

The Role of Pathology in the Era of Personalized (Precision) Medicine: A Brief Review

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Abstract

This review provides a brief overview of the state-of-the-art molecular pathology approaches emphasizing the increasingly important pathology role in clinical precision cancer medicine. Recent advances in molecular biology and genetics have tremendously affected the practice of anatomic pathology, gradually transforming it from a morphology-based into a molecular-based discipline. Molecular diagnostics has a long tradition in pathology, especially in clinical pathology. The improvement of methodology for genomic testing in recent years has made it one of the cornerstones of precision cancer medicine. The decisions related to cancer treatments are no longer solely based on the histopathological diagnosis. Various genomic analyses of human cancers are being incorporated into diagnostic and decision-making algorithms. **Conclusion.** The pathologists continue to play an essential role in developing and implementing molecular and genomic tests in practice and communicate the results and their relevance with clinicians. Such activities are of utmost importance for successfully translating scientific advancements into a benefit to patients (“next-generation pathologists”).

Key Words: Cancer Therapy ■ Precision Medicine ■ Pathology ■ Diagnostics ■ Companion Diagnostics.

Introduction

In the early 19th century, diagnostic pathology was predominantly restricted to a postmortem assessment, and description of macroscopic tumor spread in the dissection room (1). However, pathology's principles and practice changed dramatically with the establishment of light microscopy and histopathologic investigations in the second half of the 19th century by the renowned German pathologist Dr. Rudolf Virchow (1821–1902), who is considered the ‘father of modern pathology.’ Thanks to these advances, it became possible to investigate different microscopic characteristics of various human diseases, including cancer (1).

Microscopic classification of tumors has, over the last 100 years, helped in improving the pathologic diagnosis of neoplastic diseases, predicting tumor behavior and thus formulating the prognosis for each case and thus facilitating clinical decision-making. Traditional cancer classification is based on microscopic evaluations, focusing on various clinicopathological features of tumors, such as tumor morphology (type), grade, surgical margins, vascular/lymphatic invasion, lymph node assessment and routine analysis of biomarkers. Pathologists traditionally utilize frozen sections that enable a rapid gross and microscopic tissue analysis and guide surgeons' hands during surgery (e.g., providing provisional diagnosis, assessing surgical margins, and identifying tissue of origins) (2) (Figure 1). However, none of those mentioned above

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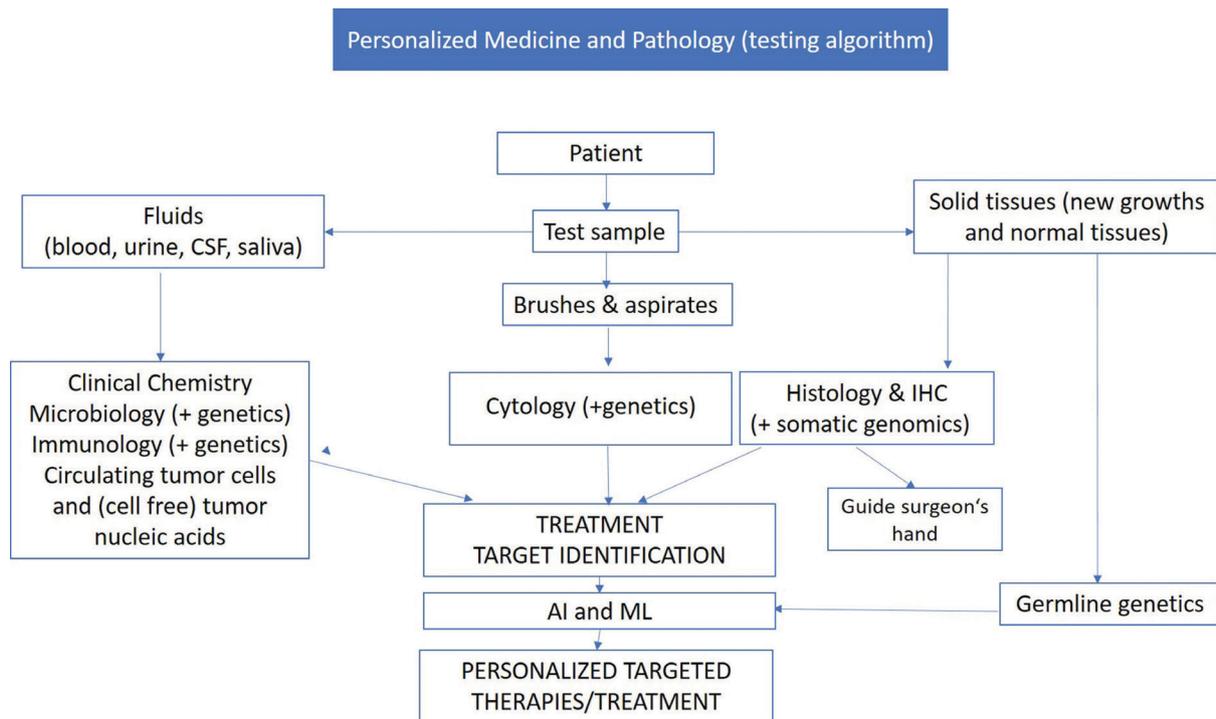


