The role of digital and integrative pathology for the detection of translocations: a narrative review

Carlo Beretta[#], Stefano Ceola[#], Fabio Pagni, Vincenzo L'Imperio

Department of Medicine and Surgery, Pathology, ASST Monza, San Gerardo Hospital, University of Milano-Bicocca, Monza, Italy
Contributions: (I) Conception and design: V L'Imperio; (II) Administrative support: V L'Imperio, F Pagni; (III) Provision of study materials or patients: C Beretta, S Ceola; (IV) Collection and assembly of data: C Beretta, S Ceola; (V) Data analysis and interpretation: C Beretta, S Ceola, F Pagni; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.
[#]These authors contributed equally to this work.
Correspondence to: Vincenzo L'Imperio. Department of Medicine and Surgery, Pathology, ASST Monza, San Gerardo Hospital, University of Milano-Bicocca, 20900 Monza, Italy. Email: vincenzo.limperio@unimib.it.

Background and Objective: Digital pathology represents an invaluable source of information and a long-term investment with high returns, with the possible deployment of artificial intelligence (AI) tools for both the clinical and research activity. Moreover, the rising nosological complexity of the oncologic diseases, e.g., lung cancer, is stressing the need of integration among different subspecialities (e.g., radiology, molecular biology, and immuno-oncology) for the final characterization of cancer. In this setting, digital pathology can play a pivotal role in the "integration" of these different competencies, and the application of AI for prognostic/predictive purposes can represent a further "third" revolution in pathology. The objective of the present review is to provide an updated overview of the possible role of digital and integrative pathology in detecting gene mutations and, especially, translocations in different types of tumors, focusing on the promising implications that this advancement can have in lung cancer characterization.

Methods: A systematic literature search was conducted on different research engines (PubMed, IEEE Xplore, dblp, ACM digital library, Inspec) over a 15-year interval from January 1, 2006 to October 31, 2021 selecting only English-language articles to highlight the possible diagnostic and prognostic role of digital pathology tools in detecting gene mutations and, especially, translocations in different types of tumors.

Key Content and Findings: Digital pathology tools already demonstrated a role in the detection of specific mutations and translocations in different types of cancer, both in a targeted (e.g., liver/thyroid carcinoma) and in an agnostic (e.g., MSI) setting. In lung cancer, AI showed the capability of highlighting specific subset of mutations (*STK11*, *EGFR*, *FAT1*, *SETBP1*, *KRAS*, and *TP53*) from whole slide imaging (WSI), with the translocation field representing a promising frontier, with some gene rearrangement (e.g., *NTRK*) more prone to be detected with this approach as demonstrated in other organs.

Conclusions: Digital pathology is becoming a valuable tool to predict gene aberrations in different types of tumors, with a promising role in lung cancer. Digital and integrative tools for the detection of translocations are progressively changing the paradigm of molecular testing, contributing to the switch from a manual to a fully automated workflow, requiring further investigation.

Keywords: Digital pathology; artificial intelligence; translocations; non-small cell lung cancer (NSCLC); integrative pathology

Introduction

Digital pathology consists of different technologies capable of transforming the "classic" workflow into a fully automated and tracked path (1) that culminates with the employment of whole slide imaging (WSI) for primary diagnosis, consultation or multidisciplinary discussions (2-4). Although it can represent an invaluable source of information and a long-term investment with high returns, the diffusion of digital pathology is still slowly progressing, leading some authors to postulate possible reasons for this reluctance (5). To address these perplexities and provide a handy and step-by-step guidance for this transition, some international societies released detailed recommendations and guidelines for the progressive implementation of digital pathology (6,7). These could represent the basis for the deployment of a further, "third revolution" in pathology (8) represented by the application of artificial intelligence (AI) tools for both the clinical and research activity (9). In line with the necessary integration among different subspecialties for the final comprehensive characterization of cancer (e.g., radiology, molecular biology, and immuno-oncology) (10), digital pathology can play a new emerging pivotal role. Thus, the present review will focus on the advancements of digital and computational pathology in prediction oncology for the detection and prevision of translocations, which can be relevant as specific targets for candidating patients to molecular therapies. We present the following article in accordance with the Narrative Review reporting checklist (available at https://pcm.amegroups.com/article/view/10.21037/pcm-21-56/rc).

