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REVIEW

POINT-OF-CARE TESTING: AN ALLY FOR PRECISION ONCOLOGY

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ABSTRACT: the future of cancer care will be based on precision oncology, which uses individual tumor molecular profiles to provide the correct drug to the appropriate patient at the appropriate time. This approach might deliver precise results with minimal side effects and enhanced treatment success rates. However, the vision fails to materialize in reality because current tools remain centralized and needs advanced infrastructure together with specialized/trained staff and prolonged procedural time. The lack of laboratory capabilities in healthcare settings can be addressed through Point-of-Care (POC) testing which enables diagnostic methods to be performed near or at the site of patient care thus linking laboratory capabilities to practical healthcare delivery. The technology is capable of delivering specific diagnostic tests at bed-side, and in particular in remote areas. The implementation of POC testing enables precision oncology to become practical allowing for prompt medical decisions. POC systems allow for continuous tracking of relapse, resistance and response. POC testing serves as an essential component of precision oncology because it enables personalized care more quickly and directly to patients. This review synthesizes current and emerging POC platforms for oncology, evaluates their analytical performance, clinical readiness, and regulatory landscape, and identifies unmet needs that must be addressed to enable routine adoption for diagnosis and monitoring.

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Impact statement: The possibility of reaching precision oncology solutions cannot be considered apart from a quick monitoring of therapeutic efficacy. In order to tailor therapies for cancer patients, the development of point-of-care devices would open to easy and quick response by specialists and patients, also strengthening the concept of telemedicine.

Key words: *precision oncology; POC testing; molecular diagnostics; liquid biopsy; sensors.*

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INTRODUCTION

Cancer treatment followed under a "one-size-fits-all" model for many decades because it relied on standardized protocols that used tumor histology, anatomical site, and clinical staging as guidance (1). These techniques show effectiveness for treating initial-stage cancer, yet fails to handle molecular complexity, heterogeneous nature and its dynamic evolutionary changes (2).

The field of tumour-genome profiling has experienced significant advancement during the last twenty years. The first wave started with commercial next-generation sequencing (NGS) in 2005 (3).

The implementation of hybrid-capture panels in 2013 enabled clinical-grade whole-exome/large-target sequencing to become a standard practice in oncology (4). Single-cell RNA/DNA sequencing followed in 2015, revealing intratumoral heterogeneity at cellular resolution (5). The landscape evolved further with the introduction of Long-read high-fidelity (HiFi) sequencing technology in 2019 that enables the detection of intricate structural variations which short reads fail to identify. Ultra-deep error-suppressed assays which started in 2021 provide part-per-million sensitivity for plasma-based minimal residual disease monitoring (6, 7). The conventional cancer classification system's limitations led to a new approach of molecular profiling which triggered successive genomic innovations that shifted oncology from histology-based treatment to biology-driven precision care.

Rapid advancements in genomic research have accelerated the adoption of precision oncology as a standard treatment approach for cancer patients. Precision oncology uses multiple biomarkers to determine the appropriate therapy intensity based on tumor biology: (i) The use of genomic markers like activating Epidermal Growth Factor Receptor (EGFR) mutations in non-small-cell lung cancer (NSCLC) leads patients to receive tyrosine-kinase inhibitors (TKI) instead of standard chemotherapy treatment (8); (ii) the presence of Human Epidermal Growth Factor Receptor 2 (HER2) overexpression as a proteomic marker enables doctors to identify breast cancer patients who need trastuzumab treatment while preventing its use in patients without HER2-positive tumors (9); (iii) the presence of MGMT promoter methylation in glioblastoma serves as an epigenetic marker to predict improved temozolomide response thus requiring more intense treatment (10) and (iv) multi-analyte expression panels such as the 21-gene Oncotype DX test stratify early-stage, hormone-receptor–positive breast cancer so that low-risk patients safely omit adjuvant chemotherapy, reducing overtreatment without compromising outcomes (11, 12). Such advancements demonstrate how precision tools both direct treatment escalation and provide safe de-escalation treatment which establish a foundation for individualized care.

Precision medicine has transformed oncology by moving away from standard treatments to personalized care which has reshaped both the objectives and organization of the field and improved treatment effectiveness through better response rates, reduced unnecessary treatment, and individualized choices (13). Yet the real-world implementation of precision oncology practices exists in a state of significant inequality and operational inefficiency. Centralized diagnostic workflows that need advanced laboratory infrastructure and expensive sequencing platforms and highly specialized personnel create delays of up to three weeks between biopsy and therapeutic decision-making (14-16). The time spent waiting for test results is crucial for patients with fast-moving cancers because tumor biology changes, patient health worsens, and treatment opportunities decrease with each passing hour. Cancer diagnostic facilities exist mainly in urban high-income countries which prevents their use by rural populations and low and middle-income countries where cancer cases are increasing quickly (17, 18). Even in well-resourced settings, the process of sample collection, transport, sequencing and analysis creates delays that result in therapeutic decision delays of days to weeks especially for aggressive or late-stage cancers (19).

Researchers have previously addressed the translational gap through laboratory-based molecular innovations. Liquid biopsy stands out as a minimally invasive and repeatable tissue biopsy alternative which allows researchers to study circulating tumor DNA (ctDNA), RNA, extracellular vesicles (EVs) and circulating tumor cells (CTCs) from biofluids, including blood, urine and (20–22). EVs are broadly classified by size and origin into exosomes (30–150 nm, endosomal origin), microvesicles (100–1000 nm, plasma membrane budding), and apoptotic vesicles (50–5000 nm, released during cell death) (23). However, liquid biopsy offers real-time insights and operational flexibility, the analytical accuracy depends on biological and pre-analytical variability which can hide true tumor signals.

Recent evidence shows that the absolute amount of circulating biomarkers can fluctuate substantially within the same individual, even when tumour burden is biologically constant, because of short-term physiological factors. Acute shifts in plasma volume caused by dehydration or strenuous exercise produce multi-fold transient rise in total cell-free DNA (cfDNA) concentration that ctDNA assays report (24). Independent time-series studies have also revealed diurnal oscillations: CTC counts in mouse and human models peak at the onset of the rest/night phase, suggesting endocrine regulation of tumour-cell egress (25). Finally, pre-analytical variables-plasma *vs* serum matrix, occult haemolysis,

and delays in tube processing - can shift total cfDNA or EVs yield by an order of magnitude, with direct consequences for mutation calling and quantitative trending (24). Collectively, these hydration, circadian and matrix-driven effects underscore the need for active normalisation strategies whenever liquid biopsy is decentralised to the point of care (POC).

The clinical adoption of liquid biopsy faces challenges because it depends on complex centralized laboratory infrastructure and sophisticated assay platforms (26). Liquid biopsy technologies need adaptation to achieve their maximum potential for POC implementation. The POC testing model transforms healthcare by providing decentralized rapid and clinically actionable diagnostics directly at or near patient care locations (27, 28). The POC systems provide quick biomarker results which enable fast diagnostic-treatment intervals and immediate therapeutic alignment (29). The time advantage is essential in oncology because urgent medical interventions have major effects on patient outcomes. The promise of precision oncology requires technological innovation to develop compact diagnostic tools that are both sensitive and clinically adaptable for POC settings.

The detection of cancer biomarkers has undergone a transformation through new smart tools that combine compact design with ultra-sensitivity and decentralized adaptability. Electrochemical biosensors now enable the real-time detection of ctDNA at femtomolar concentrations using small sample volumes (30). By integrating nanostructured electrodes with surface-functionalized aptamers or DNA probes, these platforms can achieve analytical performance similar to that of centralized laboratories through rapid POC testing (31, 32). The integration of multiple functions, including isolation, enrichment, and downstream biomarker analysis, onto a single microfluidic platform enables microfluidic lab-on-chip systems to perform multiplexed analysis of liquid biopsy. The integrated design of these systems decreases the complexity of sample handling and reduces both bioanalyte loss and contamination risks when compared to traditional benchtop laboratory procedures (33). CRISPR-based diagnostics offer programmable nucleic acid detection through user-friendly readouts, including colorimetric, luminescent and lateral-flow assays for rapid POC testing (34). The clinical adoption of nanoparticle-enhanced sensors depends on solving manu-

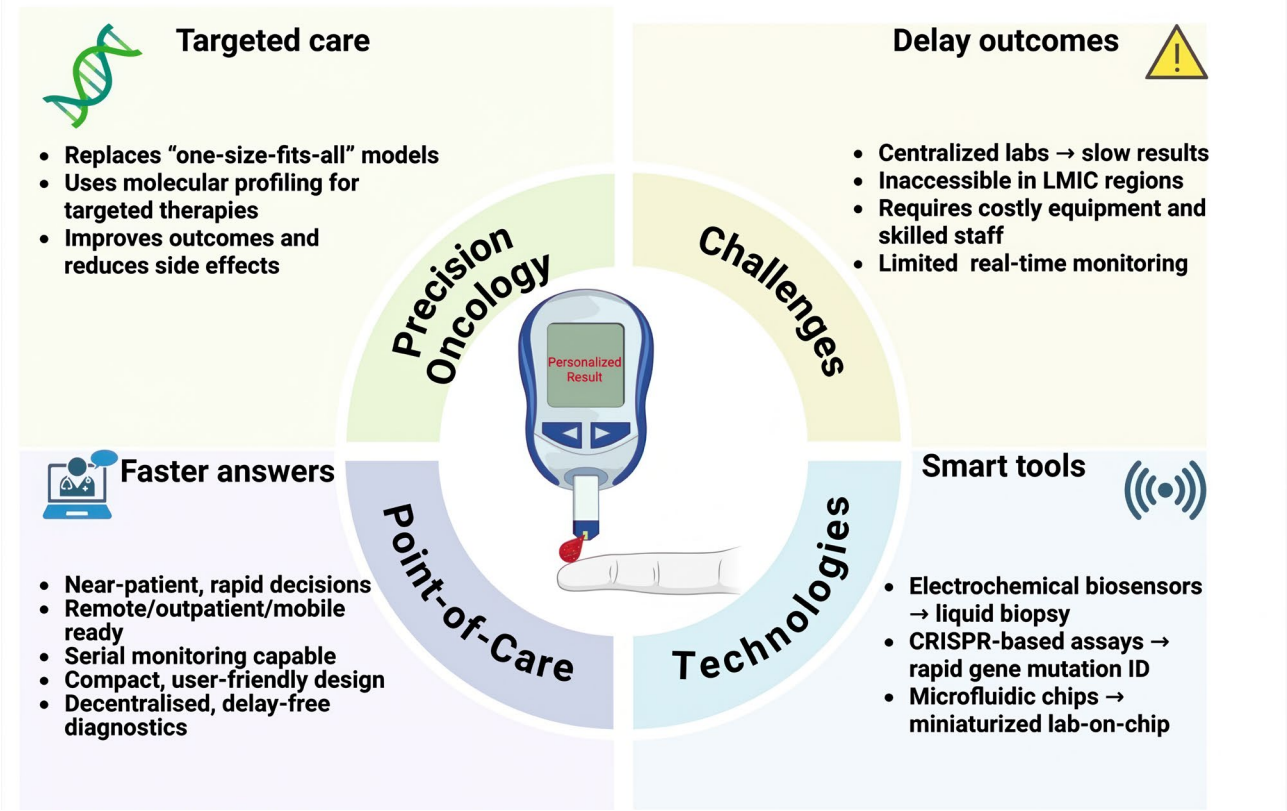

Figure 1. *From bottlenecks to bedside, smart tools accelerate precision oncology.*

facturing scalability issues, cost reduction, regulatory approval, and workflow integration challenges (35). These innovations create a vital pathway for implementing precision oncology outside clinical laboratory settings. The conceptual framework in **Figure 1** demonstrates how POC testing functions as a vital component of real-time patient-centered precision oncology.

The evidence from this review demonstrates that POC technologies possess the technical ability to detect molecular signatures for precision oncology; however, their full potential requires coordinated action. The combination of nanomaterial-enhanced electrochemical sensors, CRISPR diagnostics, and fully integrated microfluidic "lab-on-a-chip" platforms enables bedside assays to reduce the biopsy-to-decision window from weeks to minutes, thus enabling therapeutic choices that match the speed of tumor detection/management. The implementation of global POC precision oncology demands essential steps, including the development of affordable devices that are validated in the field and the establishment of adaptive regulations that match innovative approaches with context-based validation. Further implementation of scalable training programs and ethical safeguards is required to protect privacy and ensure equitable access. Collectively, these measures will establish bedside genomics as a standard medical practice.