Figure 1. A proposed algorithm for the pathology role in personalized (precision) medicine. CSF=Cerebrospinal fluid; IHC=Immunohistochemistry; AI=Artificial intelligence; ML=Machine learning.

may capture individual cancers' variable clinical courses (heterogeneity).

The underlying basic biology of various cancer and their development and progression are still poorly understood but undoubtedly depend on each tumor's genetic background. The fact that cancer is a genetic disorder was suspected surprisingly early (1914) in the history of cancer research by Dr. Theodor Boveri. He presented a systematic somatic mutation theory of cancer (3). According to Boveri's hypothesis, chromosomal changes caused the transition from normal to the cells' malignant proliferation (3). However, the detailed oncogenesis model was first described by Nowell some 50 years later (4). Dr. Nowell provided evidence that the accumulation of mutations in cancers over time makes them more advanced and aggressive, increasing their metastatic potential. In 2000, Drs. Hanahan and Weinberg elegantly explained detailed cancer evolution in their seminal papers "The hallmarks of cancer" (5, 6). The authors outlined the principal biological characteristics of carcinogenesis ("hallmarks of cancer").

These features include not only cell proliferation/replication, apoptosis, (neo)angiogenesis, down-regulation of growth suppressors, invasion/metastasis (5) but also the altered metabolic properties, genomic instability/mutations, escape from immune surveillance, and tumor-promoting inflammation (6). This evolutionary model of carcinogenesis can efficiently explain the complexity and the marked heterogeneity that characterize the vast majority of human cancers (7). Recent findings from high-throughput, multiplex/massively parallel technologies (assays) added important information about the underlying genomic alterations and human cancers' biological events. These findings provide insights into novel treatment modalities and allow for patient stratifications that impact cancer patients' management (7).

In the previous two decades, with the increasing understanding of human cancer's molecular genomic drivers, the number of novel biomarkers and the subsequent development of targeted pharmaceutical treatments have dramatically increased. These changes have strongly influenced

diagnostic pathology practice, gradually transforming it from morphology-based into molecular-based discipline.

This brief review will discuss and critically assess molecular biology's contributions to diagnostic pathology and precision cancer medicine.

Precision Medicine and Pathology

The concept of personalized medicine stems from the advances in biotechnology, molecular biology, and genetics. It affects the diagnostic tests that may guide the therapeutic options based on individual (specific) characteristics (1). Using massively parallel, high-throughput technologies, these advances enable detecting minute and precise changes at different molecular (DNA, RNA, protein) levels in enormous quantity and variety and at an ever-increasing speed. The methods include DNA/RNA sequencing, mass spectrometry, microarray technologies, comparative genomic hybridization, digital polymerase chain reaction (PCR), each of which generates an enormous quantity of information called "big data" (8, 9).

