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# Pathology and clinical practice: A review of the latest diagnostic techniques and tools in cancer diagnosis

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***Abstract*---**Background: The advent of molecular biomarkers has revolutionized cancer diagnosis and treatment, enhancing the precision of therapeutic strategies for solid tumors. However, the complexity of clinical decision-making has escalated with the increasing number of prognostic and predictive biomarkers. The integration of deep learning (DL) in histology image analysis promises to streamline these processes. Aim: This review aims to evaluate the latest diagnostic techniques and tools in cancer diagnosis, focusing on the role of molecular biomarkers and deep learning in enhancing clinical outcomes. Methods: A comprehensive review of recent studies and clinical trials was conducted, examining the impact of molecular biomarkers on cancer treatment and the application of DL in histology image analysis. The review covered fundamental DL applications in tumor identification, grading, subtyping, and advanced applications in predicting genetic mutations, treatment responses, and survival outcomes. Results: DL-based methods have shown high accuracy in automating histopathology workflows, matching or surpassing human performance in tumor detection and classification. Advanced DL applications offer new insights by predicting genetic alterations and clinical outcomes directly from histology images, which could significantly impact clinical decision-making. Conclusion: The integration of DL into cancer histology analysis presents a transformative approach to cancer diagnostics, offering both efficiency in routine tasks and novel predictive capabilities that could reshape personalized treatment strategies. Despite the promise, further validation in diverse patient cohorts is necessary to ensure the generalizability and clinical utility of these DL applications.

***Keywords*---**Cancer diagnosis, molecular biomarkers, deep learning, histology image analysis, predictive biomarkers, clinical decision-making.

## Introduction

Decision-making procedures in modern oncology have developed outside of simple, linear processes. The development of an increasing range of biomarkers has turned these processes into intricate, branching decision trees that complicate solid tumor therapy recommendations. As of right now, oncology uses molecular biomarkers either predictive or prognostic. By helping to stratify patients depending on their risk of illness progression or death, prognostic indicators enable tailored changes in therapy intensity. In stage II colorectal cancer (CRC), for example, microsatellite instability (MSI) is a prognostic biomarker; its presence predicts a better prognosis, which allows the use of less intense adjuvant treatment [1]. Conversely, predictive biomarkers direct the choice of focused therapy for particular patient groups. For immune checkpoint inhibitor-based immunotherapy in treatment-resistant stage IV CRC, for instance, MSI is an FDA-approved biomarker whose detection corresponds with a greater likelihood of a good therapeutic response, therefore providing a strong predictive value [2]. In breast cancer, too, HER2 positive makes anti-HER2 drugs possible

and functions as a robust predictive biomarker [3, 4]. Numerous molecular biomarkers, including mutations in the epidermal growth factor receptor (EGFR) gene, anaplastic lymphoma kinase (ALK) gene fusions, and the overexpression of programmed cell death ligand 1 (PD-L1), which are integral components of the molecular panel used in the standard care of advanced or metastatic disease, shape the treatment of non-small-cell lung cancer (NSCLC). Thus, the increasing number and therapeutic relevance of molecular biomarkers in daily practice help to enable exact tailoring of cancer treatments based on the genetic profile of individual tumors. In daily operations, this also results in longer turnaround times, higher tissue demands, and more expenses [8, 9].

Predictive indicators are becoming ever more important in the design of clinical trials for new treatment medicines in solid malignancies. multiple Phase 2 and 3 studies over the past five years have focused on unusual genetic subpopulations of solid tumors, notably those defined by MSI [10], homologous repair deficiency [11], and fusion-driven malignancies spanning multiple cancer types [12, 13, 14]. While poly ADP-ribose polymerase (PARP) inhibitors essentially target cancers with homologous repair deficit, MSI is employed as a predictive biomarker for immunotherapy and shows remarkable responses to molecularly targeted therapies. Screening possible volunteers for these studies is expensive and limited by the availability of molecular assays, though, since these genotypes are found in just 1% to 10% of real-world populations. Therefore, even if the growing number of prognostic and predictive biomarkers allows more exact cancer treatment, the complexity of clinical decision-making procedures presents major difficulties in regular clinical practice and clinical trial recruitment. Although most new biomarkers in oncology are acquired from molecular biology assays, developments in deep learning (DL) are allowing the extraction of once secret information straight from routinely available data. Using artificial neural networks, DL, a subtype of artificial intelligence (AI), finds trends in challenging data. Analyzing picture data—rich in information and fit for DL methods—is especially benefit from this.