PRECISION ONCOLOGY AND THE URGENCY OF DECENTRALISED TESTING

Modern precision oncology depends on continuous measurement of highly dynamic biomarkers like single-nucleotide variants, gene fusions, circulating microRNAs, exosomes, oncoproteins, and even intact circulating tumor cells (27, 33). The foundational idea is that treatment is most effective when tailored to the unique molecular profile of a patient's cancer (36). However, this vision is difficult to realize due to several limitations of current centralized diagnostic systems. These systems are labor-intensive, slow, and often fail to capture the spatial and temporal heterogeneity of malignancies (37). Traditional assays lack the sensitivity, speed, and multiplexing capabilities required for early detection, continuous monitoring, and precise treatment stratifi-

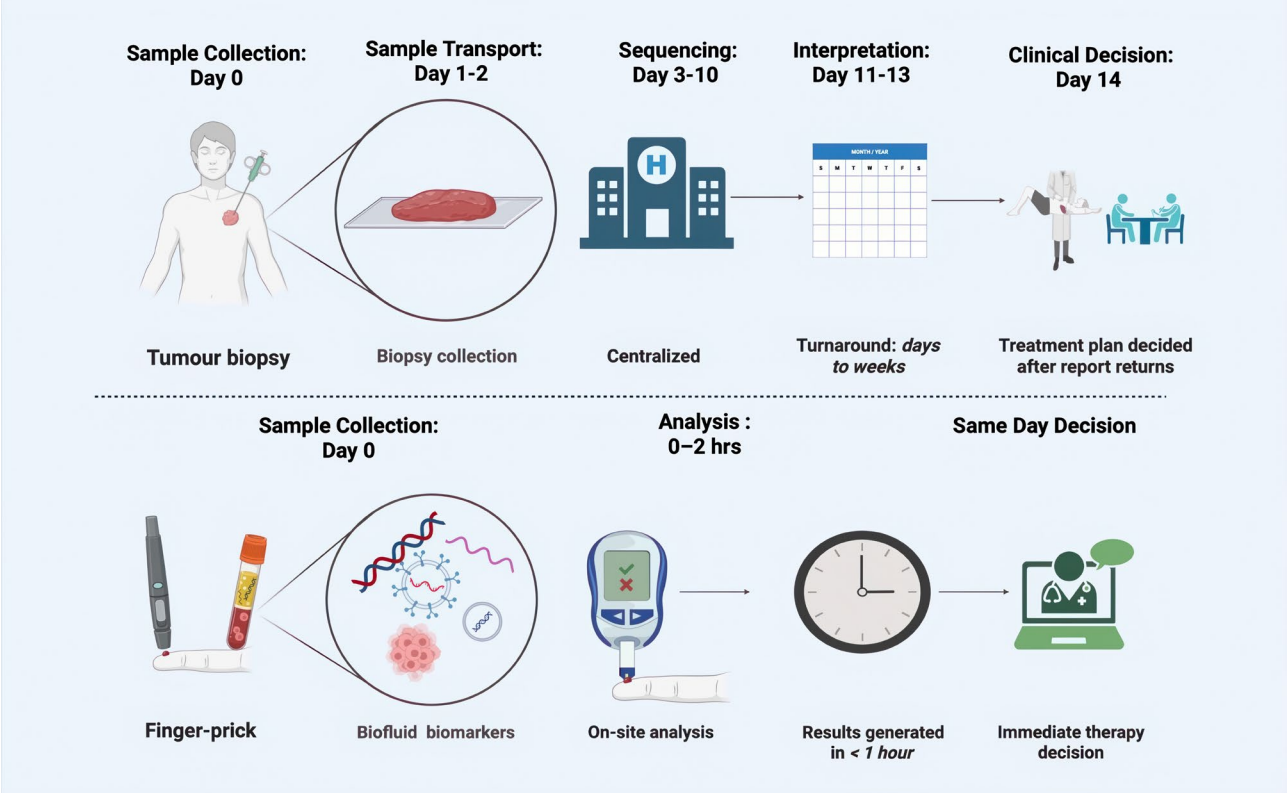

Figure 2. *Comparative timelines for centralized laboratory testing versus on-site diagnostics in precision oncology.*

cation-critical needs, especially for fast-progressing cancers. As a result, actionable biomarkers often cannot be exploited effectively in clinical practice due to logistical delays, infrastructure deficits, and access inequities inherent in the central-lab (38, 39). To overcome this translational gap, diagnostic platforms must not only deliver high analytical precision but also function with operational flexibility across diverse healthcare settings. The transition from centralized laboratory workflows to rapid near-patient testing is illustrated in **Figure 2.**

To this, POC diagnostics are designed to move molecular testing from centralized laboratories to locations where patients receive care i.e. infusion suites, operating rooms, outpatient clinics, or even the home. In oncology this decentralization is uniquely valuable because actionable biomarkers (mutations, miRNAs, proteins, circulating tumour cells/exosomes) can evolve rapidly under therapeutic pressure; short "sample-to-answer" times therefore translate directly into faster treatment adjustments and, potentially, improved outcomes (40).

POC liquid-biopsy technologies are beginning to close the "temporal gap" between sample collection and clinical decision-making by generating actionable molecular read-outs fast enough to guide therapy adjustments in real time. A good illustration is the integrated exosome isolation and detection system (EXID system) microfluidic cartridge that isolates tumor-derived exosomes, labels the immune-checkpoint protein PD-L1 on-chip, and quantifies the signal in <2 h. In a pilot cohort of 16 lung-cancer patients the assay distinguished post-treatment from pre-treatment samples and from healthy controls, with a limit of detection of 10.76 exosomes μL^{-1}-demonstrating its utility for tracking emerging resistance to anti-PD-1/PD-L1 therapy at the chair-side rather than in a distant reference laboratory (41). Researchers have used a herring-bone microfluidic chip to monitor 24 patients with metastatic pancreatic ductal adenocarcinoma over multiple chemotherapy cycles. The device captured over 80% of samples as CTC-positive and produced per-patient "CTC/CSC trajectories" that mirrored radiological progression or response, providing a quantitative relapse signal weeks before routine imaging results were available (42). A 2025 study in triple-negative breast cancer introduced a disposable, pen-printed paper chip that detects exosomal miRNA-21 directly in serum. The self-contained strip, coupled with enzyme-free signal amplification, reaches a 1.2 nM limit of detection and delivers results in 30 minutes using a handheld potentiostat. This low-cost, home-based monitoring of treatment response and recurrence between clinic visits is particularly useful in aggressive TNBC (43). Taken together, these sensor shows how decentralised testing can capture rapidly evolving oncologic biomarkers at the POC, enabling much earlier detection of relapse than is possible with traditional, centrally run assays.

SMART TOOLS: TECHNOLOGICAL CONVERGENCE IN PRECISION ONCOLOGY

A new generation of "smart" POC devices is emerging from the synergistic fusion of four previously independent innovation streams: (i) microfluidic lab-on-chip architectures that automate sample-to-answer workflows on disposable cartridges (33); (ii) biosensor transduction schemes-electrochemical that now achieve femtomolar-attomolar limits of detection for circulating proteins, exosomes, and nucleic acids (27). Further the integration of nanomaterial-enhanced signal amplification with CRISPR-based molecular recognition, microfluidic automation and miniaturized electronics to create deployable POC systems (44–46).

Electrochemical biosensing has become a cornerstone of molecular precision oncology due to its high analytical sensitivity with low-power, chip-scale instrumentation that can be mass-manufactured at minimal cost. By transducing the binding or cleavage of tumor-derived analytes-circulating-tumor DNA fragments, exosomal RNA cargoes, or oncoproteins-into voltammetric or impedimetric signatures, these platforms provide linear quantitative readouts across at least five orders of magnitude, with limits of detection routinely (47–49).

Such modular devices are operable in outpatient infusion suites, peri-operative theaters, or resource-constrained field clinics, thereby eliminating the geographic and temporal separation between specimen collection and molecular insight. The result is a compressed diagnostic–treatment loop that recasts precision oncology as a real-time discipline rather than a retrospective laboratory exercise, enabling clinicians to adjust targeted therapies at the pace of tumor evolution.

The integration of nanomaterials boosts sensor performance through faster electron transfer rates, increased biomarker capture surface area, and enhanced signal-to-noise ratio. The development of

highly sensitive electrochemical biosensors has been made possible by recent advances in nanostructured material fabrication techniques. The effective surface area of the electrodes increases through the use of gold nanoparticles (AuNPs), which also enhances conductivity and provides a dense platform for stable biorecognition element immobilization (48, 50). Research findings from recent studies confirm that electrochemical biosensors show great potential for POC oncology testing. Raucci *et al.* (2024) demonstrated that acid treated commercial gold electrodes and AuNPs modified paper-based screen-printed electrodes can detect the lung-cancer biomarker miR-2115-3p with a methylene blue based electrochemical biosensor. The commercial gold platform achieved a slightly lower detection limit (≈1 nM), but the paper-based alternative offered comparable analytical performance at a much lower cost and with a more sustainable material profile. Both configurations maintained high selectivity against non-target miRNAs and functioned directly in human serum (**Figure 3A**) (50).

Nagdeve *et al.* created a sensor that measures microRNA-31 which serves as a recognized oral-cancer biomarker, while achieving detection limits of 70 pg mL^{-1} in buffer solutions and 700 pg mL^{-1} in diluted serum solutions, thus meeting the requirements for early cancer screening and diagnosis (53). This study demonstrates how electrochemical biosensors can transform precision oncology by detecting clinically relevant biomarkers in small sample volumes with high precision. These devices possess compact dimensions, affordable prices, and smartphone-readable functionality, making them appropriate for decentralized healthcare operations in limited-resource environments. The successful clinical implementation of these devices requires addressing three main challenges which include biofouling, signal drift and calibration stability through systematic materials development and thorough validation procedures (54). The integration of electrochemical sensors into wearable devices would allow for the continuous tracking of circulating tumour DNA which could serve as an early warning system for cancer relapse in colorectal and other cancer types. The analyte detection range of electrochemical devices is mainly limited to predefined targets, although they show high sensitivity for detecting proteins and small-molecule biomarkers. A complete real-time molecular surveillance system for oncology can be developed by combining CRISPR-based assays with electrochemical devices because CRISPR-based assays provide sequence-specific amplification-free nucleic acid detection.

CRISPR-based diagnostics, such as the SHERLOCK platform, detect nucleic acid biomarkers through Cas enzyme sequence-specific cleavage activity at single-molecule resolution for point-of-care oncology testing. Gootenberg *et al.* demonstrated in their research that SHERLOCK detects KRAS oncogenic mutations at attomolar concentrations through Cas13a recognition, which leads to collateral reporter cleavage, thus enabling non-invasive mutation detection in bodily fluids (55). SHERLOCK demonstrated 88.1% sensitivity and 100% specificity in detecting EGFR T790M mutations from NSCLC liquid biopsies, which led to osimertinib therapy decisions in clinical practice (**Figure 3B**) (51). This technology allows for the rapid detection of BRAF V600E mutations in melanoma plasma samples in a short time, supporting the timely selection of targeted treatments (56). CRISPR tools serve dual purposes beyond diagnostic applications, as they help track drug responses and monitor drug resistance. A research study showed that CRISPR/Cas13 technology enables the evaluation of the biological role of vlincRNAs in drug response, thus demonstrating CRISPR's capability for monitoring treatment effectiveness (57). CRISPR-based screening platforms identify essential protein-drug interactions, leading to the discovery of novel therapeutic targets. CRISPR-based systems work alongside traditional biosensors to detect ctDNA and RNA sequences with high sensitivity, which expands the capabilities of POC testing in oncology. The proposed cloud-based CRISPR analytics system would simplify the process of mutation profiling for tracking treatment resistance. The advanced detection capabilities of CRISPR diagnostics require microfluidic platforms to integrate multiple detection methods for complete POC testing applications.

Microfluidic devices or lab-on-a-chip platforms operate with nanoliter fluid volumes to perform sample preparation, amplification, and detection functions, making them suitable for low-sample-volume applications, such as blood or saliva analysis. Microfluidic systems have been used in cancer diagnostics to detect various cancer-diagnostic factors while creating suitable nanoparticles for drug delivery, demonstrating their dual role in cancer diagnosis and treatment (58). The detection and characterization of CTCs represent a fundamental application of microfluidics technology because it helps monitor metastasis and treatment response. Fachin *et al.* developed a microfluidic chip to detect and ana-

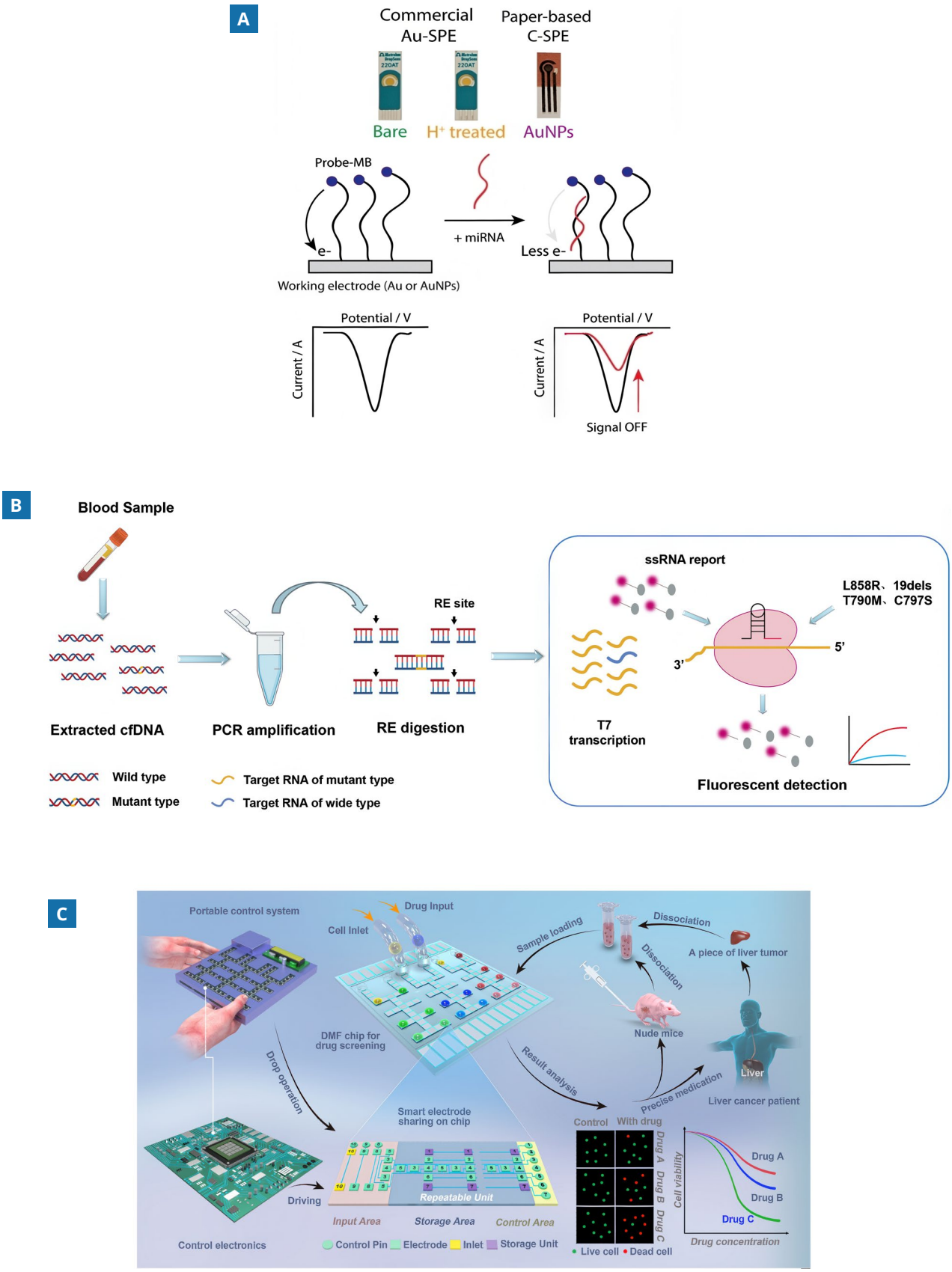

Figure 3. *Schematic overview **(A)** the electrochemical assay for miRNA detection was performed using a commercial gold electrode, an acid treated gold electrode, and a paper based carbon electrode decorated with gold nanoparticles (50); **(B)** the HiCASE assay for the detection of cfDNA sample (51); **(C)** the digital microfluidic (DMF) system was used for drug screening of biopsy samples from MDA-MB-231 breast cancer xenograft mouse model and patients with liver cancer (52).*