In the U.S., the Personalized Medicine Coalition defined personalized medicine as the "tailoring of medical treatment to each patient's characteristics. It does not mean that drugs (or medical devices) are developed to be unique to a patient but rather the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment. Therefore, the preventive or therapeutic interventions can be concentrated on those who will benefit, sparing expense, and adverse effects for those who will not" (10). On the other hand, the U.S. National Cancer Institute (NCI) defines personalized medicine as "a form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease" (11). The key features of personalized medicine include identifying populations (or individual patients) who are candidates for treatment, either by identifying patient/disease characteristics that are likely to benefit or show no improvement/develop adverse effects from treatment (12). Von

Hoff and collaborators at TGen and Caris Life Sciences were the pioneers in the precision medicine treatment approach, demonstrating that molecular profiling of patients' tumors is an efficient approach to identifying potential targets and selecting the best treatments for their refractory cancers (13). Since then, numerous studies have confirmed the clinical relevance of molecular profiling and personalized medicine in cancer treatment leading to the paradigm shift in cancer treatment (9).

Even though personalized medicine's potential benefits are already evident (e.g., enhanced therapeutic efficacy and increased safety of targeted drugs), its uptake by health care systems varies across the globe. Generally, it remains limited at this time (14). However, it is anticipated that, as a scientific understanding of cancer progresses at the molecular level, personalized medicine approaches to cancer diagnosis and treatment will become more common. For example, the number of personalized medicine devices that have been commercialized in Europe quadrupled between 2006 and 2011 (10). The development of therapeutic products (devices) that are paired with diagnostic tests is also rapidly increasing. In December 2013, the Food and Drug Administration (FDA) approved the first next-generation sequencer (NGS) (Illumina's MiSeqDx) for commercial use in the United States (15). This platform's approval made possible the dozens of genomic information in clinical practice, and markedly expanded this technology's clinical utility to guide patients' care (15). In 2017, the FDA approved the FoundationOne CDx (Foundation Medicine, Inc.) as the first comprehensive, FDA-approved tissue-based companion diagnostic (CDx) assay that is validated for all solid tumors (16). In August 2020, the FDA granted approval for the first liquid biopsy-based NGS CDx test, The Guardant360 CDx (The Guardant Health, Inc.) (17). The Guardant360 CDx utilizes liquid biopsy and NGS technology to detect 55 different genomic alterations, including *EGFR* gene mutations in patients with NSCLC from circulating cell-free DNA (cfDNA). Besides, several comprehensive NGS-platforms are currently available (e.g., Caris MI profile, Tempus xT assay,

Invitae Multi-Cancer Panel) (18) and are being increasingly used in cancer decision-making and treatment (19) (please refer to the next paragraph for more details). Recently, the European Society of Medical Oncology (ESMO) Precision Medicine Working up came out with their recommendations for the use of NGS testing for patients with metastatic cancers (20). Using the ESMO Scale for Clinical Actionability of molecular Targets (ES-CAT), the ESMO group found concrete evidence. It strongly recommended routine NGS testing for patients with metastatic NSCLC, prostate, ovarian cancers, and cholangiocarcinoma. Besides, they recommended NGS testing for several other common cancers such as colorectal cancer, small cell lung cancer (SCLC), endometrial cancer, neuroendocrine tumors, salivary, thyroid, and vulvar cancers (mainly for the tumor mutational burden (TMB) assessment as a predictive biomarker for immune checkpoint inhibitors) (20).

The Concept of Companion Diagnostic Tests (CDx)

The rise of precision medicine in pathology accelerated in the 1980s with the diagnostic use of automated immunohistochemistry (IHC) (21, 22). Since that time, IHC has been substantially improved, standardized, and its clinical utility in cancer diagnostics has been markedly expanded, particularly in recent years (23-27). IHC is an essential tool for cancer diagnostics and tumor typing (Figure 1). However, it has been increasingly used as a reliable and affordable method for precision medicine purposes. The vast majority of diagnostic tests are developed in the individual laboratories and are designated as a laboratory (or in-house) developed tests (LDT). However, several IHC tests are now designated as “companion diagnostic” or “complementary diagnostic” tests to indicate their status concerning the FDA approval/clearance status. Although IHC is a relatively simple and widely utilized method, frequently fully automated in most pathology laboratories, its interpretation is entirely subjective; hence a strict training/validation processes must be applied.