Especially in domains involving imaging data, DL-based image analysis finds extensive use in contemporary medicine. In radiology, for instance, DL does repeated tasks with accuracy either matching or surpassing human performance, such tumor detection or organ segmentation on computed tomography (CT) images. More than a dozen DL techniques for clinical application in radiology have been already approved by the FDA. Especially, a 2019 lung cancer screening experiment [15] made use of DL-based CT data analysis; evidence supporting the therapeutic relevance of these techniques is fast expanding. Additionally appropriate for DL-based analysis are magnetic resonance imaging (MRI) data, which provide more information than CT data [16]. Moreover, DL has shown strong performance on non-radiology tasks like skin cancer identification in dermoscopy pictures [19, 20] and real-time analysis of endoscopy images [17, 18]. But histology offers a rich supply of pictures with high information density that can be routinely produced in clinical practice as compared to other imaging modalities. In terms of pixel count, histological images have more information than radiological images; millions of distinct cells can be seen on a histology slide and their shape and spatial arrangement transmit more information than in other medical images. Histology pictures are a very important source for DL-based

biomarker extraction since the dataset from a single histology whole-slide image of a tumor is far greater than that of a whole chest CT dataset from the same patient

**Deep Learning-Based Histology Image Analysis**

Tissue samples are normally taken by biopsy or surgical resection, followed by pathological preparation and, most frequently, staining with hematoxylin and eosin (H&E) during the diagnostic evaluation of patients with solid tumors. As such, almost all cancer patients have regular access to H&E slides, which makes them a conveniently available and richly informational data source for evaluation through deep learning (DL) techniques. This accessibility helps to explain why so much earlier research concentrated on these kinds of images. Nevertheless, alternative histological stains including immunohistochemistry (IHC) [21] and periodic acid-Schiff [22] can also be used with DL.

**Steps in Image Processing**

For DL-based image analysis, the large data size of scanned whole-slide histology images poses practical hurdles. DL operations require graphics processing units (GPUs), but loading them entirely into GPU memory is unfeasible due to their high file sizes. Furthermore, a significant amount of non-tumor tissue is frequently present in histology photos, which reduces the amount of information overall. It takes substantial preparation to handle the problems these huge and varied photos present. A uniform image analysis pipeline has been created as a result. This methodical procedure consists of tessellation, image tile preprocessing, and training and testing of a deep learning network, or DL classifier, which can then be used with external validation cohorts. A computer software that can classify comparable data after being taught on a collection of examples is referred to as a "classifier". A classifier can identify "tumor" and "non-tumor" tissue in small image patches when used in histology image analysis. It may also identify patients as "potential responders" or "potential non-responders." When it comes to classifiers in general, DL networks are the most popular and effective kind.

**DL's Basic and Advanced Uses in Cancer Histology**

Histological pictures can be used in a range of DL applications after normal preprocessing. DL processes estimate a predefined label from image data by training on a cohort of patients. A variety of labels have been investigated in earlier research, ranging from identifying the direct tumor genotype from histological images [26, 27] to anticipating the existence of invasive tumor tissue in prostate samples [23, 24, 25]. We suggest classifying these labels according to whether they are utilized in basic or advanced deep learning applications. The goal of Basic DL Applications is to automate regular processes that are currently completed only by human pathologists. Examples include the identification of tumor tissue in biopsy samples or the morphological subtyping of tumors, such as the Gleason score assigned to prostate cancer. Here, the label used to train a DL system is the Gleason score. Although they don't change the essential data

that physicians use to guide their treatment decisions, these simple DL apps offer the potential to save costs and turnaround times in pathology departments.