Table 1. *A comparative analysis of essential biomarkers together with detection principles, validated specimen, cancer applications, analytical sensitivity, specificity and LOD to demonstrate each platform's translational status and diagnostic potential.*

BIOMARKERS	PRINCIPLE OF DETECTION	CLINICAL / VALIDATED SPECIMEN	TYPE OF CANCER DETECTED	SENSITIVITY	SPECIFICITY	LOD	REFERENCES
BCR–ABL1 & PML-RARA fusions (APL/CML)	CRISPR	EDTA blood / dried spots	Leukemia	100 %	100 %	NA	(61)
Glycoprotein Tumor Biomarkers	Electrochemically controlled Atom Transfer Radical Polymerization (eATRP)	Human serum samples	General (*e.g.*, Alpha-fetoprotein)	High	High	0.32 pg/mL	(62)
EGFR L858R, T790M (Cas12a DETECTR)	Cas12a trans-ssDNA collateral cleavage	Plasma	NSCLC (lung)	100	100	0.005 % MAF	(63)
Cas13, Cas12a, and Csm6	SHERLOCK-v2 multiplex panel	Plasma & contrived cfDNA	General liquid-biopsy demonstration	High	High	2 aM nucleic acid	(55)
CEA	Probe-integrated electrochemical immunosensor	Human serum samples	Colorectal Cancer	High	High	4 pg/mL	(64)
AFP (Alpha-Fetoprotein)	Homogeneous Electrochemical Immunoassay	Diluted human sera of hepatocellular carcinoma (HCC) patients	Hepatocellular Carcinoma	High	High	5 pg/mL	(65)
QGY-7701; QGY-7703	Competitive Electrochemical Sensing	Cancer cell	General Cancer	High	High	20 cells/mL & 35 cells/mL	(66)
Soluble HER2-ECD	Screen-printed ELISA immunosensor	Patient serum	Breast cancer	NA	NA	4 ng mL^{-1}	(67)
Exosomes expressing CD63 & EpCAM	Microfabricated aptasensor combining CD63 capture, EpCAM aptamer bridging, HCR signal amplification, and HRP-TMB electrochemical readout	Serum samples from lung cancer patients (early- & late-stage), plus cultured cell-line exosomes	Lung cancer	High	High	5×10^2 exosomes/mL	(68)

lyze CTCs in the blood of cancer patients. The chip successfully captured 95% of EpCAM-positive cells, allowing genomic analysis for direct trastuzumab therapy. The microfluidic system proved superior to CellSearch systems through its enhanced sensitivity and faster operation, which shows its capability for real-time metastasis tracking (59). Zhai *et al.* developed a portable digital microfluidic platform (23 × 16 × 3.5 cm^3) that performs parallel screening of three anticancer drugs on a 4 × 4 cm^2 chip using primary tumour cells. The drugs that showed effectiveness on the chip device successfully reduced tumour growth in animal models during MDA-MB 231 breast cancer xenograft and patient-derived liver cancer specimen tests. The device demonstrated potential for precision medicine guidance through whole exome sequencing which confirmed that effective agents main-

tained their target genes (**Figure 3C**) (52). Multiplex microfluidic platforms enable the simultaneous measurement of multiple cancer biomarkers from microliter-scale samples, thereby supporting comprehensive diagnosis, early detection, and evidence-based therapy selection in precision oncology. Chen *et al.* used magnetic-bead capture with acoustic micromixing to measure prostate-specific antigen and carcinoembryonic antigen in under 20 minutes with detection limits of 0.028 ng mL^{-1} and 3.1 ng mL^{-1}, respectively. These results illustrate the feasibility of rapid, POC cancer diagnostics based on multi-analyte profiling (60). The integration of advanced diagnostic applications through microfluidics can transform precision oncology by closing therapeutic gaps. Research has demonstrated its effects on different cancer types, leading to better personalized treatments. This technology optimizes clinical operations to deliver enhanced cancer care worldwide.

To help synthesize the diverse technologies discussed, **Table 1** provides a summary of the major POC platforms mentioned, highlighting their clinical application potential. This comparative overview supports the preceding discussion by visually organizing the diagnostic scope, sensitivity, and implementation status of each tool.

REGULATORY AND OPERATIONAL CHALLENGES

Despite the spectacular analytical sensitivity now achievable in precision oncology, very few tests have been validated in prospective (*e.g.* Guardant360 CDx for EGFR mutations in NSCLC), and global regulatory harmonization is lacking. Different regions (EU IVDR, US CLIA/FDA, ISO standards) apply varied thresholds for evidence and performance, slowing global deployment. All of which inflate cost and lengthen timelines for regulatory submission.

Rigid in-vitro-diagnostic (IVD) frameworks that were originally drafted around single-analyte infectious-disease strips do not map neatly onto multimarker oncology cartridges. The next-generation POC liquid-biopsy devices must still satisfy U.S. CLIA-waiver "simple test" criteria while simultaneously proving multiplex variant accuracy that normally requires high-complexity molecular laboratories, a mismatch that slows 510(k)/De Novo submissions and has left only a handful of cancer assays cleared to date (69).

Progress is further hampered by the absence of universally commutable reference materials for ctDNA, microRNA, and extracellular-vesicle targets. The integrated lab-on-a-chip review by Surappa *et al.* notes that most groups calibrate limits-of-detection with contrived spike-ins prepared in-house, making cross-platform performance claims difficult to harmonize and complicating multi-site reproducibility studies demanded by regulators (33).

Operationally, the most consistent pain points involve pre-analytical variability and supply-chain resilience. Paper-based liquid-biopsy platforms demonstrate how hemolysis, diurnal swings in EV release, and freeze-thaw cycles can each shift electrochemical readouts by more than one standard deviation, forcing manufacturers to integrate on-cartridge normalization controls and environmental sensors, which in turn raise cost and assembly complexity.

The implementation of POC testing has the potential to transform precision oncology through bedside biomarker analysis; however, its adoption remains limited by major technical challenges. POC devices must precisely measure trace tumor-derived analytes, including circulating nucleic acids, in complex biofluids while functioning in different environmental settings. The combination of temperature changes and sample contamination along with environmental disturbances leads to assay accuracy degradation which results in unreliable results when tests are performed outside laboratory control (70).

Finally, real-world deployment in low- and middle-income countries (LMICs) encounters infrastructure limitations-intermittent power, limited cold-chain capacity, and scarce biomedical-engineering support-that can erode field accuracy by up to 20 % relative to controlled settings. A 2025 review of oncology POC implementation in LMICs calls for locally manufactured consumables, solar-powered readers, and streamlined post-market surveillance to sustain diagnostic precision outside tertiary centers (71).

The solution to these barriers requires coordinated innovation efforts. The adoption process will speed up through platforms that are accessible to all and resilient and use unified data standards and adaptive risk-based regulatory pathways. The implementation of scalable workforce training and robust ethical frameworks will protect data security and ensure equitable access. The implementation of these pillars will enable POC diagnostics to redefine precision oncology by providing fast individualized care across the world.

Finally, the ecosystem necessary for the successful implementation of POC oncology diagnostics is inherently complex. It requires coordinated efforts among diagnostic developers, clinicians, regulatory authorities, payers, and standards organizations.

FUTURE DIRECTIONS AND CONCLUSIONS

The next wave of POC precision-oncology devices is moving toward single-cartridge, ultra-sensitive and highly multiplexed platforms that couple CRISPR/Cas recognition, nanomaterial signal amplification, and fully integrated microfluidics. In the coming 3-5 years, these lab-on-chip architectures are expected to converge with wearable biosensors-flexible electrochemical patches, microneedle fluidics, or smartphone-coupled optical readers, supporting continuous or immediate, on-demand cancer-biomarker surveillance outside formal clinic walls.

For clinical integration, engineering priorities are shifting toward closed, sample-to-answer system that run on finger-stick blood, urine, or saliva and can be operated by self, nurses or community health workers after minimal training. Bluetooth/FHIR-compliant connectivity will push results straight into electronic health records and multidisciplinary tumor-board dashboards, facilitating rapid therapeutic alignment and longitudinal monitoring without centralized laboratory dependencies.

Translational success, however, hinges on standardization and regulation. Achieving global health equity remains a pressing mandate. Although most commercial POC cancer tests are currently configured for high-resource markets, the greatest diagnostic gaps exist in LMICs. Future development must therefore emphasize low-cost readers with battery or solar power, lyophilized reagents stable at tropical temperatures, and open-source firmware that can be localized for language and connectivity constraints (71).

Collectively, the literature paints a clear trajectory: POC diagnostics are poised to transform precision oncology by collapsing the temporal and geographic gap between biomarker measurement and clinical action. The technological capability to match centralized laboratories in sensitivity is emerging; the challenge now is to embed these advances into rigorous yet agile regulatory frameworks, pragmatic clinical workflows, and equity-focused distribution models. With sustained interdisciplinary collaboration and deliberate attention to global implementation, POC precision-oncology testing can redefine cancer care as a rapid, individualized, and universally accessible enterprise.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflicts of interests

The authors declare no competing interests.

Data availability

All data generated or analyzed during this study are included in this article.

Author Contributions

Conceptualization: SC and SS. Writing- Original Draft Preparation: SC and SS. Visualization and Figures: SS and AG. Critical Review and Editing: PMK, AM, AR, and GI. Scientific Input and Guidance: SC, MDL, and CM. Funding Acquisition: SC, SS, and AG. All authors reviewed and approved the final version of the manuscript for submission.

Publications ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data corresponds to the real.

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REVIEW ARTICLE

GENERATIVE INTELLIGENCE IN PRECISION ONCOLOGY: PRIORITIES IN INFORMATICS ENGINEERING, PATHOLOGY AND ONCOLOGY

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ABSTRACT: Generative artificial intelligence (GAI) applied to clinical diagnostics and research is reshaping the panorama of precision oncology. Combining hematoxylin-eosin-stained whole slide images with computational algorithms opens new avenues in digital pathology. GAI allows for extracting molecular, immunological, and prognostic information based on routinely processed histological sections and removes the need for additional molecular testing.

In oncology, GAI models excelled in cancer histotyping, malignancy ranking, molecular profiling, identification of prognostic and predictive biomarkers, and inference of immune gene signatures. The latest foundational models provide additional opportunities to develop generalizable, scalable tools that can be consistently leveraged in line with pathology missions.

However, several challenges must still be addressed to optimize GAI performance and encourage its clinical application. These include data quality, algorithm bias, generalizability across institutions, and validation through robust multicenter trials. This strategy is crucial for increasing clinical confidence, ensuring reproducibility, and facilitating the routine use of AI in precision oncology.

This review focuses on the operational application of computational pathology within the broader context of precision oncology. It addresses the most significant technical innovations in biomarker assessment and critically examines the priorities to enhance the reliability, scalability, and performance of AI-driven tools in precision oncology.

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Impact statement: Generative artificial intelligence applied to digital histopathology offers novel strategies for biomarker identification and tumor classification, advancing precision oncology and diagnostic accuracy.

Key words: *precision oncology; computational pathology; deep learning; machine learning; whole slide images; generative artificial intelligence.*

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INTRODUCTION

Histology is regarded as the gold standard for diagnosing human diseases, including cancer (1). In recent years, the rapid emergence of artificial intelligence (AI)-driven models in digital and computational pathology (2) has revolutionized cancer histopathology, significantly advancing both cancer research and clinical oncology (3, 4).

The integration of AI into surgical pathology is accelerating progress across various oncological domains, including cancer subtyping (5), survival prediction (6), and detection of nodal metastasis (7). Moreover, deep learning (DL) models have shown the ability to identify clinically relevant genetic alterations, such as microsatellite instability (MSI) (8) and multiple gene mutations (9), from hematoxylin and eosin-stained (H&E) sections (10, 11). Furthermore, AI-based tools have been developed in oncology, such as grading in prostate cancer (12) and, more recently, predicting DNA methylation profiles from histology sections (13).

In this review, we describe the emerging role of artificial intelligence in oncology, with a particular focus on computational histopathology (**Figure 1**). We aim to highlight the transformative potential of AI-driven models in shaping the future of precision oncology, ultimately supporting more accurate and high-quality cancer diagnoses.

THE INTRODUCTION OF SCANNERS IN PATHOLOGY DEPARTMENTS: THE ROLE OF WHOLE-SLIDE IMAGES (WSIS) IN COMPUTATIONAL PATHOLOGY

The introduction of slide scanners for digitizing glass slides in pathology, along with the growing use of AI for research and diagnostics, signifies a pivotal shift in precision oncology. Despite the promise of AI in clinical workflows, several challenges persist (14).

Adopting new technologies often necessitates rethinking established practices. In pathology, slide scanners gradually replace the optical microscope, the pathologist's primary tool, with digital workflows. Routine slide digitization generates WSIs of cancerous tissues, serving as a crucial entry point for incorporating digital tools in diagnostics (15).

WSI technology enables the application of machine learning (ML) and dep learning (DL) algorithms to histopathological images, allowing for clinically relevant data extraction to aid in cancer diagnosis, prognosis, and treatment decisions (16-18). The broader implementation of WSI is expected to significantly influence diagnostic pathology, facilitating AI-supported precision diagnosis (19).