A companion diagnostic device is defined as “an in vitro diagnostic device (IVD) or an imaging tool that provides information that is essential for the safe and effective use of a corresponding therapeutic product” (28). The use of an IVD companion diagnostic test with a specific therapeutic product is stipulated in the instructions for use in the labeling of both the diagnostic device and the corresponding medicinal product and the labeling of any generic or biosimilar equivalents of the therapeutic product. In other words, a CDx test is required before a specific treatment to determine eligibility. On the other hand, although predictive, a complementary diagnostics test is not needed for the drug’s prescription. The concept of precision medicine is accompanied by various CDx tests that have become increasingly approved and used for targeted treatment purposes (29) [a full list of companion diagnostic devices that have been approved by the FDA is available at its website (28)].

One of the earliest CDx relied on the identification of *HER2/erbB2* gene amplification (in-situ hybridization) or HER2 protein overexpression (immunohistochemistry) in breast and later in gastric/gastroesophageal junction (GEJ) cancers for the treatment with various anti-HER2 therapeutics such as trastuzumab and pertuzumab (monoclonal antibodies) and lapatinib (a small molecule tyrosine kinase inhibitor that inhibits HER2 specifically) (30-32) (Table 1).

Additional examples of commonly used CDx assays include the assessment of *C-KIT* (CD117) and *PDGFR α* mutations in gastrointestinal stromal tumors (GIST) (33), tyrosine kinase mutations affecting the Epidermal growth factor receptor (*EGFR*) gene, and *EML-ALK* gene rearrangements in non-small cell lung cancer (NSCLC) (34-36), and mutations in the *BRAF* gene in malignant melanoma (37). Notably, most of these genetic alterations have a substantial potential to render tumors susceptible to specific inhibitors mentioned above. In addition to diagnostic PCR or in-situ hybridization-based assays, IHC testing, performed by pathologists, is robust, genomic alteration-specific, cheap, and consequently utilized in a clinical setting. For instance, the ALK 5A4 IHC assay has

Table 1. Some of the Commonly Used Single Diagnostic Tests [Diagnostic Antibodies (Immunohistochemistry) and Probes (in Situ Hybridization)] with an Approved Status As “Companion Diagnostic” Assays

Biomarker/diagnostic assay (manufacturer)	Scoring system	Companion diagnostic (CDx) Thresholds	Indication (cancer subtype)	Drug(s)
Immuno-Oncology (I-O) Biomarkers				
22c3 (DAKO Agilent)	CPS	<1, ≥1, ≥10	Gastric/GEJ carcinoma, cervical carcinoma TNBC	Pembrolizumab
22c3 (DAKO Agilent)	TPS	0, 1-49, ≥50	NSCLC	Pembrolizumab
SP142 (Ventana)	IC scoring	IC ≥1%, IC≥5%	TNBC, Urothelial carcinoma (bladder)	Atezolizumab
Other companion diagnostic (CDx) single tests				
ALK Testing ALK D5F3 Ventana (IHC)	Positive/ negative	Any percentage of positive cancer cells	NSCLC	Crizotinib or ceritinib
Vysis ALK Break Apart FISH Probe Kit (Abbott) (FISH)	-	Identification of <i>ALK</i> gene rearrangements with its partners (e.g., <i>EML4</i> , <i>TFG</i> , <i>KIF5B</i>)	NSCLC	Crizotinib or ceritinib
HER2 testing anti-HER2 (4B5) (Ventana) (IHC)	Score 0-3+	Score 3+ in cancer cells	Breast cancer	Trastuzumab, pertuzumab, adotrastuzumab emtansine
INFORM HER2 Dual ISH DNA Probe Cocktail (Ventana) (ISH)	<i>HER2/CEP17</i> ratio	<i>HER2/CEP17</i> ratio ≥2 (positive)	Breast cancer	Trastuzumab, pertuzumab, adotrastuzumab emtansine
DAKO c-Kit PharmDx (IHC)	Positive/ negative	Any expression in cancer cells	GIST	Imatinib mesylate
DAKO EGFR PharmDx (IHC)	Positive/ negative	Any membranous expression in cancer cells	Colorectal cancer	Cetuximab, panatumumab