Methods

A systematic literature search was conducted on different research engines (PubMed, IEEE Xplore, dblp, ACM digital library, Inspec) using the terms: "digital pathology AND translocations"; "digital pathology AND mutations"; "artificial intelligence AND translocations"; "deep learning AND translocations"; "deep learning AND mutations"; "digital pathology AND lung cancer", over a 15-year interval from January 1, 2006 to October 31, 2021 (*Table 1*). The research led to 2,216 results, from which 118 duplicates and 11 articles in non-English language were removed (*Figure 1*). For the obtained 2087 articles, abstracts were evaluated by two of the authors (CB and SC) and screened for manuscripts focused on the detection of mutations/translocations from digital pathology tools. Discordances

on the selection of manuscripts have been resolved through the re-evaluation by a third author (VL or FP). For these articles (n=17) the full manuscript has been revised and from the references additional articles have been added to the present review.

Digital pathology workflow: essential requirement for AI implementation

The implementation of WSI as a surrogate of the glass slides is just one of the steps required for the transition towards a digital pathology workflow (11). Actually, this represents the last stage of the progressive modifications that should affect the anatomic pathology laboratory, starting from the accessioning to the reporting and archiving phases, as stressed by the most recent recommendations (7). One of the most important advancements in this direction is represented by the introduction of a completely tracked and fully automated laboratory workflow, aided by the implementation of barcodes to uniquely identify each case/patient along the diagnostic process. This is further enhanced by the employment of dedicated barcode readers and cameras at grossing, embedding, and sectioning stations (1), helping to significantly reduce the rate of errors due to missing/under-represented material on the WSI. This is further facilitated by the complete integration with the laboratory information system (LIS), an essential interface that plays different roles in all phases of patient testing, including specimen and test order entry, specimen processing and tracking. Moreover, the adoption of a LIS-centric approach would allow an easy integration of the WSI in the diagnostic routine of every pathologist independently from the vendors of scanners and supplies used in the laboratory. These are the main and basic requirements to lay the foundations for the adoption of AI tools for the detection of mutations/translocations in human cancer.

Adding a molecular dimension to digital pathology: the detection of mutations in cancer

The application of AI tools coupled with digital pathology already demonstrated a complementary role in traditional histology diagnoses (12-14). Moreover, a prognostic application was evaluated, predicting a metastatic aggressive behavior from hematoxylin and eosin (H&E) stained sections of the primary tumor (15). The most fascinating aspect is related to the possibility of predicting specific

Table 1 The search strategy summary

Items	Specification
Date of search (specified to date, month, and year)	November 15, 2021
Databases and other sources searched	PubMed, IEEE Xplore, dblp, ACM digital library, Embase, Inspec.
Search terms used	"Digital pathology AND translocations"; "digital pathology AND mutations"; "artificial intelligence AND translocations"; "deep learning AND translocations"; "deep learning AND mutations"; "digital pathology AND lung cancer"
Timeframe	January 1, 2006 to October 31, 2021
Inclusion and exclusion criteria (study type, language restrictions, etc.)	From the original literature search have been removed: duplicates, non-English articles, review/non-original data, paper based on unclear methods, manuscripts focused on the assessment of mutation/translocation applying digital tools on other sources (no WSI).
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	Two of the authors (CB and SC) conducted the literature research, independently evaluating the abstracts and screening for manuscripts focused on the detection of mutations/translocations from digital pathology tools. Discordances on the selection of manuscripts have been resolved through the re-evaluation by a third author (VL or FP).