In oncology, DL algorithms have shown the capability to extract vital information from H&E-stained WSIs alone, such as tumor classification and treatment

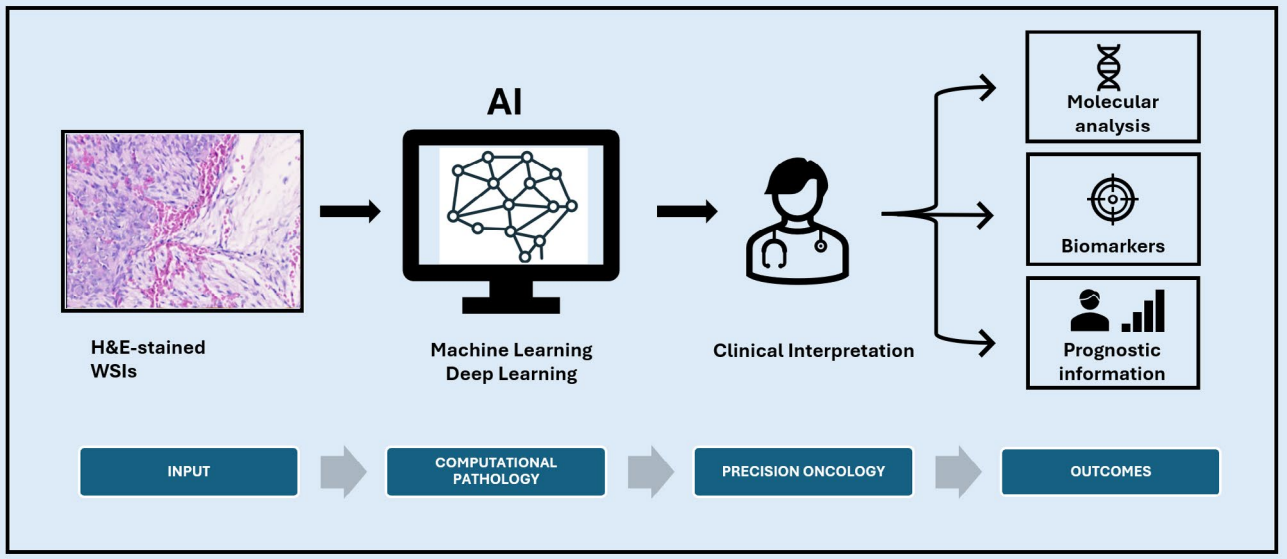

Figure 1. *Workflow of AI-Based Computational Pathology for Precision Oncology.*
Machine learning and deep learning models extract molecular and prognostic insights from H&E-stained whole slide images, supporting clinical interpretation and outcome prediction. H&E = Hematoxylin and Eosin; WSI = whole-slide images; AI = Artificial Intelligence.

selection (20), metastatic potential prediction (21), and identifying primary sites in cancers of unknown origin (22). Moreover, WSIs allow the extraction of molecular-level data, including immunohistochemical and histochemical markers, directly from H&E slides. This includes predictions of PD-L1 and PD-1 expression (23) and mutational status across cancer types (24-26). Such tools could soon offer fast, cost-effective methods to inform personalized treatments. The integration of slide scanners and routine WSI use, combined with AI-driven models, presents a significant opportunity for both healthcare institutions and research centers (27). Digital pathology through WSI technology has the potential to transform can-

Table 1. *Summary of published studies applying AI models to histopathology for biomarker prediction and clinical tasks.*

STUDY	YEAR	CANCER TYPE/TASK	AI METHODOLOGY	MAIN FINDINGS	REF.
Coudray *et al.*	2018	NSCLC – mutation classification	CNN on H&E	Predicted mutations (EGFR, STK11, TP53, etc.) and PD-L1 status	(24)
Skrede *et al.*	2020	Colorectal – outcome prediction	Deep learning on WSIs	DL model predicted prognosis with high AUC	(6)
Kather *et al.*	2020	Pan-cancer – actionable mutations	AI on H&E	Detected multiple genetic alterations from histology	(10)
Fu *et al.*	2020	Pan-cancer – mutation & composition	CNN on H&E	Inferred mutations, cell types and prognosis	(25)
Qu *et al.*	2021	Breast – pathway prediction	Deep learning on WSIs	Predicted mutations and signaling pathways	(26)
Lu *et al.*	2021	Cancer of unknown primary	AI on H&E + weak supervision	Predicted tissue of origin with high accuracy	(22)
Saldanha *et al.*	2023	Pan-cancer – mutation prediction	Self-supervised DL	Accurate prediction of genomic alterations	(11)
Shamai *et al.*	2022	Breast – PD-L1 prediction	DL on H&E	AI matched IHC PD-L1 expression	(52)
Wang *et al.*	2022	NSCLC – PD-L1 scoring	Multimodal DL	Fusion model predicted PD-L1 & survival	(55)
van Eekelen *et al.*	2024	NSCLC – PD-L1 scoring	Cell-level DL	AI showed better reproducibility *vs* pathologists	(56)
Jin *et al.*	2024	Pan-cancer (20 types) – PD-L1	Multiple instance learning	AUC 0.83 on >12k slides, mRNA correlation	(57)
Saillard *et al.*	2023	Colorectal – MSI screening	AI-based MSI detection (MSIntuit)	Validated model for MSI prediction on H&E	(36)
Arslan *et al.*	2022	Multi-cancer – multi-omic prediction	DL on H&E	Predicted mutations, expression, MSI, CNAs	(37)
McCaw *et al.*	2024	Pan-cancer – digital biomarkers	ML on histology	Predicted multiple digital biomarkers from WSIs	(38)
Nakatsuka *et al.*	2025	NASH – HCC prediction	DL on liver biopsies	Predicted HCC development years in advance	(69)
Hoang *et al.*	2024	CNS tumors – DNA methylation	DL on histology	Inferred methylation subtype from slides	(13)
Amgad *et al.*	2024	Breast – prognostic biomarker	Population-level digital pathology	Created a histological biomarker for prognosis	(75)
Chen *et al.*	2024	Pan-cancer – general model	Foundation model (UNI)	Predicted 108 cancer types from WSIs	(78)

AI = Artificial Intelligence, CNN = Convolutional Neural Network, DL = Deep Learning, H&E = Hematoxylin and Eosin, IHC = Immunohistochemistry, ML = Machine Learning, MSI = Microsatellite Instability, NSCLC = Non-Small Cell Lung Cancer, PD-L1 = Programmed Death-Ligand 1, PD-1 = Programmed Cell Death Protein 1, WSI = Whole Slide Image, CNS = Central Nervous System, NASH = Non-Alcoholic Steatohepatitis, HCC = Hepatocellular Carcinoma, CNA = Copy Number Alteration, mRNA = Messenger Ribonucleic Acid, MSI = Microsatellite Instability, UNI = Universal foundation model for computational pathology, AUC = Area Under the Curve.

cer diagnosis and research by converting conventional slides into digital data, laying the groundwork for computer-assisted diagnostics (28).

WSIs provide the foundation for fully digitized pathology workflows, backed by AI-powered decision-support systems. These tools leverage computational histopathology to enhance diagnostic accuracy and consistency (29). The ongoing digitalization of pathology departments, alongside advances in ML and DL, is poised to accelerate oncological research and foster the development of AI-assisted diagnostic tools for various malignancies (30).

ML-MODELS ALLOW THE PREDICTION OF MULTIPLE BIOMARKERS FROM WHOLE SLIDE HISTOPATHOLOGY IMAGES

One of the most intriguing aspects of AI in digital pathology is its ability to predict multiple biomarkers, including mutation status, from H&E-stained WSIs (2, 11). Recent AI-driven models can now predict diagnostic and predictive biomarkers such as immunohistochemical, genetic, epigenetic, and *in situ* hybridization markers. Traditionally, identifying these biomarkers requires manual assessment by trained pathologists, a time-consuming and costly process that can delay diagnosis and treatment.

The progressive adoption of digital pathology has been complemented by the development of an alternative approach, whereby AI models analyze routinely acquired H&E-stained WSIs to extract multiple predictive biomarkers. These include key molecular features that are instrumental in-patient stratification for targeted therapies (31-33). This paradigm shift has revealed that H&E-stained sections, long considered tools primarily for morphological assessment, contain a rich reservoir of latent molecular information.

WSIs can now support automated disease detection, histological and molecular subtyping, and tumor grading, as well as prognostic evaluation, survival prediction, and treatment planning (33). AI models trained on H&E-stained WSIs have demonstrated the ability to predict a range of molecular biomarkers across different cancer types (34-36). Additionally, emerging studies suggest that WSIs may also be used to infer other molecular alterations, such as RNA expression patterns and protein abundance (37). While initial efforts focused on models trained to predict a single biomarker in a specific cancer type, newer frameworks now predict multiple biomarkers, including copy number alterations and RNA-derived signatures, across various malignancies (38). These findings emphasize the vast, clinically relevant data embedded in standard H&E-stained slides. **Table 1** provides an overview of significant studies utilizing AI for biomarker prediction, tumor classification, and outcome forecasting across diverse cancers.

Given the widespread use of H&E-stained slides in pathology laboratories globally, digitizing these images could enable the deployment of AI-driven biomarker prediction models even in low-resource settings, potentially benefiting a broader patient population.

DL-MODELS APPLIED TO THE PREDICTION OF PD-1 AND PD-L1 EXPRESSION BASED ON H&E-STAINED SECTIONS

The immune system maintains a balance between eliminating harmful pathogens and preserving self-tolerance, regulated by immune checkpoints like PD-1 (39). PD-1, a key checkpoint receptor, modulates T-cell activity to maintain peripheral tolerance, preventing autoimmune responses (40, 41).

The identification of PD-1 and its ligand PD-L1 in tumor cells, first reported in 2002, unveiled a critical mechanism of immune evasion by which tumors exploit immune checkpoint pathways to evade immune surveillance (42). PD-L1 is predominantly expressed on the surface of tumor cells but can also be released in the tumor microenvironment via exosomes, amplifying immune suppression (43, 44). Given its role in promoting tumor immune escape, PD-L1 has become a major target in cancer immunotherapy (45-48).

Traditionally, PD-L1 expression is assessed through immunohistochemistry (IHC), which remains the standard in clinical practice (49-51). However, despite its widespread use, IHC presents several challenges: it is time-consuming, costly, and may deplete limited tissue samples, particularly in small biopsies. Furthermore, interpretation of PD-L1 staining is prone to significant variability due to differences in staining protocols, subjective interpretation of staining intensity, and interobserver variability, especially in borderline cases (52, 53). This inconsistency can critically impact clinical decision-making, potentially misclassifying patients and affecting their eligibility for immune checkpoint inhibitors.

Digital pathology and AI-driven models offer a promising alternative, providing a more standardized and reproducible assessment of PD-L1 expression by analyzing WSIs across multiple tumor regions (54). Unlike manual scoring, AI models can systematically quantify PD-L1 expression across heterogeneous tumor regions, reducing variability and enhancing diagnostic accuracy. For instance, AI algorithms applied in lung cancer demonstrated high accuracy in predicting PD-L1 status, aligning closely with pathologist assessments even in challenging cases (55, 56). A pivotal study by Jin *et al*. introduced a pan-cancer AI model capable of predicting PD-L1 expression directly from H&E-stained WSIs, analyzing over 12,000 slides from 20 tumor types and achieving a mean area under the curve (AUC) of 0.83 (57). The model's predictions were validated against conventional IHC and mRNA expression, underscoring the potential of AI to standardize biomarker assessment and minimize interobserver variability.

Table 2 summarizes selected studies comparing AI-based digital pathology approaches with conventional immunohistochemistry for PD-L1 assessment across different tumor types.

This shift toward AI-driven PD-L1 evaluation reflects the emerging paradigm of "intelligent digital pathology", where AI augments conventional diagnostics, potentially accelerating therapeutic decision-making, expanding access to precision oncology, and ensuring more consistent biomarker assessment across diverse clinical settings (32, 58, 59).

The clinical integration of this approach is particularly relevant in the context of therapeutic decision-making. By predicting multiple biomarkers, including immune checkpoint-related proteins and mutational profiles, from routine H&E-stained slides, AI-driven pathology can guide the selection of targeted therapies or immunotherapies. For instance, in advanced non-small cell lung cancer or gastric cancer, accurate prediction of PD-L1 expression or MSI status directly from histology can streamline treatment eligibility decisions and reduce dependence on costly or time-consuming molecular assays (24, 36, 52, 55, 57). Additionally, in multidisciplinary oncology settings, integrating AI-generated outputs into tumor board discussions may enhance personalized care planning, particularly when biopsy material is limited or when rapid turnaround is needed.

Table 2. *Comparison between immunohistochemistry (IHC) and AI-based digital pathology for PD-L1 assessment.*

FEATURE	IMMUNOHISTOCHEMISTRY (IHC)	AI-BASED DIGITAL PATHOLOGY (ON H&E WSIS)	REF.
Sample requirement	Requires additional antibody-based staining	Uses routine H&E-stained slides already available in pathology labs	(49-51)/ (24, 57)
Tissue consumption	Consumes precious tissue, critical in small biopsies	No additional tissue required; preserves material for other tests	(52)/ (23/37)
Cost	High costs due to antibodies, reagents, and specialized equipment	Lower long-term costs after digitization infrastructure is in place	(53)/ (24, 57)
Turnaround time	Time-consuming due to staining and manual interpretation	Faster analysis after digitization and model deployment	(52)/ (23, 55, 57)
Expertise required	Requires experienced pathologists for accurate interpretation	AI supports interpretation; reduces reliance on specialist expertise	(52, 53)/ (54, 56)
Interpretation variability	High inter- and intra-observer variability	Provides standardized, reproducible results	(53)/ (54, 56)
Accessibility	Often unavailable in peripheral or low-resource settings	H&E-based AI tools are scalable and suitable for resource-limited contexts	(52)/ (24, 57)
Multiplexing capability	Generally limited to one biomarker per slide	Potential to predict multiple biomarkers from a single H&E image	(49-51)/ (25, 26, 36-38)
Molecular correlation	Direct protein expression detection	Can predict mRNA expression, mutation status, and other molecular features	(50)/ (37, 38, 57)
Scalability and automation	Manual, slow, and hard to scale	Fully automatable and scalable across large datasets	(52)/ (23, 54, 57)

IHC = Immunohistochemistry, AI = Artificial Intelligence, H&E = Hematoxylin and Eosin, WSI = Whole Slide Image, mRNA = Messenger Ribonucleic Acid.

DEEP LEARNING APPLIED TO DIGITAL PATHOLOGY IN THE PREDICTION OF HCC

Non-alcoholic steatohepatitis (NASH), a progressive form of non-alcoholic fatty liver disease (NAFLD), is now recognized as the leading cause of chronic liver disease and a key risk factor for hepatocellular carcinoma (HCC) (60). Histologically, NASH is marked by macrovesicular steatosis, lymphocytic infiltration, hepatocellular ballooning, apoptotic bodies, and varying degrees of fibrosis (61). Traditionally, fibrosis has been considered the strongest predictor of adverse outcomes, including cirrhosis and HCC (62, 63). However, over 50% of NASH-related HCC cases arise in non-cirrhotic livers, indicating that other histological and molecular features beyond fibrosis may drive carcinogenesis (64, 65).

Recent AI-based models have been applied to the automated assessment of liver fibrosis and other NASH-related histological changes, demonstrating ML techniques' key advantage in providing objective, quantitative evaluations that reduce interobserver variability and support more consistent longitudinal disease monitoring (66, 67). DL approaches, in particular, have shown significant promise in identifying subtle histological markers associated with early carcinogenesis that may be missed in conventional assessments, extending predictive capabilities beyond fibrosis and nodular regeneration (68).