On the other hand, Advanced DL Applications go beyond the typical reporting that pathologists already do. One illustration is the direct prediction of genetic alterations and survival from tissue slides stained with H&E. When it comes to genetic alterations, the genotype—which is identified by established diagnostic processes utilizing a molecular biology assay or other gold-standard methods—is the picture label. The kind of test that is used to label photos during training is referred to as "ground truth". Thus, by examining histological image data, the DL classifier can be trained to mimic the "ground truth" (also referred to as the "gold standard method"). These sophisticated DL apps, in contrast to basic ones, can give physicians access to supplementary data that isn't usually gleaned from standard sources in today's clinical processes. DL is consequently a powerful tool for information extraction from solid tumor histology images, capable of automating existing workflows or producing new insights not used in clinical practice yet. The present status of basic and advanced DL applications in cancer histology image analysis is summarized in the following sections.

**Fundamental Uses of DL: Subtyping, Grading, and Tumor Identification**

A qualified pathologist often performs a thorough study on each solid tumor sample to verify the presence of tumor tissue and to provide additional information such tumor grade and subtype. DL has shown its ability to automate repetitive operations in diagnostic pathology in these fundamental yet important diagnostic procedures.

**Using DL to Automate Histopathology Workflows**

Digital pathology research has spent many years concentrating on and improving upon fundamental image analysis tasks, such as cell number quantification [30], tumor identification [28], tumor subtyping [29], and cell type categorization [31]. These methods usually entail feeding the DL system and the ground-truth method with the same image data. For instance, invasive tumor tissue in prostate cancer biopsy samples is often evaluated by a pathologist using H&E-stained tissue slides. Using the identical H&E histology images, a rudimentary deep learning system duplicates this work and is trained to detect the presence of invasive cancer. DL-based tumor detectors can thereby automate laborious activities that are usually done by hand.

The accuracy with which a deep learning classifier predicts a predefined endpoint is known as classification performance, and it is commonly assessed using the area under the receiver-operating curve (AUROC). AUROC values above 0.99 are frequently attained by DL-based tumor detectors, demonstrating nearly perfect agreement between pathologists' findings and DL networks' conclusions. Subtyping based on histological traits and reproducing tumor identification are two more possible fundamental image analysis tasks. For example, the most important morphological biomarker for prostate cancer patient categorization is the Gleason system. Expert pathologists typically grade gleason specimens manually using H&E tissue slides, however DL systems have been effectively used

to automate this process [23]. Similar to this, it is clinically significant to categorize non-small-cell lung cancer (NSCLC) as either squamous cell carcinoma or adenocarcinoma; this can be done fast and accurately by DL systems as well as skilled pathologists [26].

Clinicians see little use for basic Deep Learning (DL) systems for tumor diagnosis, grading, and subtyping because they don't substantially change oncology clinical procedures. These technologies rarely perform better in terms of sensitivity and specificity than human specialists, despite the possibility that they could save costs or provide quicker turnaround times than expert pathologists. As a result, although new DL systems have the potential to transform pathology practice, from an oncologist's point of view, they have little direct effect on clinical workflows or treatment recommendations for cancer patients.

**Validation of Basic DL Methods at the Clinical Grade**

Achieving clinical validation is the most difficult part of establishing fundamental DL systems in digital pathology. When a DL system is developed and validated on a single dataset, overfitting may occur, which can cause the system to perform well within the particular patient cohort but not well enough to generalize to other cohorts. For regular use and regulatory approval, external dataset validation—ideally from multicenter studies—is therefore essential. There has been a surge in large-scale, multicenter studies concentrating on foundational DL systems during the 2 years. For example, DL systems for prostate cancer grading and detection have been shown in three separate trials to attain pathologist-level performance with external validation in sizable patient cohorts [23, 24, 25]. One important conclusion drawn from these extensive efforts is that the performance of deep learning (DL) systems increases with the number of patients in the training set. Performance reaches a plateau after training on 10,000–15,000 histological whole-slide images [23]. This suggests that a large volume of images and data is required to develop DL systems that function well. These initiatives run concurrently with extensive global research projects that employ different imaging modalities, such as mammography imaging [32]. Thus, in the near future, DL algorithms may lessen the workload for human experts on simple picture identification jobs.