WSI, whole slide imaging.
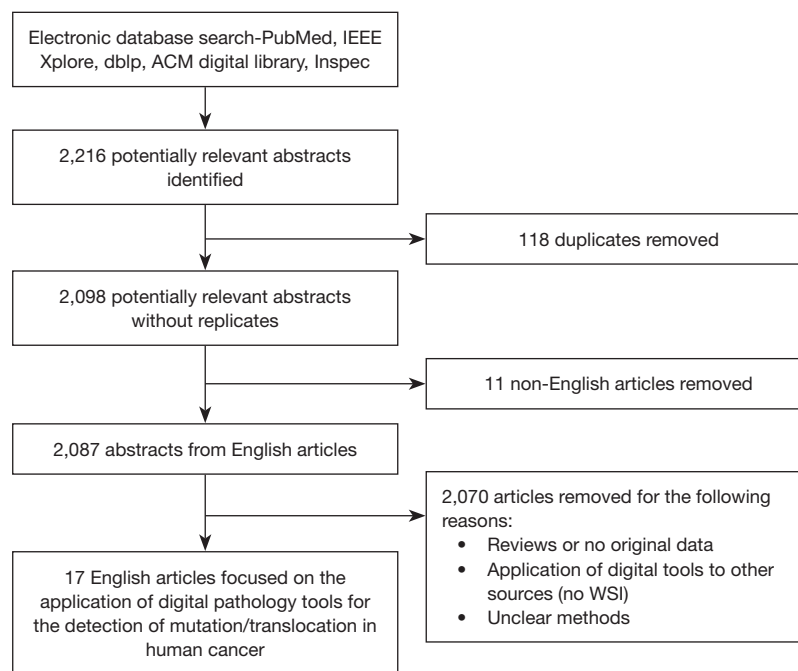
Figure 1 Flowchart of the review process reporting inclusion/exclusion criteria. WSI, whole slide imaging.

cancer-related mutations from phenotypic data. This is not surprising since the neoplastic cell phenotype (nuclear and cytoplasmic texture, size, shape) is, after all, conferred by genetic alterations, so individual gene mutations can directly be predicted by AI from H&E slides observing morphological patterns. It should be emphasized that imaging techniques can only identify genetic variants whom directly imprinted tissue morphology; AI algorithms cannot be applied in cases where actual variant allele frequencies of selected mutations impact the classification and prognosis

of individual nosological entities, as some hematologic neoplasms (16). With a convolutional neural network (CNN) trained with WSIs of H&E stained hepatocellular carcinoma (HCC), Chen *et al.* demonstrated how four of the ten most common prognostic and mutated genes in HCC (*CTNNB1, FMN2, TP53*, and *ZFX4*) were correctly identified by AI (17). Their method demonstrated high performances at recognizing tumors from normal liver tissue (AUC =0.961; 95% CI: 0.939–0.981) in the validation set. Moreover, testing the mutation prediction capabilities of the model on the region/tiles-level, five of the ten most significant gene mutations, including *ARID1A* (P=0.036), *CTNNB1* (P<0.0001), *FMN2* (P=0.0003), *TP53* (P=0.0011) and *ZFX4* (P=0.0054), showed significant differences between mutated and wild type groups, with the AUCs from 0.71 to 0.89 in the external validation set. Tsou *et al.* trained a CNN to predict the most common oncogenic drivers in papillary thyroid carcinoma (PTC), *BRAF* V600E and *RAS*, based selected ROI on H&E slides. Their work showed good results and, interestingly, the model performed better in predicting *RAS* than *BRAF* mutations with a rate of successful prediction from 63.6% and 90% for the *BRAF* group and *RAS* group, respectively (18). Indeed, future potential applications of this approach could lead to a reduction of the turnaround times that are generally delayed by the need of genetic testing and to implement a reflex-test approach, limiting the molecular confirmation only to those cases highlighted by the algorithm. The possibility of acting in a completely agnostic fashion, by approaching the molecular assessment of the disease irrespective of the cancer site of origin could be the final goal. For microsatellite instability (MSI) status, deep learning (DL) has been used in different malignancies, distinguishing features predictive of MSI in gastric and colorectal cancer samples (19), even demonstrating high performance at predicting MSI on H&E-stained WSIs (AUC of 0.865 *vs.* 0.605), with ground truth microsatellite status determined using mismatch repair protein immunohistochemistry (IHC) and MSI-PCR (20).