A significant study by Nakatsuka *et al.* explored a DL model to predict HCC development using only H&E-stained WSIs of liver biopsies from steatosis patients (69). The model aimed to identify individuals at higher HCC risk solely based on liver steatosis analysis, achieving an AUC of 0.80 for predicting HCC onset within seven years post-biopsy. Notably, the model identified at-risk patients without advanced fibrosis, underscoring the role of additional histological features in liver tumorigenesis.

Through saliency map analysis, the model highlighted key predictors of HCC development, including a high nuclear-to-cytoplasmic ratio, nuclear atypia, lymphocytic infiltrates, and the absence of large lipid droplets. These findings suggest that AI models can detect subtle histological changes predictive of liver cancer risk in routine biopsies, potentially without expensive molecular assays (69).

This work emphasizes two critical points: first, AI algorithms can extract complex histological signals indicative of future disease progression; second, integrating AI with digital pathology, or computational pathology (CPath), may revolutionize liver histopathology by enhancing diagnostic accuracy, aiding prognostic stratification, and supporting preventive strategies in NASH-related HCC (69).

TOWARDS A GENERAL FOUNDATION MODEL FOR COMPUTATIONAL PATHOLOGY

In routine clinical practice, pathologists are responsible for a broad spectrum of diagnostic tasks, including cancer detection, subtyping, grading, and staging. These tasks require consideration of thousands of potential differential diagnoses. To address these challenges, a wide range of AI models have been developed in recent years, particularly within the domains of digital and computational pathology (70, 71). Among the most promising innovations is the development of AI-driven models capable of multimodal data integration, which should combine clinical, genomic, epigenomic, radiomic, pathomic, and microbiological data to provide a more comprehensive view of the oncologic landscape (72). Computational pathology (CPath) has demonstrated the potential to predict molecular alterations directly from histopathological images, including microsatellite instability (MSI) (8, 73, 74), patient prognosis (75), and treatment response (76). However, most of these models are trained for a specific cancer type and are limited to predicting a narrow set of molecular or immunohistochemical features, which restricts their applicability in diverse clinical contexts. To overcome these limitations, a new class of AI tools has emerged: multi-cancer, multi-biomarker models designed to simultaneously predict a wide range of molecular alterations across various tumor types using standard H&E-stained slides (39). These systems, defined as "foundation models," are characterized by their scalability, versatility, and adaptability to multiple diagnostic tasks and cancer types (77). In this direction, a general-purpose foundation model for computational pathology, defined as *UNI*, has been recently introduced by Chen TJ and colleagues (78). Pretrained on over 100 million images, the UNI model demonstrated the capacity to classify up to 108 cancer types, marking a significant advancement toward the integration of AI into routine workflows in anatomic pathology Labs.

COMPUTATIONAL PATHOLOGY IN ONCOLOGY

Artificial intelligence has emerged as a revolutionary tool for the discovery of predictive biomarkers in human cancers. AI-based methods are redefining the landscape for researchers, pathologists, and oncologists, demonstrating the potential of well-trained algorithms to extract clinically relevant molecular information directly from routinely stained H&E sections.

When applied to clinical practice, the advantages of this paradigm shift are numerous. One of the most significant is the speed of analysis: the average computational time to generate a PD-L1 probability map has been reported at approximately 40 seconds, with a range from 7.9 to 66 seconds (79). This indicates that, with a robust and validated DL model, pathologists could provide near-instantaneous estimates of PD-L1 expression, facilitating timely and personalized therapeutic decisions for oncologists (87).

In addition to rapidity, AI-based approaches offer substantial cost-saving opportunities. The reliance on conventional immunohistochemistry, dependent on specialized reagents, equipment, and trained personnel, may be significantly reduced or even replaced. The possibility of identifying genes and immune-related biomarkers, such as PD-L1, directly from H&E sections without antibody-based detection opens intriguing transformative possibilities, particularly for decentralized and resource-limited settings.

Furthermore, AI-driven histopathological analysis enables the extraction of novel insights beyond PD-L1 expression, potentially enhancing clinical decision-making. Immune pathology, a key foundation for immune checkpoint inhibitor (ICI) therapies, remains a relatively underexplored area within diagnostic pathology. AI methodologies could facilitate the identification of novel "metabiomarkers", complex, integrative features predictive of ICI therapy response (82). This hypothesis is supported by recent evidence showing that DL models can predict immune and inflammatory gene signatures in hepatocellular carcinoma directly from histological images (83).

Taken together, these findings underscore the potential of AI, particularly DL algorithms, to extract multiple molecular and immunological biomarkers from standard histology, enabling the discovery of novel predictive features and advancing the goals of precision oncology. Computational pathology (also referred to as pathomics) thus represents a unique opportunity: to serve as a rapid, cost-effective, and integrative diagnostic tool for clinicians, oncologists, and surgeons alike, delivering morphological, genetic, and molecular data in near real time.

Another major strength of computational pathology lies in its ability to generate large-scale datasets of digitized slides, which can be integrated with complementary clinical (real-world data), genomic, epigenomic, microbiologic, radiologic (radiomics), and laboratory information. This multimodal integration offers the potential to define novel metabiomarkers, which can outperform unimodal models in terms of predictive accuracy, as measured by improved AUC metrics (76).

Moreover, computational pathology can address a long-standing challenge in diagnostic histopathology: interobserver variability. This is particularly relevant for PD-L1 scoring, which is known to vary significantly among both expert and generalist pathologists (84-86). While DL models can provide more consistent and standardized assessments of PD-L1 expression, their capacity to directly infer molecular and transcriptomic features from histology offers a far more transformative leap than simply resolving variability issues.

For successful adoption in clinical practice, AI-based computational pathology systems must be integrated into existing digital workflows within pathology departments. This includes embedding AI models into slide viewers and laboratory information systems (LIS), allowing pathologists to access real-time predictions directly from digitized H&E slides. Additionally, the deployment of AI tools should be supported by intuitive, clinician-oriented interfaces that facilitate interpretation and integrate seamlessly into the diagnostic process. Real-world implementation also requires rigorous prospective validation studies and standardized protocols to demonstrate clinical utility. Importantly, AI-driven solutions should be designed to complement rather than replace human expertise, acting as decision-support tools that enhance diagnostic accuracy, reproducibility, and efficiency in oncology care.

NEXT CHALLENGES

Along with its unquestionable advantages, the real-world implementation of computational histology entails several major issues that need to be addressed before AI models can be safely and effec-

tively integrated into clinical practice, particularly within the field of immuno-oncology. The major current limitations hindering clinical implementation are summarized below:

1. Data Quality and Availability: robust algorithm performance requires access to large volumes of high-quality, well-annotated data. However, oncologic datasets are often incomplete, heterogeneous, biased, and inherently complex, limiting model generalizability and reproducibility.

2. Model Selection Complexity: the proliferation of ML and DL algorithms, often promoted through marketing strategies emphasizing innovation rather than practical limitations and clinical safety, can make it challenging for researchers and clinicians to select the most appropriate model for specific applications. While advanced DL models are widely marketed as cutting-edge solutions, classical ML models may outperform them in low-data scenarios and should not be overlooked, particularly when sample sizes are limited (87).

3. Regulatory Certification: certification is a critical prerequisite for clinical adoption. At present, there is no universally accepted regulatory pathway for the validation and certification of AI-based tools in pathology. The establishment of worldwide (or at least continental-wide) standardized, harmonized processes for model certification should be encouraged to ensure safety and efficacy.

4. Lack of Guidelines and Protocols: clear protocols and guidelines for conducting rigorous, clinically meaningful studies on AI model applicability are currently lacking. This gap hinders reproducibility and delays the translation of research findings into clinical practice.

5. Lack of Trust and Interpretability: a significant barrier to clinical implementation is the skepticism among healthcare professionals, including pathologists, regarding the reliability and transparency of AI tools. Improving model interpretability is essential to foster trust. Techniques from the field of explainable artificial intelligence (XAI) may help to demystify algorithmic decision-making and reduce the "black box" effect (88, 89).

6. Insufficient External Validation: AI models that perform well on internal datasets often fail when applied to external, real-world data. To ensure clinical robustness, models should be validated using diverse, multi-institutional datasets. One proposed strategy is divergent validation, which evaluates model performance across various independent datasets to enhance generalizability and transparency (90, 91).

6. Bias and Variability: algorithmic biases can result from inconsistencies in slide staining, errors in labeling the data sets used for training, scanner calibration, or demographic imbalances in training data. These factors can significantly impair model performance and reliability. Reducing such biases is crucial to enable fair and accurate deployment of AI models in clinical settings.

Despite these several interconnected limitations, the primary obstacle hindering the widespread use of AI strategies in clinical practice is the lack of standardized and universally accepted pathways for validation and certification. Without clear regu-

Table 3. *Current challenges and proposed solutions for the clinical integration of AI in computational pathology.*

CHALLENGE	DESCRIPTION	SUGGESTED SOLUTIONS	REF.
Data Quality	Incomplete, biased, heterogeneous datasets	Centralized data curation and federated learning	(1, 92)
Model Selection	Difficult choice among ML/DL models	Model comparison guidelines, model benchmarking	(89)
Certification	Lack of standard regulatory pathways	International consensus on AI model validation	–
Trust and Interpretability	Lack of clinician trust due to black-box nature	XAI, transparent algorithms	(90–91)
Interobserver Variability	Variability in human assessment (*e.g.*, PD-L1 scoring)	Algorithmic standardization, model calibration	(86–88)
External Validation	Limited generalizability across datasets	Multi-institutional validation, divergent validation	(90-91)
Staining and demographic biases	Bias in data acquisition and population representation	Dataset balancing, domain adaptation techniques	–

ML = Machine Learning, DL = Deep Learning, AI = Artificial Intelligence, PD-L1 = Programmed Death-Ligand 1, XAI = Explainable Artificial Intelligence.

latory guidance and robust multicenter validation studies, many AI-based models remain restricted to research settings. This uncertainty, coupled with a lack of transparency in algorithmic outputs, continues to undermine clinician trust and delays the full integration of AI into routine oncologic diagnostics. The key limitations currently hindering the implementation of AI in clinical workflows, along with proposed solutions, are summarized in **Table 3**.

CONCLUSIONS

The introduction of AI-driven models has triggered a true revolution in oncology, with applications spanning from the interpretation of medical imaging to the enhancement of diagnostic and prognostic accuracy, including prediction of overall survival and response to various therapeutic strategies (92). Among these tools, convolutional neural networks (CNNs) have emerged as indispensable new tools for the recognition and classification of both histological and radiological images. CNNs can detect subtle and complex patterns that may escape even the most experienced pathologists and radiologists. A key strength of CNNs lies in their ability to autonomously learn from data, particularly when trained on large, high-quality datasets. This has enabled a shift from traditional machine learning towards deep learning in medical image analysis. CNNs have demonstrated outstanding performance in tasks such as cancer detection, histological classification, and subtype recognition.

More recently, advanced CNN-based architectures have achieved notable success in cancer diagnostics. For instance, CNNs combined with Long Short-Term Memory (LSTM) networks have shown promise in predicting cancer prognosis by capturing temporal patterns in patient data. Spatially Constrained CNNs (SC-CNNs) have proven effective for nuclei classification in colorectal cancer, enhancing precision in histopathological assessment. Moreover, the integration of CNNs with Fourier Transform Infrared (FTIR) spectroscopy has yielded promising results for accurate cancer detection in biopsy specimens. Taken together, these developments highlight the transformative role of AI in advancing precision oncology, in which context pathology assumes a pivotal role. Manual interpretation of medical images remains susceptible to human error and interobserver variability. In this context, AI-based methodologies, particularly those leveraging CNNs, offer robust solutions to improve diagnostic consistency and uncover patterns beyond human perception. These innovations pave the way for more refined, data-driven approaches to cancer detection, classification, and treatment selection, ultimately supporting the realization of a truly personalized oncology. This constellation of technological advancements fosters a more data-driven, patient-centered approach to precision oncology. It creates a new medical universe that aligns with tailored cancer care's ethical and scientific mission. Pathology plays a pivotal role in this evolving "computational" landscape, echoing the transformative impact once initiated by Virchow's microscope.

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GF, MR and MS collectively conceived and designed this comprehensive review. All authors contributed to the initial draft of the manuscript. GF, MR, MS, LS, MC, PZ, AP provided supervision, graphics support, editing, and finalized the manuscript. All authors actively participated in the revision of the manuscript, carefully reviewed it, and approved the final version for submission.

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Publications ethics

Plagiarism

The article provides a comprehensive review of the latest studies in the field, with accurate citations.

Data falsification and fabrication

The writing and contents of the article are entirely original and were developed entirely by the authors.

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SHORT REPORT

LIQUID BIOPSY AND DIAGNOSTIC OF IMAGING (LIDIA) FOR THE DEFINITION OF PROGNOSTIC BIOMARKERS AND PERSONALISED THERAPIES IN LUNG CANCER: A CLINICAL TRIAL

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ABSTRACT: in recent years, imaging techniques have been successfully used to deliver diagnostic biomarkers with even greater accuracy. In particular, radiomic analysis methods (application of artificial intelligence on radiological images), which describe a segmented tumor region, using various quantitative characteristics derived from radiological images, have shown great potential in the identification, characterisation/classification of different types of cancer and in evaluating the response to radiotherapy and chemotherapy. Liquid biopsy is used for both early screening of malignancy and diagnosing minimal residual disease. It is also performed to assess and monitor the response to pharmacological treatments for a personalised therapeutic strategy. The analysis of morphostructural data obtained by imaging, correlated with the genetic/molecular results of liquid biopsy, could provide useful predictive factors for early diagnosis and predicting the response to anti-cancer drugs. The study aims to design and develop a report structured in CT with contrast media, which includes, in addition to the subjective evaluation of the radiologist, a quantitative/objective assessment of lung cancer (LC) with features that describe the texture and morphology of the lesion. Therefore, we present a workflow aimed at extracting the DICOM images acquired with CT using contrast medium from a significant number of patients, and to evaluate their accuracy in characterising the LC lesions. Furthermore, these data will be correlated to gene mutations and epigenetic changes (DNA methylation) evaluated in circulating tumour DNA derived from peripheral blood with a liquid biopsy approach. The correlation between radiomic characteristics, quantitative analysis of tumours performed by CT, structured lesion reports, and liquid biopsies could help avoid many unnecessary biopsy procedures and enable personalised treatment of LC patients.

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Impact statement: An integrated radiomics–liquid biopsy model is proposed to support non-invasive molecular assessment and guide personalised therapy in patients with stage III lung cancer.