All the listed assays have been approved by the Food and Drug Administration/FDA/ (28). ALK=Anaplastic lymphoma kinase; CEP17=Centromere of chromosome 17; CPS=Combined positive score is defined as the number of PD-L1 positive cells (cancer cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells, multiplied by 100; EGFR=Epidermal growth factor receptor; FISH=Fluorescent in situ hybridization; GEJ=carcinoma Gastroesophageal junction carcinoma; GIST=Gastrointestinal stromal tumor; HER2=Human epidermal growth receptor 2; IC (immune cells) score=A proportion of the tumor area occupied by PD-L1 staining of any intensity; IHC=Immunohistochemistry; ISH=In situ hybridization; NSCLC=Non-small cell lung cancer; TC=Tumor cells; TNBC=Triple-negative breast cancer; TPS=Tumor proportion score. Defined as the percentage of viable cancer cells showing a partial or complete membrane staining at any intensity.

been confirmed as a reliable screening diagnostic test for ALK-rearranged NSCLCs and is associated with treatment response and survival (38). Similarly, c-Kit expression using c-Kit PharmDx (Agilent) is indicated in both differential diagnosis of gastrointestinal stromal tumors (GIST) (95% positive) and in selecting the GIST patients that are eligible for treatment with imatinib mesylate (Gleevec). EGFR pharmDx™ is another FDA-approved IHC-based assay that was supposed to identify colorectal cancer patients who are eligible for anti-EGFR-treatment modalities such as cetuximab or panatumumab (Table 1). However, later studies revealed that EGFR protein expression is

not a clinically useful predictive biomarker to cetuximab response in patients with CRC (39, 40).

Specific biomarkers can also indicate when patients are less likely to respond to a specific therapy, e.g., *KRAS* or *NRAS* mutations make colorectal cancers unresponsive to anti-epidermal growth factor receptor (anti-EGFR) therapies such as cetuximab or panatumumab (40-42). Similarly, specific *EGFR* gene mutations in NSCLC (e.g., point mutation T790M) are responsible for approximately 50% of acquired resistance to the EGFR tyrosine kinase inhibitors (43).

In contrast to those mentioned above single genomic assays, FoundationOne CDx is the first FDA-approved comprehensive tissue-based CDx

that has been validated in a variety of solid tumors. It includes a broad panel of tested genes (n=324) and two genomic signatures. It has already been utilized in treatment decision-making for various solid cancers, including NSCLC, colorectal, breast, ovarian cancer, and malignant melanoma (44) (Table 2). The test also provides microsatellite instability (MSI) and tumor mutational burden (TMB) status, both of which are essential predictive biomarkers in immuno-oncology (I-O) treatment regardless of the tumor histology (“tumor agnostic approach”) (please refer to the next paragraph). The FoundationOne CDx is also a CDx for identifying the neurotrophic receptor tyrosine kinase (NTRK) genes (*NTRK1*, *NTRK2*, and *NTRK3*) fusions. All the patients whose cancers harbor any of these fusions and regardless of the tumor histotype are eligible for the treatment with an NTRK inhibitor (entrectinib or larotrectinib) (Another “tumor agnostic approach”) (45). FoundationOne Liquid CDx is a similar FDA-approved CDx that explores potentially targetable genes using a simple blood sample (liquid biopsy, which is based on peripheral blood analysis of cell-free circulating tumor DNA). It is along with the Guardant360 CDx, the only comprehensive FDA-approved blood-based test that analyzes > 300 genes, making it the most comprehensive FDA-approved liquid biopsy CDx currently available (Table 2). Besides, FoundationOne Liquid CDx provides blood tumor mutational burden (bTMB), MSI, and tumor fraction values. Another FDA-approved comprehensive molecular genomic assay is the MSK-IMPACT™ test developed by the Memorial Sloan Kettering Cancer Center. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) covers 468 different genes, many of which are potentially targetable with currently available drugs (46). MSK-IMPACT has been shown to reliably profile tumor DNA for somatic mutations in various solid malignancies with high accuracy and sensitivity (47). In contrast to the FoundationOne CDx, this assay is mainly used in the United States.