Adding a molecular dimension to digital pathology: a possible role in the detection of translocations

One of the first fields in which the detection of translocations has been investigated with digital/integrative pathology tools is probably represented by hemato-oncology, in which different entities are characterized by specific and sometimes pathognomonic chromosome rearrangements. Acute promyelocytic leukemia (APL) carries in 95% of cases a balanced chromosomal translocation between chromosomes 15 and 17 t(15;17)(q24; q21) resulting in the formation of the PML-RARA fusion protein (21,22). Molecular techniques require time and are not available in many countries with limited healthcare resources. In contrast, the peripheral smear is universally available, and easily and rapidly obtained in all healthcare settings. For these reasons, some authors investigated whether DL approaches could find morphological features predictive of the molecular translocation status for a correct identification of APL from other subtypes of myeloid leukemias from cellular morphology (23). The proposed model on a discovery cohort of 82 patients and tested on an independent prospective validation cohort of 24 patients had a ROC AUC of 0.822 in the discovery cohort and 0.739 in the validation cohort with analysis based only on the assessment of immature myeloid cells, with lower performances when considering the whole cellular sample. Interestingly, for the phenotypic classification the AI focused on cytoplasmic pixels in non-APL leukemias and nuclear pixels in APL, suggesting some differences in the dispersion of chromatin on Wright staining which could be more focused at the edge of non-APL leukemias cells and more condensed at the center of the APL cells. In Diffuse Large B-cell Lymphomas (DLBCL), the evidence of rearrangement of *MYC* oncogene demonstrated a poor prognostic value, defining a subset of high-grade lymphomas when in association with rearrangements of *Bcl2/Bcl6* (24). Although MYC IHC has been proposed as a surrogate marker, a reliable cut-off to reduce the interobserver reproducibility and to improve the performances of this marker is still missing (25). For this reason, Swiderska-Chadaj *et al.* developed a DL model to detect *MYC* rearrangement in WSI from DLBCL on a first multi-center data cohort of 91 cases and a test set of 66 WSIs (26). This approach led to an accuracy of 0.77, with 0.88 and 0.66 of sensitivity and specificity, respectively. They further validated their findings on a larger cohort (245 patients) additionally using CD20 IHC to define the tumor area, with a 0.90/0.95 and 0.52/0.53 of sensitivity and specificity for the internal and external validation sets, respectively (27). The algorithm gives a fast and cheap pre-screening that can direct the application of genetic tests for *MYC* rearrangement as compared to MYC IHC that shows a lower sensitivity (0.86 for IHC versus 0.93 for the algorithm) and a lower specificity (0.36 for IHC *vs.*

0.52 for the algorithm) (28). One of the most interesting and stimulating aspects of this approach would be the investigation of the "false negative" results with MYC IHC, which could be compensated by the higher sensitivity of the AI model allowing it to detect a further segment of patients with *MYC* rearrangements. The introduction of AI tools for the detection of translocation has not been restricted to the hemato-oncology field, with some solid tumors already experiencing the benefits of such digital/integrative pathology techniques. Some specific subtypes of renal cell carcinoma (RCC), in example, are characterized by specific chromosomal translocations (e.g., Xp11.2 leading to the fusion transcript involving TFE3 protein), which can show indistinguishable morphological features from the most common clear cell histotypes (29,30). In this setting, Cheng *et al.* applied a machine learning model to identify Xp11.2 translocation based on WSI comparing TFE3-RCC and clear cell RCC (ccRCC) (31). Starting from two different dataset of H&E stained WSIs (148 images in total) with a 1:1 TFE3-RCC—ccRCC ratio (TFE3-RCC diagnosis confirmed by FISH), they found an AUC ranging from 0.842 to 0.894, stressing the ability of this model to capture subtle morphological differences between TFE3-RCC and ccRCC. Finally, the application of such digital pathology tools can even facilitate the assessment of routine tests (e.g., fluorescence *in situ* hybridization FISH) for the detection of some chromosomal abnormalities, as in the case of breast cancer, in which *HER2* gene amplification is found in almost 20% of invasive forms and patients with this alteration could benefit from targeted therapy (32). Prediction of *HER2* rearrangement is determined by assessing *HER2* status with IHC and reflex testing of doubtful IHC (2+) with ISH. Although some oncology societies stressed the need of standardization through automation of the FISH procedure (for *HER2* as well as for *ALK*, *ROS1* and *NTRK*) (33), these slides are still mainly analysed manually by an epifluorescent microscope. Some authors already tried to validate the standardized detection of *HER2* gene status using a fully automated ISH procedure, obtaining an overall agreement after revision of 99.4% (κ=0.97) between Leica FISH (automatic) and Abbott FISH on a cohort of 328 invasive breast cancers (34). Moreover, the application of further AI algorithms on FISH digitized slides can provide good picture quality, reducing the analysis time and allowing easy picture archiving and remote diagnostics compared to manual FISH, making *HER2* FISH evaluation more efficient (35).