Key words: *radiomic; liquid biopsy; texture analysis; stage III lung cancer; biomarkers.*

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BACKGROUND

Lung cancer (LC) represents a leading cause of death worldwide, contributing to a high percentage of cancer-related deaths in both sexes (1). However, over the last decade, novel targeted therapies and immunotherapies have been developed due to the discovery of different mutations and aberrations in driver oncogenes (2, 3). Based on this, molecular testing and clinical biomarkers are now routinely used in clinical practice for the management of advanced LC, including the search for activating mutations

of epidermal growth factor receptor (EGFR), BRAF, HER2, MET, ERBB2 and KRAS, rearrangements of anaplastic lymphoma kinase (*ALK*) and ROS proto-oncogene1 (*ROS1*) as well as fusions of *NTRK1-3* genes (4, 5). Unfortunately, a large proportion of patients have an advanced disease, especially stage III, at diagnosis, thus excluding tumor resection opportunities (6, 7). In these cases, the available tumour tissue that can be used for molecular testing is often limited, being represented by small needle core biopsies or cytology specimens due to the risks associated with biopsy procedures involving the lung. However, the collection of sufficient material for diagnosis, subtyping, and characterisation is mandatory, and when not available, repeating the diagnostic procedures can lead to delayed decision-making in creating an algorithm. This can lead to detrimental effects on the clinical outcome of the patients, especially in stage I, II LCs in which the molecular diagnosis is essential for treatment decisions. In fact, in stage III LC, concurrent chemoradiotherapy (CRT) associated with the immunotherapy represents the preferred treatment, being proven to increase the overall survival of the patients if compared to radiotherapy alone. The rapidity of the best therapy choice is essential in these cases, considering that the optimal and personalised treatment of stage III LC patients is critical for achieving disease downstaging, which allows for subsequent surgical resection. The diagnostic delay can lead to a loss of the therapeutic window and subsequent opportunities for neoadjuvant treatment.Therefore, the availability of a diagnostic technique that enables a rapid definition of the diagnostic workflow is essential for informed treatment decision-making. Methodologies based upon liquid biopsy fulfil this role by allowing a wide range of molecular assessments through a minimally invasive procedure (8, 9). Different body fluids can be used for liquid biopsy, including saliva, cerebrospinal fluid, and, more often, peripheral blood. The latter is collected to obtain intact circulating tumour cells or their products, including circulating cell-free DNA (cfDNA), circulating tumour DNA (ctDNA), circulating miRNA, exosomes, extracellular vesicles, and others (10, 11). These products could subsequently be used in diagnosis, prediction of response, monitoring of treatment, and assessment of mutational status before and during the various treatments to which patients are subjected. Therefore, liquid biopsy is highly attractive for assessing both the tumour biology and molecular status of LC, both at single or multiple time points (*e.g.*, at diagnosis or relapse). For instance, it is established that LC demonstrates genomic instability with the progressive acquisition of genetic alterations (including point mutations, chromosomal instability and epigenetic alterations), although at varying rates, resulting in the development of genetic changes during the clinical evolution of the disease, as well as due to the effects of the different treatments. In stage III LC, these alterations can contribute to clonal evolution and resistance development, emphasizing the need for the

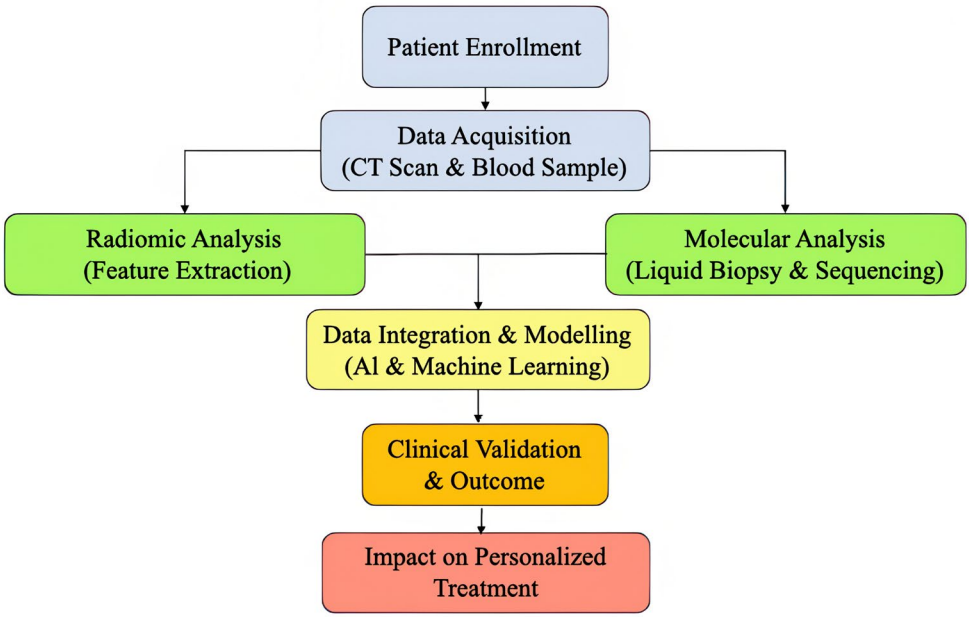

Figure 1. *Workflow of the LIDIA project, from patient enrollment to data analysis, AI modelling, clinical validation, and personalized treatment.*

continuous tracking of LC molecular profile during treatment. The selection of resistant clones during therapy represents a significant mechanism for the development of treatment resistance and disease progression. Moreover, given the increasing role of targeted therapy, the monitoring of the molecular profile of the disease is of paramount importance to identify resistance mechanisms. Therefore, all testing strategies that offer a safe modality for assessing LC biology are likely to be of significant clinical interest. An example is provided by the observation that approximately 15% of LC, particularly advanced non-small cell lung cancers (NSCLCs), display activating EGFR mutations, which can be targeted by tyrosine kinase inhibitors (TKIs). Liquid biopsy can be used not only to determine the presence of activating EGFR mutations in treatment-naïve patients for whom the tissue sample is insufficient or inadequate for molecular analysis, but also in patients who, after disease progression to first- or second-generation TKIs, develop resistance mechanisms.

The identification of EGFR mutations is also crucial for the management of early-stage NSCLC patients who may be candidates for adjuvant therapy with the latest-generation TKI, Osimertinib. Moreover, the potential incorporation of liquid biopsy techniques into screening algorithms, both for routine population screening and for therapy monitoring, represents an extremely attractive approach and an area of active investigation with promising early results. Liquid biopsy has definite and clinically relevant applications for the management of LC, particularly in stage III and other advanced stages, as well as in early-stage disease; however, its use is limited by cost, technical challenges, and availability. Therefore, although it is highly predictable that liquid biopsy will play a significant role in diagnosis, response assessment, and ongoing surveillance in the future, the available data are still inconclusive. Liquid biopsy techniques offer an excellent combination of convenience and safety for molecular profiling, reducing the need for invasive and technically complicated tissue sampling. This information can be combined with data from imaging of tumour lesions to improve the diagnostic definition of the disease, allowing for molecular subtyping and predicting response to therapies.

Another critical challenge of the present study is determining the epigenetic alteration of cfDNA based on its methylation profile. Epigenetic modifications are considered a hallmark of cancer and are found in early stages of disease, tumour progression, and metastasis formation. DNA methylation is a tissue- and cancer-specific modification and, in contrast to the heterogeneity of gene mutations, appears to be similar in cancer cells of the same type and tissue origin (12, 13). Genome-wide methylation analysis using the bisulfite conversion method of cfDNA has been previously employed for cancer diagnosis (14). However, this method is expensive, time-consuming, and requires large amounts ofcfDNA. An innovative and highly sensitive alternative is offered by using cell-free methylated DNA immunoprecipitation with anti-5mC antibodies and subsequent high-throughput sequencing (cfMeDIP-seq) (15) to assess the methylation profile, even with low cfDNA input. Differentially methylated regions (DMRs) have been used to construct classifiers that can identify patients with several cancers (15, 16). Therefore, one of the objectives of the present study will be to use cfMeDip for the early diagnosis, determination of minimal residue disease, and histological subtyping of patients with LC, and to correlate these results with radiological imaging.

Based on these advances, this study aims to evaluate the diagnostic accuracy of chest CT in the morpho-structural characterisation of stage III LC. By extracting radiomic capabilities related to the structure and morphology of the lesions, the observation aims to correlate this information with the results of genetic, epigenetic, and molecular analyses obtained through liquid biopsy.

METHODS/DESIGN

The concept of a single-site biopsy to monitor disease dynamics during therapy is practically unfeasible, as it is invasive and may result in an underestimation of heterogeneity. On the other hand, a liquid biopsy based on the analysis of circulating tumour cells or tumour macromolecular products reflects the mutational status of the overall disease sites, allowing for the identification of emerging subclones responsible for treatment resistance. Additionally, radiomics has emerged as a novel field of research dealing with the extraction and analysis of specific features from diagnostic images, potentially reflecting the pathophysiological processes and the heterogeneity of tumour genetics.

The combined approach of radiomics and liquid biopsy has the potential to elucidate the dynamics of molecular lesions, thereby supporting informed clinical decision-making (17, 18).

Table 1. *Overview of Study Phases and Methodologies.*

PHASE	DESCRIPTION	TECHNIQUES / TOOLS
Data Collection	Collection of clinical data, imaging, and biopsy samples	Clinical Records, CT Imaging, Liquid Biopsy
Radiomic Analysis	Extraction of radiomic features from imaging data	Pyradiomics, ITK-SNAP
Genetic Analysis	Study of genetic mutations through liquid biopsy	PCR, Sequencing
Prediction of Outcomes	Combination of data to predict therapeutic outcomes	Machine Learning Models, AUC

Aims and objectives

The project aims to create a structured report in CT with contrast media, which includes, in addition to the subjective evaluation of the radiologist, a quantitative/objective assessment of the lung tumour with several features that describe the texture and morphology of the lesion (19, 20).

Therefore, as shown in **Figure 1**, the project aims to extract the DICOM images acquired with CT with contrast medium, from a significant number of patients, with a particular focus on stage III LC, for the subsequent evaluation of their accuracy in the characterisation of LC malignancy. Furthermore, in the same patients, the molecular analysis of the genes involved in LC will be performed on DNA extracted from a peripheral blood sample (21, 22).

The correlation between radiomic characteristics, quantitative analysis of tumours performed by CT, structured lesion reports, and liquid biopsies could help avoid many unnecessary biopsy procedures (23, 24).

Study design

This section outlines the techniques and protocols of our experimental study, aimed at integrating scientific, molecular, and imaging records to assess the diagnostic accuracy of CT scans and liquid biopsy in LC management, with a selected emphasis on stage III cases.

Inclusion criteria:

- Age ≥18 years
- Full understanding of the study and signed informed consent
- Presence of a neoplasm requiring further diagnostic evaluation
- Availability to undergo liquid biopsy.

Exclusion criteria:

- Allergies to contrast media
- Inability to maintain immobility during the exam
- Pregnancy or breastfeeding
- Risk factors for contrast nephropathy (GFR <60 ml/dl)
- Known allergy to contrast agent.

Recruitment Process

In the first year, from the first bimonthly period to the sixth, the two Diagnostic Imaging Units will be responsible for enrolling hospitalised patients who undergo CT-guided biopsy for suspected lung cancer. The CT investigation will be performed before histopathological sampling, to obtain information regarding morpho-densitometric characteristics of the lesion and to plan the subsequent biopsy procedure (25-28).

Prior to treatment administration and molecular pathology assessments, all patients provided written informed consent. The study was approved by the Ethics Committee "Comitato Etico Università degli Studi della Campania Luigi Vanvitelli" (approval No. 24997/2020) on 11th November 2020.

The recruitment and collection phase of clinical anamnestic data will be performed in a specific DICOM file (structured report) and will start after Informed Consent has been signed by the patient. Informed Consent will be accurately prepared for this study by the PI and substitute PI.

Imaging Acquisition and Analysis

Once the CT imaging has been acquired, the DICOM images will be evaluated by the PI and the Deputy PI from the first to sixth bimonthly period of the first year. From the second to the sixth bimonthly period of the first year a quantitative analysis of the lung lesion will be performed with an artificial intelligence system capable of identifying the tumor on the CT image, calculating its diameters and volume in a semi-automatic way.

Subsequently, the radiologists assisted by the engineer of the second research unit, will export the CT images. This phase will take place from the third

to the sixth bimonthly period of the first year. The images exported by the individual research units will be archived using a 'GDPR compliant' Cloud system that will be developed ad hoc for the study.
The cloud platform comprises:

- Storage Section: Secure archiving of DICOM files, structured reports, and genetic data.
- Computing Section: An online application for structured reporting, integrating clinical and anamnestic information, ensuring standardized procedures and simplifying radiomics/radiogenomics analysis.

Following the data extraction, during the period from the fifth to the fourth two-month period of the first year to the second year, the Department of Electrical Engineering and Information Technologies at the University of Federico II will carry out the computational analysis of the tumour volume to extract the radiomics features. Subsequently, from the sixth bimonthly period of the first year to the fourth bimonthly period of the second year, the same Department will carry out the classification of radiomic features with Machine Learning techniques. Finally, from the sixth bimonthly period of the first year to the third bimonthly period of the second year, these data will be processed and analysed to predict tumour characteristics.
Peripheral venous blood samples can be gathered throughout imaging acquisition to evaluate liquid biopsy molecular data. Plasma samples will be stored in two laboratories to maintain ctDNA integrity:

- Molecular and Precision Oncology Laboratory (Vanvitelli University and Biogem scarl)
- Cytology and Predictive Molecular Pathology Laboratory (Federico II University).

Furthermore, from the fifth two-month period of the first year to the third two-month period of the second year, the extraction of the ctDNA and the preparation of the genetic library will be performed by using OncomineTM Lung ctDNA Assay (Thermofisher, Massachusetts, USA). Afterwards, from the first two-month period of the second year to the fourth two-month period of the second year, the sequencing by Next Generation Sequencing (NGS) technique on the Ion Torrent GeneStudio S5Plus system (Thermofisher, Massachusetts, USA) will be run. Regarding data analysis, NGS technology involves various processes, which are very expensive from the point of view of the computational resources used. Gene sequencing of cfDNA samples using the NGS technique will be analysed on ThermoFisher systems and software.
The analysis of the characteristic driver mutations of lung cancer, as included in the OncomineTM Lung ctDNA Assay, provides sequencing of 11 genes (*ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MAP2K1*, *MET*, *NRAS*, *PIK3CA*, *ROS1*, and *TP53*) and more than 150 hotspots. The analysis has a high specificity and sensitivity, along with an efficient workflow that enables the rapid generation of results. In addition to being inclusive of clinical-laboratory information, patient data will also contain information obtained from genetic analysis and will always be archived within the same cloud platform created *ad hoc* by the Department of Biomedical Engineering of Federico II.
Subsequently, from the third two-month period of the first year to the sixth two-month period of the second year, the two Research Units will undertake to correlate the data obtained from radiomic analysis with the data obtained from genetic analysis.
Finally, from the sixth two-month period of the first year to the sixth two-month period of the second year, the Engineering department of the second Unit will carry out the Radiomic analysis of the segmented volume using Imaging with the aim of obtaining a number of significant features that can be correlated with the genetic data of the liquid biopsy.