Immunotherapy, based on immune checkpoint inhibitors against programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) axis,

has dramatically changed the cancer treatment paradigm and improved the outcomes of several common cancers such as melanoma, NSCLC, renal, urothelial, cervical, gastric/gastroesophageal (GEJ), head and neck squamous cell and triple-negative breast carcinomas (TNBC) (48). The therapeutic breakthroughs have been followed by the development of predictive biomarkers of response to immune checkpoint inhibitors. Several of these biomarkers have been validated and achieved a companion diagnostic status, including PD-L1 expression in tumor (T.C.) or immune cells (IC) tested by IHC. Tumor mutational burden (TMB) and microsatellite instability-high (MSI-H) status, determined by NGS-based assays, have also been approved as predictive biomarkers to immune checkpoint blockade (e.g., pembrolizumab) (49) (Table 2). Other potential predictive biomarkers [e.g., PD-1 status, PD-L1 (*CD274* gene) amplification], although extensively studied, have not been fully validated and consequently approved as CDx assays.

Multiple IHC assays with different scoring algorithms have been approved for various immune checkpoint blockade therapies and associated cancers (Table 1). Several of these IHC assays have been approved as CDx, such as VENTANA SP142 assay for selecting NSCLC, bladder, and TNBC patients for the treatment with immune checkpoint inhibitor atezolizumab. DAKO 22C3 pharmDx assay has been approved for testing eligibility of NSCLC, TNBC, gastric/GEJ, cervical, urothelial, head and neck squamous cell (HNSCC), and esophageal squamous cell carcinoma patients for the treatment with pembrolizumab while DAKO 28-8 pharmDx clone is utilized for the selection of NSCLC patients for the combined treatment with two immune checkpoint inhibitors: nivolumab (against PD-1) + ipilimumab (targeting cytotoxic T-lymphocyte-associated protein 4/CTLA-4/) (28). Notably, each of these assays has different scoring algorithms, e.g., tumor proportion score (TPS), combined positive score (CPS), and immune cells (I.C.) score, all of which have different thresholds for positivity. These issues make the current PD-L1 IHC testing landscape very compli-

Table 2. Overview of the Commonly Used and FDA-Approved Comprehensive Companion Diagnostic Tests

Assay [*]	Technology	Gene panel [†]	Indications [‡]	Drugs
FoundationOne CDx [§]	NGS-based	324 targetable Genes MSI status TMB status	Multiple cancers (NSCLC, colon, breast, ovary, melanoma)	21 FDA-approved targeted therapies
FoundationOne Liquid CDx	NGS-based	300 targetable genes MSI status Blood TMB status	Multiple cancers	Multiple targeted drugs
MSK-IMPACT [§]	NGS-based	468 targetable genes MSI status	Multiple cancers	Multiple targeted drugs
The Guardant360 CDx	NGS-based	55 targetable genes, including <i>EGFR</i>	NSCLC and other cancers	TAGRISSO (Osimertinib) and other targeted drugs

^{*}Manufacturer; [†]Predictive biomarkers; [‡]Cancer subtypes; [§]Tissue-based assay; ^{||}Based on peripheral blood analysis of cell-free circulating tumor DNA (cfDNA) (=liquid biopsy); EGFR=Epidermal Growth Factor Receptor; FDA=Food and Drug Administration; MSI=Microsatellite instability; NGS=Next-generation sequencing; NSCLC=Non-small cell lung cancer; TMB=Tumor mutational burden.

cated. They require pathologists to be specifically trained for each of the proposed assays and their respective scoring systems.

Besides, PD-L1 status in cancer is substantially challenged by its complex regulation within the tumor parenchyma and microenvironment (immune cells), its dynamic clonal and proteomic changes to therapy, heterogeneous host immune defects, and markedly variable standardization among sample preparation and reporting (48). Despite all the advances mentioned above, there is still an unmet need to optimize predictive I-O biomarkers given their limited clinical utility (a low response rate/resistance in most cancers) and highly demanding testing and interpretation algorithms (20).

