker analyses used to reliably inform patient treatment decisions.

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References


35. Palmieri G, Colombino M, Cossu A, Marchetti A, Botti G, Ascieto PA: Genetic instability and increased mutational load: which
diagnostic tool best direct patients with cancer to immunotherapy? J Transl Med 2017, 15:17


64. Grzywa TM, Paskal W, Wlodarski PK: Intratumor and intertumor heterogeneity in melanoma. Transl Oncol 2017, 10:956–975

Yelensky R: A computational approach to distinguish somatic vs. germline origin of genomic alterations from deep sequencing of cancer specimens without a matched normal. PLoS Comput Biol 2018, 14:e1005965


75. Douglas MP, Rogers SO: DNA damage caused by common cytotoxic drugs 2010, 48:389