**Advanced Uses: Using Histology to Predict Mutations, Survival, and Treatment Response**

Beyond the fundamental uses of DL in histology image analysis, histology images include a wealth of information that is not yet routinely used to assist cancer treatment decisions, even though DL systems can match human performance in tumor detection, grading, and subtyping. Several studies have shown that DL can infer high-level labels directly from H&E images, which are outside the scope of normal histopathology and cannot be reliably identified by human experts. In particular, there is an increasing interest in using histology to predict clinically meaningful labels directly in three main domains: treatment response prediction, genetic change inference, and survival prediction. Research in these three important DL applications has expanded quickly in recent years, much like the area of digital pathology as a whole. These sophisticated DL applications in

histological image analysis, in contrast to simpler methods, have the ability to directly impact clinical judgments made on the treatment of solid tumors. Here, we examine the current status of DL pathology that is practically useful, its effects on clinical processes, and the planning and enrollment of clinical trials.

**Genotype and Gene Expression Prediction**

Through modifications to cellular machinery and a fundamental shift in cellular function, oncogenic driver mutations convert healthy cells into malignant cancer cells [33, 34]. As a result, within a histological image, these genetic driver mutations cause alterations in the morphology of cancer cells, including nuclear and cytoplasmic texture, size, and shape. Furthermore, malignant cells have the ability to trigger reactions in nearby non-cancerous cells, like lymphocytes and fibroblasts, which can result in secondary morphological changes in tumor tissue on a millimeter or micrometer scale [35]. Studies have demonstrated that DL is capable to accurately identifying various morphological alterations, even if each one of these traits may be slight due to a single oncogenic driver mutation. In fact, routine histology photos can be used to estimate the genotype of particular genes just by looking at these morphological patterns in H&E images. The first comprehensive DL-driven investigation in this field showed that the histological phenotype of lung adenocarcinoma reflected the cancer genotype: With AUROC values as high as 0.85, which they validated in an external cohort, Coudray et al. demonstrated that, in addition to automated tumor detection and classification, certain genetic mutations, such as those in serine/threonine kinase 11 (STK11), tumor protein p53 (TP53), and epidermal growth factor receptor (EGFR), could be predicted from histology alone [26]. Although with lower classification ability, a different study found that the genotype of the oncogene speckle-type BTB/POZ protein (SPOP) could be predicted from H&E-stained pictures of prostate cancer [36]. Similar to this, it was possible to determine the mutational status of the B-Raf proto-oncogene (BRAF) and NRAS proto-oncogene (NRAS) in melanoma directly from H&E pictures [37].

Accurately predicting these genes' mutational status is essential for tailored treatment. Multiple tyrosine kinase inhibitors (TKI) of the mutant EGFR protein are used to treat lung cancer based on the patient's genotype; in melanoma, mutated BRAF can be directly targeted with a serine/threonine kinase inhibitor. Consequently, there may be significant effects on clinical procedures if mutations in these genes are immediately discovered via regular histology. Cancer immunotherapy is another example that is clinically useful. The only genetic biomarker for immune-checkpoint inhibition therapy that has been approved by the FDA is MSI, the genetic correlate of mismatch-repair deficit (dMMR). It is the only diagnostic that can be used for any kind of cancer. In gastric, colorectal, and endometrial cancer, MSI can be accurately identified through histology alone. It causes notable morphological alterations in the tumor and its surroundings [27]. Numerous investigations have corroborated these results and expanded the scope of DL-based genotyping to encompass a variety of other mutations and gene expression markers spanning various tumor types. A "pan-cancer pan-mutation" strategy has been used in studies released in the last 1-2 years to predict any genetic change in any kind of solid tumor straight from H&E histology [38,39,40]. To assess the robustness of these approaches in pan-cancer applications, large-

scale validation in genomically characterized cohorts beyond TCGA is required, as these studies have primarily relied on one specific dataset, "The Cancer Genome Atlas (TCGA)" provided by the National Cancer Institute (NCI).