Techniques for the Analysis of Liquid Biopsy

A liquid biopsy will be performed only for patients who have previously undergone a CT study for diagnosis and staging. All patients enrolled in the study will undergo a peripheral venous blood sample collection in two test K2 tubes.
The ctDNA will be extracted from the plasma for molecular analysis, which will be performed using NGS technology, based on Ion Torrent technology. Unlike other fluorescence-based platforms, Ion Torrent uses an electrochemical approach to detect nucleotides, eliminating the need for optical labels and thereby increasing sequencing speed and accessibility. After genomic library preparation, DNA molecules are fragmented and ligated to oligonucleotide adapters, allowing immobilization onto specific beads. Each bead is then placed into an oil droplet containing emulsion PCR (emPCR) reagents, ensuring that each bead carries a single amplified DNA molecule. After amplification, the beads are loaded into a semiconductor chip, with each well containing a single bead with multiple copies of the same DNA fragment. Sequencing occurs through the sequential introduction of nucleotides. When a

complementary nucleotide is incorporated by DNA polymerase, a proton (H+) is released, causing a pH shift. This change is detected by chip sensors, which convert the electrochemical signal into digital data.

Routine Sample Processing Strategy

Circulating free nucleic acids are purified from 1 mL of clarified plasma. In particular, cfDNA is isolated by using the Qiamp Circulating Nucleic Acid Kit (Qiagen) and eluted with 50µL of Nuclease-free Water, following manufacturer instructions. The extracted cfDNA is stored at -20°C. The concentration of cfDNA is evaluated using a Qubit 4 fluorometer (ThermoFisher) with the Qubit 1X dsDNA High Sensitivity (HS) kit.

Extracted cfDNA samples are tested on Genexus (Thermo Fisher Scientific) system. The platform enables entire NGS workflows (from library preparation to data interpretation) within 24 hours. The OPA assay includes the most clinically relevant actionable genes for solid tumour patients. Firstly, samples are created on a dedicated server and assigned to a new run. Then, the Genexus platform is loaded with OPA primers, strip solutions, strip reagents, and supplies according to manufacturer instructions. A total of 10ng is required by the OPA assay on the Genexus platform. Accordingly, each sample is dispensed on a 96-well plate, following manufacturer instructions. Finally, nucleic acids are sequenced on a GX5™ chip that allows for the simultaneous processing of n = 4 samples in a single line with an OPA assay, for a maximum of 4 lanes (16 samples) in a row. Data analysis is performed using proprietary IonTorrent Genexus software (6.8.2.0). Particularly, detected alterations are annotated by adopting Oncomine Knowledgebase Reporter Software (Oncomine Reporter 5.0).

In addition, BAM files are also visually inspected with the Golden Helix Genome Browser v.2.0.7 (Bozeman, MT, USA) in hotspot regions in *EGFR*, *KRAS*, and *BRAF* lung cancer-addicted molecular alterations.

cfMeDIP-seq

cfMeDIP-seq is conducted following previously published protocols. In short, cfDNA libraries are generated using the Kapa Hyper Prep Kit (Roche) according to the manufacturer's guidelines. After performing end-repair and A-tailing, adaptors from the NEBNext Multiplex Oligos for Illumina (NEB) are ligated to the samples, followed by purification using AMPure XP beads.

To achieve a final quantity of 100 ng, Lambda DNA—comprising both methylated and unmethylated amplicons with varying CpG content—is added to the libraries. 0.3 ng of methylated and unmethylated *Arabidopsis thaliana* DNA is added for quality control purposes (Diagenode). One small part of the library is kept aside for input control (IC), and the remaining part was used for immunoprecipitation (IP).

MeDIP is carried out with the MagMeDIP Kit (Diagenode) and Antibody anti5mC* (33D3 clone) as per the manufacturer's protocol. The efficiency of the immunoprecipitation is verified via qPCR by detecting the recovery of the spiked-in Arabidopsis thaliana DNA (both methylated and unmethylated), following Diagenode's instructions. All samples with a specificity of reaction are sequenced at the resolution with a mean of 54.7 million reads per sample, resulting in ~5.1X depth per sample.

Processing of cfMeDIP-seq data

The quality of raw reads is evaluated using FastQC version 0.11.9 and MultiQC version 1.11. Low-quality reads and adaptors are removed with Trim Galore version 0.6.6. The trimmed reads are aligned to hg38 with Bowtie2 version 2.3.4.3. SAMTools version 1.9 is used to convert the SAM alignment files to BAM files, sort and index reads, and remove duplicates. Samples with <10M mapped reads are excluded. Tumour fraction is estimated using IchorCNA on the low-pass WGS of IC samples.

Processing of cfMeDIP-seq data

The quality of raw reads is evaluated using FastQC version 0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc) and MultiQC version 1.11 (29). Then, low-quality reads and adaptors are removed with Trim Galore version 0.6.6 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore). The trimmed reads are aligned to hg38 with Bowtie2 version 2.3.4.3 (30). SAMTools version 1.9 (31) is used to convert the SAM alignment files to BAM files, sort and index reads, and remove duplicates. Samples with <10 M mapped reads are excluded. Tumour fraction is estimated using IchorCNA (20) on the low-pass WGS of IC samples.

Identification and annotation of differentially methylated regions (DMRs)

The filtered BAM files are processed using MEDIPS (32) to identify the Differentially Methylated Regions (DMRs) between LC patients with different hystotypes and stages. The enrichment scores relH and GoGe are estimated for each sample to express the grade of CpG enrichment in the DNA fragments compared

to the reference genome. The enrichment score relH is the ratio between the relative frequency of CpGs within the regions and the reference genome. The enrichment score GoGe is the observed/expected ratio of CpGs within the regions and the reference genome. Samples with a relH value less than 2.7 and/or a GoGe value less than 1.75 are excluded. Then, the genome of each sample is binned into 300-bp windows, and the methylation status of each bin is compared between the two groups. Regions with an absolute value of log2 fold change (FC) greater than or equal to 2 and a p-value less than 0.01 are selected as differentially methylated. The identified DMRs are annotated with the annotatr (33) R package. Gene set enrichment with DAVID and gene ontologies with a p-value less than 0.05 is selected.

Base Calling

In base calling, nucleotide sequences are "extracted" from the image data generated by sequencing platforms. Base-calling algorithms convert image information into sequence data. The process also corrects for artefacts such as crosstalk and phase errors. Crosstalk occurs due to overlapping fluorescence emissions of different nucleotides, while phasing is caused by signal dispersion and diffusion between cycles. Each base is assigned a quality score, called the "Phred quality score" (Q), which indicates the accuracy of base identification.

Alignment

Short DNA reads (200–8000 bp) are sequenced from either one or both ends of DNA fragments (single-end or paired-end reads), with typical lengths around 400 bp on platforms like 454. Alignment aims to locate these reads on a reference sequence, but challenges arise in regions that diverge significantly from the reference. Using longer or paired-end reads, which sequence DNA in both 5'-3' and 3'-5' directions, can improve alignment accuracy. A critical factor for successful assembly is coverage, defined as the number of times a sequence aligns with the reference, ensuring reliability and completeness in the reconstructed sequence.

Calibration of Quality Scores

Phred quality scores derived from alignment algorithms do not always accurately reflect real errors in base calling. Therefore, recalibration is performed, considering factors such as raw quality scores, the relative position of the base within the read, and the dinucleotide context.

Clinical Applications

The clinical applications of liquid biopsy depend on the approach used to study circulating tumour cells or ctDNA. A quantitative approach provides prognostic information, while a qualitative approach enables the analysis of predictive mutations, monitoring of clonal evolution, and adjustment of therapeutic strategies. ctDNA, released by apoptotic or necrotic tumour cells, provides DNA information from both primary lesions and metastases.

Imaging Techniques

CT will be performed using multidetector equipment (GE Revolution GSI 128 MDTC).
Clinical and radiological data will be collected to correlate with molecular and genomic data.

Radiologists' Responsibilities

Radiologists will be required to:

- Collect clinical information using a structured report (see sheet).
- Obtain informed consent from patients.

Extraction of Quantitative Features for Radiomics

Textural Features

Plot features will be obtained from manually segmented ROIs on CT images. They will include first-order features (mean, mode, median, standard deviation (std), median absolute deviation (MAD), range, kurtosis, skewness, and interquartile range (IQR) and second-order characteristics. For the latter, bandpass, wavelet, isotropic resampling, discretisation length corrections and different quantisation tools will be implemented. The first three sets are based on the grey-level co-occurrence matrix (GLCM), the grey-level run-length matrix (RLM), and the size zone matrix (SZM), all of which belong to the family of statistical matrices. Once these matrices have been constructed, it is possible to derive texture features (such as Haralick features and moments).
To improve robustness, advanced techniques like bandpass filtering, wavelet transformations, isotropic resampling, and quantisation corrections will be applied. Multi-grey-level SZM variants will also be utilised to compute texture features across various quantisation levels, combining the results using weighted averages to enhance sensitivity to subtle texture variations.

The formula for calculating the multi-gray-level SZM is as follows:

$$MSZM_f(s,g) = \sum_{k=1}^{8} w_k SZM_f^{N_k}(s,g)$$

The integration of these features enables detailed and multi-scale texture characterisation, optimising the ability to differentiate and classify lung lesions in CT imaging, even across diverse morphological and pathological presentations.

Morphological Features

A set of morphological features will be considered, including mean radial length, radial length entropy, irregularity, diameter, circularity, compactness, smoothness, roughness, rectangularity, convexity, eccentricity, and eulogy.

Classification Methods

Classification involves assigning an individual (such as a lesion or patient) to a specific class based on extracted features. This is done using a feature vector $x = [x(1),x(2), \ldots ,x(N)]$, where the classifier assigns the individual to one of K possible classes.

The process includes several steps:

- Choosing the Classification Criterion:
  This decision (linear or nonlinear) depends on the problem and the available data.
- Training:
  The classifier is trained on a data subset, typically using supervised learning with cross-validation techniques.
- Validation:
  The classifier's performance is tested on a separate dataset to evaluate its generalisation ability.

The performance of a classifier depends on the combination of features, algorithms, and training methods used. In recent years, deep learning techniques have gained popularity for their ability to identify critical features from large datasets automatically. eeThe following sections examine some of the most popular classification techniques and methods. In this study, all currently available techniques will be applied with the aim of finding the best combination in terms of classification performance.

Classifier Types

Classification techniques can be essentially divided into linear and nonlinear. Linear techniques adopt a linear combination (sum) of features to try to classify the individual. Such techniques (*e.g.*, Linear Discriminant Analysis, LDA) are helpful when features are chosen such that the problem is linearly separable. More often, the problem is not linearly separable, and therefore nonlinear techniques (such as neural networks, k-nearest-neighbours, and support vector machines) are more useful. Trees are a special type of nonlinear classifier that is based on successive dichotomous processes. At each step, the algorithm creates a binary separation, and each leaf is further divided into two at the next step. This type of algorithm is generally chosen for its 'human' comprehensibility. Dichotomies are binary decisions of the yes/no type on individual features, and thus their interpretation is transparent. In contrast, classification rules generated by linear or nonlinear algorithms are generally not understandable.

Cross-Validation

Cross-validation is an essential aspect of classifier training and aims to reduce possible overfitting, i.e., the tendency of training to select parameters that make the classifier very good at classifying individuals used as a training set, while the ability to generalise, i.e., classify individuals not belonging to the training set, is limited. This issue is related to the fact that, often, as in the present case, it is not possible to examine a significant sub-population that is representative of the entire population (all possible breast cancers, in this case). Therefore, it is necessary for the classifier to be able to have reasonable performance on the entire population.

Evaluation Metrics

Performance metrics for classifiers will include standard measures like True Positives, False Positives, ROC curves, and confusion matrices. For binary classifiers, the confusion matrix provides insight into misclassifications; for multi-class classifiers, more complex metrics are used.

Implementation in the Present Protocol

All pre-processing, DICOM image handling, and feature extraction will be conducted using Matlab (The MathWorks Inc., Natick, MA) or R (R Core Team, 2018). Matlab is widely used for scientific data processing and classification. At the same time, R is an open-source statistical analysis tool that has grown to support advanced techniques in machine learning and radiomics.

Statistical Analysis

The characteristics of the study population and other relevant variables will be described using the appro-

priate descriptive statistics for both continuous and categorical data.
Data will be presented with absolute frequencies and percentages, reporting the respective confidence limits. The mean and standard deviation will be reported for discrete parameters, following a Gaussian curve. Medians and interquartile ranges will be reported in cases where parameters are not distributed according to a Gaussian curve. Parametric and nonparametric tests for paired and unpaired data will be used, regardless of whether the data distribution is Gaussian or non-Gaussian, to detect statistically significant differences between groups. For continuous variables, the difference between median values for different groups will be calculated and tested using a two-sided Student t-test (if the differences are normally distributed) or the Mann-Whitney test (if the differences are generally not distributed. Assessment of inter-observer variability will be performed by calculating Cohen's Kappa index. Mixed-effects regression models will adjust for covariates in longitudinal data. Multivariate analysis, including linear classifiers, support vector machines, and decision trees, will explore feature combinations to optimise classification accuracy of lung lesions.
A p-value <0.05 will indicate statistical significance, with Bonferroni correction for multiple comparisons. Analyses will use Matlab Statistics Toolbox and R.