Challenges Ahead: “Next-Generation Pathologists” and Precision Medicine

Molecular pathology has become one of the cornerstones of precision cancer medicine. This change has required enormous efforts from pathologists to gain and demonstrate expertise and skills in this rapidly evolving era (50). Simultaneously, the paradigm shift allowed for the discipline of pathology to reinvent itself as a leading diagnostic discipline in the precision medicine era. Indeed, this would imply additional and continuous efforts to educate pathologists in using genomic and molecular data and interpret novel diagnostic tests and proce-

dures. Therefore, the dissemination of knowledge on molecular pathology among pathologists by incorporating the courses in postgraduate training programs for trained pathologists and improving pathology residency training programs are critical for the future of diagnostic molecular pathology (51). In this regard, several countries have already taken the necessary measures. Thus, the Dutch Society of Pathology has launched a 2-year training program in molecular pathology, while the Royal College of Pathologists has a fellowship in molecular pathology for clinical scientists. In the United States, the Association of Molecular Pathology has also developed a molecular pathology curriculum for medical laboratory scientists (52). At the same time, the American Board of Pathology offers certification for the pathologist completing at least 12 months of training in an ACGME (The Accreditation Council for Graduate Medical Education) accredited molecular genetic pathology program (53).

Another essential aspect in which the pathologists play a crucial role is developing and implementing molecular tests in clinical practice and communicating the obtained results with other diagnostic disciplines (e.g., microbiology, clinical chemistry, genetics, and immunology) (15, 54-56) and clinicians (Figure 1). Pathologists also play a crucial role in optimizing the samples for molecular profiling. They are also responsible for the proper and selective use of the available specimens

to enhance predictive tests' clinical utility. New, complementary diagnostic methods have also been launched, e.g., the sequence analysis of cell-free tumor DNA isolated from plasma or urine (=liquid biopsy) (Figure 1). Liquid biopsy and the traditional tissue samples (biopsy, cytology) are of utmost importance for identifying biomarkers for personalized medicine. Increased availability of diagnostic tests and a growing emphasis on personalized medicine have been approached by the novel, revolutionary technologies such as machine learning (ML) and artificial intelligence (A.I.), both of which could greatly help in analyzing the "big data" and identifying the proper biomarkers for precision medicine (treatment) and diagnostics ("personalized diagnosis") (57, 58) (Figure 1). Both methods are rapidly evolving and are believed to contribute to precision medicine and other healthcare fields.

All of the activities mentioned above contribute to a successful translation of the scientific advancements into a benefit to patients ("next-generation pathologists"). In this regard, the European Society of Pathology (ESP) and the Royal College of Pathology (RCPATH) groups proposed the guidelines for laboratories performing molecular pathology for cancer patients (52). The guideline encompasses all the essential issues related to molecular pathology testing, including preanalytical considerations, sample receipt and handling (fixation and processing), DNA and RNA extraction, selection of appropriate analytical method(s), and quality control (both internal and external) (52). College of American Pathologists (CAP) has also been actively developing numerous evidence-based guidelines about preanalytical and analytical aspects of diagnostic and molecular pathology. These guidelines also include precision medicine biomarkers and CDx assays such as estrogen receptor (ER), progesterone receptor (PR) and HER2 (breast cancer), various predictive biomarkers in NSCLC and CRC, biomarkers for prostate cancer, Human Papillomavirus (HPV) testing in HNSCC, etc. (59). Similar initiatives have been taken by international external quality assurance (EQA) agencies [e.g., UK NEQAS ICC & ISH,

NordiQC, and European Molecular Quality Network (EMQN)], who put joint efforts to publish the guidelines for EQA. These guidelines aim to improve molecular tests' performance for precision medicine purposes (60).

Conclusions and Future Directions

The practice of diagnostic pathology has been substantially changed in the previous years due to the advances in molecular diagnostics and targeted treatment (precision medicine). Molecular pathology has become one of the cornerstones of precision cancer medicine. Although "the next-generation pathologists" have already been launched, further and continuous educational efforts must fully implement the paradigm shift into diagnostic molecular pathology practice and reinvent it as a leading diagnostic discipline in the precision medicine era. Most of the approved and validated predictive biomarkers in precision medicine still require further optimization and standardization. There is an unmet need for novel and more reliable predictive tests (biomarkers) given a low response rate and common resistance for most approved targeted treatment modalities.

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