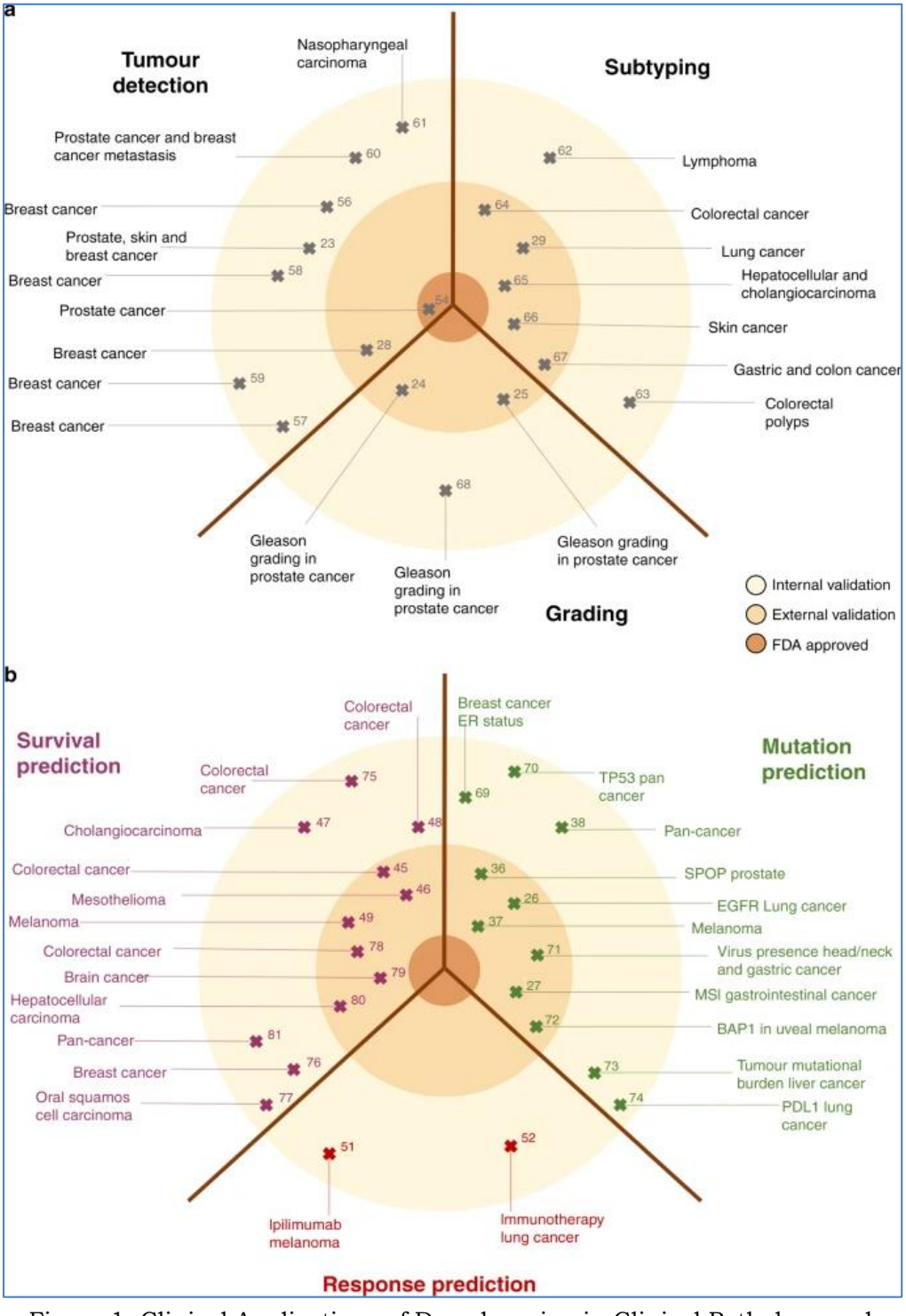


Figure 1: Clinical Applications of Deep learning in Clinical Pathology and Diagnosis

As things stand right now, routine histology sample evaluations like tumor subtyping and grading must be combined with wet-lab assays like immunohistochemistry (IHC), in situ hybridization (ISH), polymerase chain reaction (PCR), or next-generation sequencing (NGS) to detect any genetic changes in tumor tissue in a clinical setting. While the sensitivity and specificity of various wet-lab assays vary, they all have the same drawbacks, which include being time-consuming, expensive, and not being accessible at all stages of cancer therapy. On the other hand, DL-based assessment of routinely scanned histology slides doesn't require a lot of time or money, and it can be used with mobile gear as well [39]. Notably, however, performance (measured by AUROC) has continuously lagged behind the gold standard wet-lab tests in all DL-based investigations carried out to date, with variations occurring based on the size of the training cohort's sample and the specific genetic target's phenotypic strength. Performance improvements are anticipated as a result of increased dataset training and technological breakthroughs. Furthermore, as will be mentioned below, patients could be prescreened for genetic changes of interest with even subpar DL-based techniques.