Adding a molecular dimension to digital pathology: focus on lung cancer and its translocations

Over the last 20 years, significant insights into the heterogeneous nature of non-small cell lung cancer (NSCLC), particularly adenocarcinoma, have motivated new research areas endeavoring to elucidate additional actionable characteristics of tumor cells and the surrounding tumor microenvironment. In the era of precision medicine, the identification of targetable somatic mutations is an essential step in determining optimal systemic therapy in patients with adenocarcinoma of pulmonary origin. During these years, different efforts have been made to decipher subtle morphological differences that can underpin specific genetic alterations of NSCLC (36,37). In this setting, the application of innovative AI techniques represent a promising source of information that can complement the "classic" histopathology, as already demonstrated by the capability of these tools to correctly detect (38) and classify NSCLC subtypes (39), predicting its prognosis (40) and the presence of metastatic lymph nodes (41). Recently, Coudray *et al.* using a deep CNN correctly classified lung tumors as adenocarcinoma and squamous cell carcinoma, with an AUC of 0.97 (*vs.* human eye), and predicted 6 out of 10 mutations (*STK11*, *EGFR*, *FAT1*, *SETBP1*, *KRAS*, and *TP53*) by the analysis of WSIs with AUCs from 0.733 to 0.856 (42). This can be strategically applied in the future in delicate cases with scant material insufficient to perform additional mutational analysis in frail patients that cannot undergo further bioptic procedures as a possible alternative to the liquid biopsy. Although in the last decade different automated systems have been proposed to scan and score FISH samples for the assessment of *ALK* (43), *ROS1*, *RET* and *NTRK* aberrations (44), leading to a change in the paradigm of the optimal workflow for digitized FISH analysis (35), attempts to predict the presence of chromosomal aberrations through AI tools from scanned H&E slides is still substantially lacking. However, the field is promising, as can be demonstrated by the possibility of detection of some of these fusions in other districts, such as in thyroid cancer. Indeed, starting from a cohort of 802 thyroid cancer H&E stained WSIs, of which 23 belong to tumors with *NTRK* fusions, a proposed CNN model to predict *NTRK* status demonstrated an AUC of more than 0.8 on images from the same cohort that was used for training (45). However, the model performance dropped significantly in external cohorts, A further promising
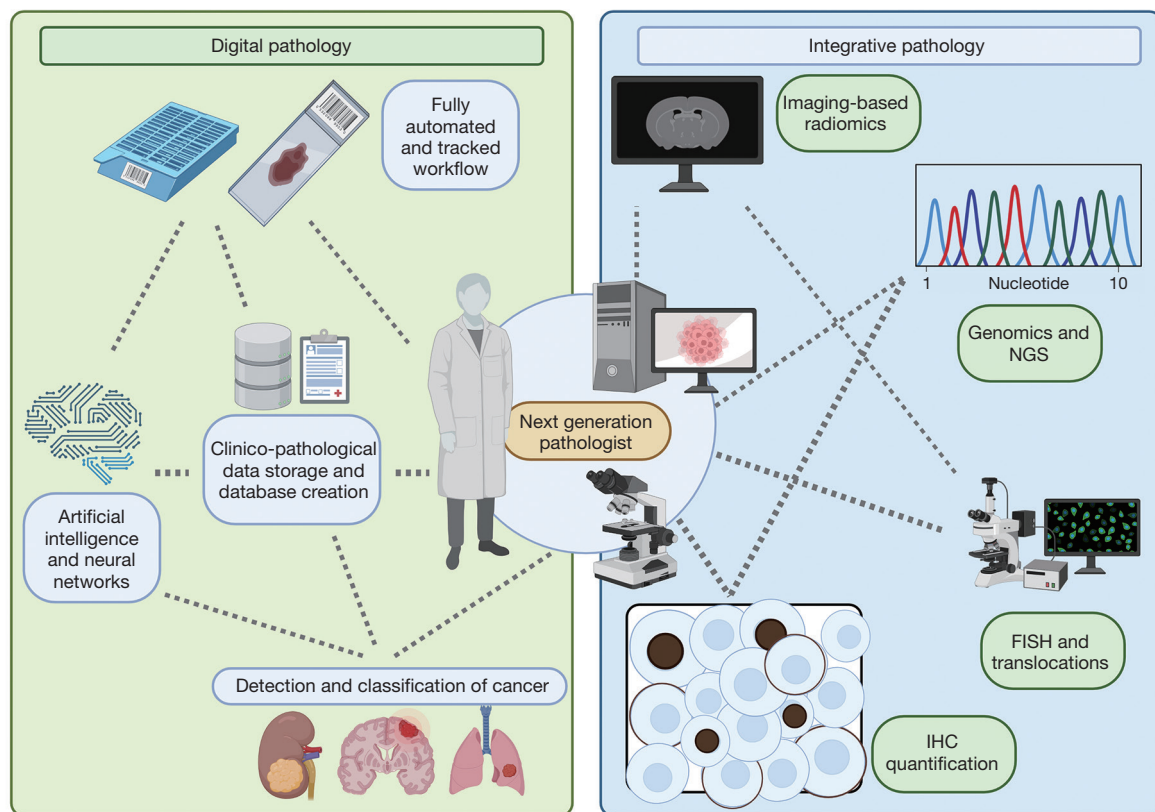
Figure 2 Schematic representation of the complex interplay between digital and integrative pathology and the pivotal role of the next generation pathologist in this setting. Credit: created with BioRender. NGS, next generation sequencing; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