**Predicting Survival Using DL Biomarkers**

The risk of death or relapse is a factor in almost every oncology treatment decision-making branch. For instance, a high risk of relapse in patients with stage II or III colorectal cancer (CRC) may support adjuvant chemotherapy after surgery [41], and a high risk of death in stage IV CRC may lead patients and oncologists to choose more aggressive systemic therapy than what is currently advised in guidelines [42]. Clinical characteristics such age, gender, cancer stage, pre-existing diseases, genetic changes, and histological risk factors are currently used to determine survival. Tumor cell differentiation, stromal abundance, lymphocyte percentage, lymphatic vessel invasion, vascular invasion, perineural invasion, and necrosis in almost all solid tumors are among the many histological risk factors. Beyond these known risk factors, prognostic information is carried by higher-level traits. For example, a high neutrophil-to-lymphocyte ratio has been linked to a poor overall survival rate when analyzing the spatial arrangement of lymphocytes [43]; similarly, sub-visual characteristics such chromatin texture can be used as a prognostic signal in many solid tumors [44]. Several studies have shown that DL may possibly combine all these visible and sub-visual information directly from image data to predict survival. Curiously, some studies have trained DL networks for survival prediction using manually defined prior parameters [45], while others have taken an unbiased approach, letting the deep network handle all feature selection [46, 47]. This means that prognostic parameters, like tissue type or cellular aspects, were not manually identified or extracted during the process. Both methods still need to be independently verified in the future in order to be used as the basis for risk-adjustment techniques in a clinical context.

A number of important studies have investigated DL-based survival prediction for different kinds of cancer. Bychkov et al. showed that H&E-stained tissue microarrays alone can be used to predict the 5-year disease-specific survival of CRC patients [48]. Comparing CRC patients to state-of-the-art approaches, improved survival prediction was also shown by using tissue classification to

predict overall survival [45]. In a sizable cohort of patients with malignant mesothelioma, Courtiol et al. projected overall survival and visualized histological characteristics linked to extended or abbreviated survival as determined by the DL network [46]. In patients with initial melanoma, disease-specific survival was concurrently calculated using DL-based prediction of distant metastatic recurrence development [49]. This is an excellent illustration of how training deep learning networks on minimal datasets is feasible when paired with transfer learning or extensive tissue-level annotations. Even with these encouraging outcomes, there are still a lot of obstacles that survival prediction by DL must overcome, like significant cohort variability and small training sample sizes [50].

## Conclusion

The evolution of cancer diagnostics has reached a critical juncture with the incorporation of deep learning (DL) into histology image analysis. This review underscores the dual potential of DL in both automating routine diagnostic tasks and uncovering novel insights that traditional methods might overlook. Fundamental DL applications, such as tumor detection, grading, and subtyping, have demonstrated remarkable accuracy, often comparable to or exceeding the performance of human pathologists. These advancements hold the potential to streamline workflows in pathology laboratories, reducing turnaround times and potentially lowering costs. However, it is the advanced applications of DL that promise to make the most profound impact on cancer treatment. By leveraging DL to predict genetic mutations, treatment responses, and survival outcomes directly from histology images, clinicians can gain access to critical data that traditionally required time-consuming and costly molecular assays. For instance, the ability of DL to predict mutations in genes such as EGFR, BRAF, and MSI from routine histology slides could revolutionize personalized cancer therapy, enabling more precise and timely interventions. Despite these promising developments, several challenges remain. The validation of DL models across diverse and independent patient cohorts is essential to ensure their reliability and generalizability in clinical practice. Additionally, the integration of DL-based tools into existing clinical workflows requires careful consideration to avoid disruptions and ensure seamless adoption. In conclusion, the future of cancer diagnostics lies in the successful marriage of molecular biology and artificial intelligence. As DL technologies continue to evolve and gain acceptance, they will likely play a pivotal role in personalizing cancer treatment, ultimately improving patient outcomes and shaping the next generation of oncological care. Continued research, collaboration, and clinical validation will be crucial in realizing the full potential of these innovations.

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