frontier for this approach is represented by the integrative way, coupling histology-based AI with information coming from other subspecialties (46,47). In this setting, some groups already demonstrated the capability of radiomics from computed tomography (CT) (48) or positron emission tomography/computed tomography (PET/CT) (49) coupled with clinicopathological data to predict the presence of *ALK* rearrangements, stressing once again the importance of the integrative role of pathology and the enormous potentialities of digital pathology (*Figure 2*).

Limitations and challenges of the approach

The results obtained so far with the application of AI tools for the prediction of mutation/translocation in different types of cancer are exciting, but some drawbacks still exist and should be taken into account for the design of future and more rigorous studies. First of all, one of the limitations that often affect these studies is represented by

the dimension of the used cohorts, especially the validation ones, often small and enriched in the most "common" histotypes of cancer. This last point may not ensure to cover the entire tumor heterogeneity that can be encountered in the clinical practice, potentially hampering the investigation of less frequent histologies, thus limiting the performances of the algorithms when applied to these rare tumors. Moreover, it should always be noted that the model is not a complete replacement for pathologists' examination, which include the diversity and heterogeneity of tissues that pathologists typically inspect (e.g., inflammation, necrosis, and blood vessels). From the more "analytic" side of the AI algorithms, other specific challenges should be noted, starting from the need to normalize the stain of WSI (50), for which different methods exist (e.g., metric learning, domain agnostic learning, transfer learning or continual learning) that should be ideally tailored in future studies. Moreover, novel approaches in the field of explainable AI could help to better understand the visual features that

drive the model decision, which could lead in the future to the identification of specific morphological aspects that can predict the underlying genetic landscape even for the human eye. Finally, the segmentation process is still quite heterogeneous among the different studies, with some authors performing a manual ROIs identification, which is time-consuming and prone to human errors. From these considerations, as compared to studies based on the automatic segmentation (17,20), those using a "manual" approach (18,19) could potentially be affected by some bias, as the "supervised" character of the analysis could affect the final results.

Translating AI tools in the routine clinical practice

In order to be able to predict genetic abnormalities for routine clinical use, the results of these studies should follow the requirements dictated by the regulatory authorities. In this direction, in order to be approved as *in vitro* diagnostic tools by the Food and Drug Administration (FDA) (51), such AI-based devices should follow strict methodological recommendations that can be summarized as follows:

- ❖ The technical performance assessment and study protocols (e.g., ROI localization study), as well as results used to assess the device output(s) (e.g., image overlays, image heatmaps, etc.), should be clearly declared and follow the most rigorous evidences;
- ❖ The training dataset must include cases representing different pre-analytical variables representative of the conditions likely to be encountered when used as intended (e.g., fixation type and time, histology slide processing techniques, multiple sites, patient demographics, challenging diagnostic cases especially when dealing with rare genetic variants);
- ❖ The number of WSI in an independent validation dataset must be appropriate to demonstrate device accuracy in detecting and localizing ROIs on scanned WSI, and must include subsets clinically relevant to the intended use of the device.

Another critical point that can significantly affect the performances of such AI predictive tools is represented by the size and number of tiles/patches automatically retrieved from the WSI by the algorithm during the training phase of the study. Recently, breaking WSIs into many smaller manageable image patches for high-throughput sampling have allowed investigators to overcome old AI approaches that only analyzed small ROIs and were unable to deal with large WSI. While increasing image patch size for training may lead to improved algorithm performance (52), this also could increase computation time and cost. Thus, independently from the actual number of patches used, the most important step is to ensure a good representation of the diversity of samples/pictures in the training set, because there can be substantial variance present within a single target class. Regarding the dimension single patches and magnification of the WSI employed, the situation is quite heterogeneous among the studies analysed in the present review, with some dealing with scanned slides at 20× (17,19,26,42) and others at 40× (31), with extracted regions of different size at 0.5 µm/px or 0,24 µm/px (MPP): 2,000×2,000 (31), 1,500×1,500 (19), 512×512 px (18,27,42), 360×360 (23) and 256×256 (17). However, all these studies share a common approach based on the exclusion of regions affected by processing artifacts or avoiding patches with a low amount of information (e.g., all the tiles with >50% of the surface covered by background, namely with values below 220 in the RGB color space). These methods seem to agree with those proposed in the datasheets of several diagnostic *in vitro* devices already approved by the principal regulatory authorities (51) that are already in use in clinical practice to morphologically detect tumor areas in WSIs. Finally, recent experiences highlighted the possibility of performing an annotation-free WSI training approach that does not require the detection of patches/tiles, specifically tested on lung cancer specimens, that would further simplify the workflow and reduce the variability among studies (53). The progressive clarification and standardization of these technical aspects is contributing to the significant increase in the number of trials aimed at applying AI-tools for cancer prediction. In particular, ongoing studies (ClinicalTrials.gov Identifier: NCT04217044) are evaluating the possibility to predict the most common genetic alterations of gliomas (1p/19q co-deletion, *MGMT* methylation, IDH and TERTp mutations, etc.) with AI from routine H&E stains. The subsequent step of this dramatic transformation will be the implementation in the routine practice of such tools, to be utilised by pathologists and oncologists which are not always familiar with complex informatics and AI. Thus, the future challenge will be the complete integration of these predictive algorithms in the currently employed automated workflows (3), as already experienced for diagnostic AI-based tools in clinical-grade computational pathology (14,54).

Conclusions

Digital pathology is becoming a valuable tool to predict gene aberrations in different types of tumors, with a promising role in lung cancer. What is truly changing the paradigm of molecular testing from a manual to a fully automated approach is probably represented by the introduction of digital scanning systems for IHC and FISH analysis, whereas the implementation of AI in this setting, even if promising, still requires further investigation.

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Footnote

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