DISCUSSION

Precision medicine enables the targeted treatment of LC, including stage III, by applying multimodal omic strategies tailored to individual groups based on their genetics (34, 36).
Radiogenomics aims to correlate imaging phenotypes with gene and epigenetic modifications. Radiomics has recently emerged as a promising tool for discovering new imaging biomarkers. It can be applied to any field of diagnostic imaging and is used in various clinical settings. Radiogenomics is a specialised evolution of oncology radiomics that utilises imaging capabilities to non-invasively identify or predict tumour-specific genomic alterations (37-38).
The biopsy of the suspected cancer is today the gold standard for the characterisation of LC. However, it is expensive, invasive and evaluates only the sampled section of a heterogeneous tumour. The applicative and ambitious goal of the present study is to develop a new protocol and mathematical algorithm based on the imaging of the entire tumour or of a multifocal tumor load in a single patient, with the possibility of providing a non-invasive diagnosis correlating also the data derived from liquid biopsy on the gene mutations and epigenetic changes of the tumour.
Currently, there is no universal image acquisition protocol and no structured reporting standards (39) The method and application of the structured report could be adopted as a reporting method not only in LC but more generally in all cancers. The algorithm derived from the present study should be validated by scientific agencies and societies to transfer the obtained diagnostic procedures into the clinical setting and real-world practice.
Suppose the goals of the present project are successful. In that case, they will result in a significant reduction of health system expenses, allowing for highly personalised LC treatment and enabling early-stage diagnosis, thereby avoiding unnecessary treatments.
All this would bring enormous benefits to patients in terms of quality of life and social and productive contribution.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflicts of interests

The authors declare no competing interests.

Data availability

Data collection and analysis are ongoing; data will be made available from the corresponding author upon reasonable request after study completion.

Author's contributions

All authors contributed equally to this work, read and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ethics Committee "Comitato Etico Università degli Studi della Campania Luigi Vanvitelli" (approval No. 24997/2020) on 11th November 2020.

Publication ethics

The study was conducted in accordance with publication ethics standards.

Plagiarism

The authors declare that this manuscript is an original work and has not been published elsewhere.

Data falsification and fabrication

The authors declare that no data were fabricated or manipulated in this study.

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OPINION PAPER

NEW CLINICAL NEEDS IN MELANOMA STAGING: IS THERE STILL ROOM FOR SINGLE LYMPH NODE EXCISION?

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ABSTRACT: the presence of metastatic cells in the first draining lymph node is crucial for staging melanoma, traditionally treated by the removal of the regional nodal basin until few years ago. The results of some prospective studies of surgical strategy and the introduction of immunotherapy and targeted therapy has significantly changed clinical practice, reshaping the role of lymph node dissection. Single Lymph Node Biopsy (SLNB) is now used for accurate staging with less invasive surgery, aiding in identifying patients who may benefit from adjuvant therapy. The aim of this review is to enlighten the needs perceived during everyday clinical practice. Prognostication in melanoma is still a challenge, with serum lactate dehydrogenase (LDH) as the only biomarker. Elevated LDH levels correlate with worse outcomes in advanced melanoma.SLNB time and curative role are debated, with studies suggesting that the timing of SLNB may influence outcomes and that SLNB has limitations in predicting mortality, especially in different age groups. The use of precision medicine tools like circulating tumour DNA (ctDNA) tests and the emerging role of neoadjuvant immune checkpoint inhibitors (ICI) are improving outcomes.
While SLNB still remains fundamental, further research is needed to identify which patients' subgroups benefit the most from it.

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IMPACT STATEMENT: This article challenges conventional melanoma staging by reintroducing single lymph node excision as a selective tool in modern practice. It proposes refined clinical criteria for its use, aiming to guide oncologists toward more personalized and pragmatic staging decisions in the era of precision oncology.

Key words: *melanoma; SLNB; surgery; biomarkers; clinical needs.*

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INTRODUCTION

The presence of metastatic dissemination in the first draining lymph node of a melanoma is an essential element for correct staging according to international guidelines (1). For decades, the most common initial route for metastatic spread has been recognized as the lymphatic drainage of the primary lesion. Over the last few decades, studies on surgical strategy and the revolutionary therapeutic introductions of immunotherapy and targeted therapy have reshaped the role of lymph node dissection and transformed survival rates in both adjuvant and metastatic settings (1).
Results from the Multicenter Selective Lymphadenectomy Trial II (2) have clearly demonstrated that there is no survival advantage from complete lymph node dissection when compared to ultrasound surveillance of the locoregional district. The concept of the Single Lymph Node Biopsy (SLNB) was developed for

melanoma by D.L. Morton in the late 1980s, based on earlier lymphoscintigraphy studies.

The benefits of this procedure include more accurate staging of the regional node, combined with less invasive and morbid surgery. According to current guidelines, SLNB can help identify patients with at least pT1b melanoma who may benefit from adjuvant therapy. In histopathological procedures, SLNB positivity rates vary, with a reported false-negative rate as high as 10% (3).

Following the excellent results from the Checkmate 238, Keynote-054, and COMBI-AD trials in 2018, adjuvant treatment has become standard clinical practice for patients with stage III melanoma (4, 5). Furthermore, Pembrolizumab has demonstrated significant improvements in both Relapse-Free Survival (RFS) and Distant Metastasis-Free Survival (DMFS) in pivotal adjuvant trials for stage IIB/C disease, making these stages eligible for adjuvant therapy (6). New therapeutic options are emerging following the excellent results from the phase 3 NADINA trial (7) and the randomized phase 2 SWOG S1801 trial. These trials are clearing a path for the implementation of neoadjuvant (Nivolumab + Ipilimumab) or perioperative (Pembrolizumab) regimens for stage III melanoma patients with clinical evidence of lymph node dissemination or satellitosis.

This review aims to highlight the needs perceived in everyday clinical practice.

CLINICAL PATHOLOGICAL FEATURES, BIOMARKERS, AND GENE EXPRESSION PROFILING

Do we have reliable biomarkers for melanoma prognostication? Currently, lactate dehydrogenase (LDH) is the only biomarker consistently associated with prognosis in melanoma (8). Several studies have suggested that a baseline elevation of serum LDH (sLDH) is associated with poorer treatment outcomes in patients with stage IV metastatic melanoma (24). In a study by Fischer *et al.* (9), molecular and immunological characteristics were not significantly associated with sLDH status. It is possible that sLDH is associated with worse outcomes primarily as a surrogate for tumour burden, as a strong correlation was found with the number of metastatic sites (9). However, some multivariate analyses have provided evidence that sLDH is associated with poorer outcomes independent of tumour burden (9).

Dutriaux *et al.* (10) found a similar correlation between higher levels of sLDH and decreased Progression-Free Survival (PFS) and Overall Survival (OS) in patients with advanced BRAFV600-mutant melanoma and brain metastases who were treated with targeted therapy. Additionally, sLDH levels may differ among patients with stage IV metastatic melanoma due to variations in the extent of organ damage and the influence of comorbidities (8, 9).

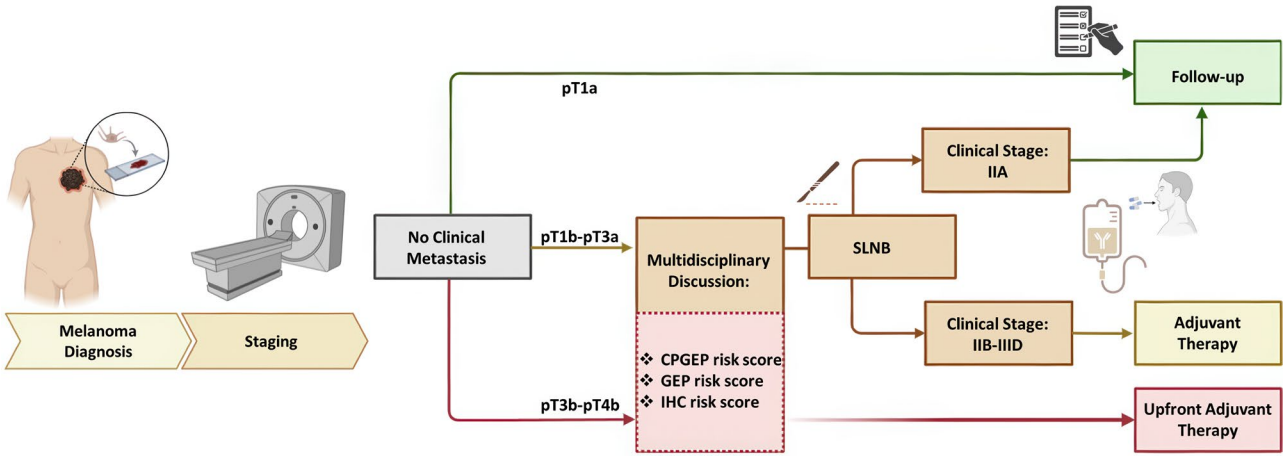

Figure 1. *Melanoma staging and treatment according to anatomopathological T stage features.*
The absence of clinical metastasis determines a pivotal juncture in the therapeutic management of the patients, to date pT1a patients are candidate to periodic follow-up, on the other hand pT1b-pT3a and pT3b-pT4b patients are multidisciplinary discussed to receive sentinel lymph node biopsy to define a clinical IIA, resulting in clinical follow-up, or IIB-IIID stage, leading to adjuvant therapy. In the recent future, it is hypothesized a different management for pT3b-pT4b patients based on the CPGEP, GEP and IHC risk scores (dashed square), which could allow to avoid SNLB and to an upfront adjuvant therapy. Immunohistochemistry (IHC), clinicopathological and gene expression profile (CPGEP) gene expression profile (GEP), sentinel lymph node biopsy (SNLB).

Some studies have also revealed that an Interferon-gamma (IFN-γ) signature can be useful in distinguishing patients at high risk of recurrence from those at low risk (11). Immunotherapies engage the immune system to target and eliminate cancer cells and can stabilize malignancies until immune escape mechanisms lead to progression. A long-term follow-up of the KEYNOTE-001 trial revealed that 12% of the 105 melanoma patients who were initially classified as having a complete response after anti-PD-1 treatment eventually had detectable disease in their serum, suggesting their tumours were held in a state of clinically undetectable equilibrium (11).

IFN-γ signalling is critical for the early response to checkpoint blockade, and inactivating IFN-γ sensing in tumour cells promotes resistance to immunotherapy (11). It is hypothesized that IFN-γ inhibits tumour growth and promotes CD8+ T cell-directed responses through improved antigen presentation. However, the long-term role of IFN-γ remains unknown because biopsies cannot be obtained when patients have clinically undetectable disease (11). Moreover, IFN-γ has negative feedback mechanisms that can, in some cases, promote tumour growth (11).

In a preclinical study, the viral expression of IL-12, a cytokine able to stimulate IFN-γ production and enhance the growth and cytotoxicity of natural killer (NK), CD8, and CD4 T cells, was found to "freeze" melanoma-bearing mice, with mice lasting over 120 days, neither clearing nor succumbing to their tumours (11). Consistent with the importance of IFN-γ in that model of equilibrium, transcriptomic data from The Cancer Genome Atlas (TCGA) were analysed, and a positive association was found between IL-12, IFN-γ -stimulated gene expression, and increased survival in melanoma patients. It was observed that, indeed, melanoma patients with higher expression of IFN-γ response genes fared better than patients with lower expression (11). In a study from Versluis *et al.* the role of IFN- γ signature was compared between an observation cohort and an adjuvant intention cohort. In both arms, better RFS were achieved in patients with high IFN-γ score (12). Another study from Long *et al.* (13) evaluated molecular and biochemical characteristics of patients who underwent adjuvant treatment with Nivolumab *vs* placebo in IIB/IIC stage melanoma, finding that better RFS was linked to higher IFN-γ signature, tumour mutational burden (TMB), and percentage of CD8+ T cells, and lower C reactive protein (CRP) levels. Despite what had been found in other cited studies, in this work, molecular biomarkers were not associated with RFS in patients who underwent a placebo treatment. In a study in which a biomarker-based signature was retrospectively analysed in patient treated with dabrafenib plus trametinib versus placebo in the COMBI-AD trial (14), a correlation between higher IFN-γ gene expression signature and prolonged RFS was found in both groups. Patients with low TMB had a substantial long-term RFS benefit from targeted therapy. Conversely, patients with high TMB seem to have a less pronounced benefit, especially if they had an IFN-γ signature lower than the median (14).

SURGICAL TIMING OF SLNB AND ITS CURATIVE ROLE

Given that there are no consensus guidelines on the optimal timing for performing SLNB in high-risk melanoma patients (**Figure 1**), a study involving 53,355 patients who underwent the procedure found that surgery was performed a median of 5-7 weeks after diagnosis (15). The study also revealed that for each week of delay, the probability of finding a positive node increased by 2.4%. Furthermore, patients with a higher Breslow depth index showed a significant increase in nodal positivity with increased time to surgery, although no significant trend was observed in T4 patients (15).

A study by Dixon *et al.* sought to evaluate the efficacy of SLNB in predicting mortality in melanoma patients at different ages, using data from the Tubingen University Database for patients who underwent SLNB between January 2000 and December 2014. The results showed that predicted SLNB-positive rates were significantly higher than mortality rates for 20-year-old patients, while the opposite was true for 80-year-old patients. This study highlights the limitations of SLNB in predicting mortality, suggesting it may lead to the overtreatment of younger patients and undertreatment of older patients (15).

In a multicentre international study by Moncrieff *et al.* (16), patients with pT1b-pT2a melanoma were analysed. This group has a reportedly low risk of a positive SLNB (10%), and even when a positive node is found, the 5-year survival rate for stage IIIA melanoma is 90% (16). The study, which included 3,610 patients with early primary cutaneous melanomas, found that only 11.4% had a positive SLNB, and the only clinical and histopathological characteristic associated with SLNB positivity was a mitotic rate greater than 1/mm^2. The authors concluded by sug-

gesting a re-evaluation of the indication for SLNB in early T-stage melanoma (16).

Another study from Kakish *et al.* tried to investigate the association of SLNB and survival in the elderly. What emerged from this retrospective study is that SLNB still adds prognostic information for elderly patients with melanoma and should not be eliminated in this population unless justified by poor performance status or patient preference. In the analysed cohort the decreasing in SLNB performance could correlate with a lack in the therapeutic offer for elderly melanoma patients (17). By quantifying the prognostic role of SLNB (18), Varey and colleagues found that the risk of regional node field relapse with SLNB plus adjuvant IO for T3b and T4 is around 9 *vs* 27% in all cases in which patients did not undergo surgery. Similarly, the node field recurrence rate with SLNB alone is around 14% compared to around 40% in patients in which both IO and surgery were not performed. Thus, in this setting of patients, SLNB should always be performed, improving the locoregional control of disease.

In Keynote 716 there was the possibility to undergo adjuvant therapy in stage IIB-IIC patients. This meant that even without nodal involvement, patients with melanomas characterized by a bad pathological T stage had the chance to lower the possibilities of recurrence (19).

This can lead to arguing the role of SLNB if all patients with a T stage between pT3b and pT4b, independently if with or without lymph nodal dissemination, will be recommended to undergo adjuvant treatment.

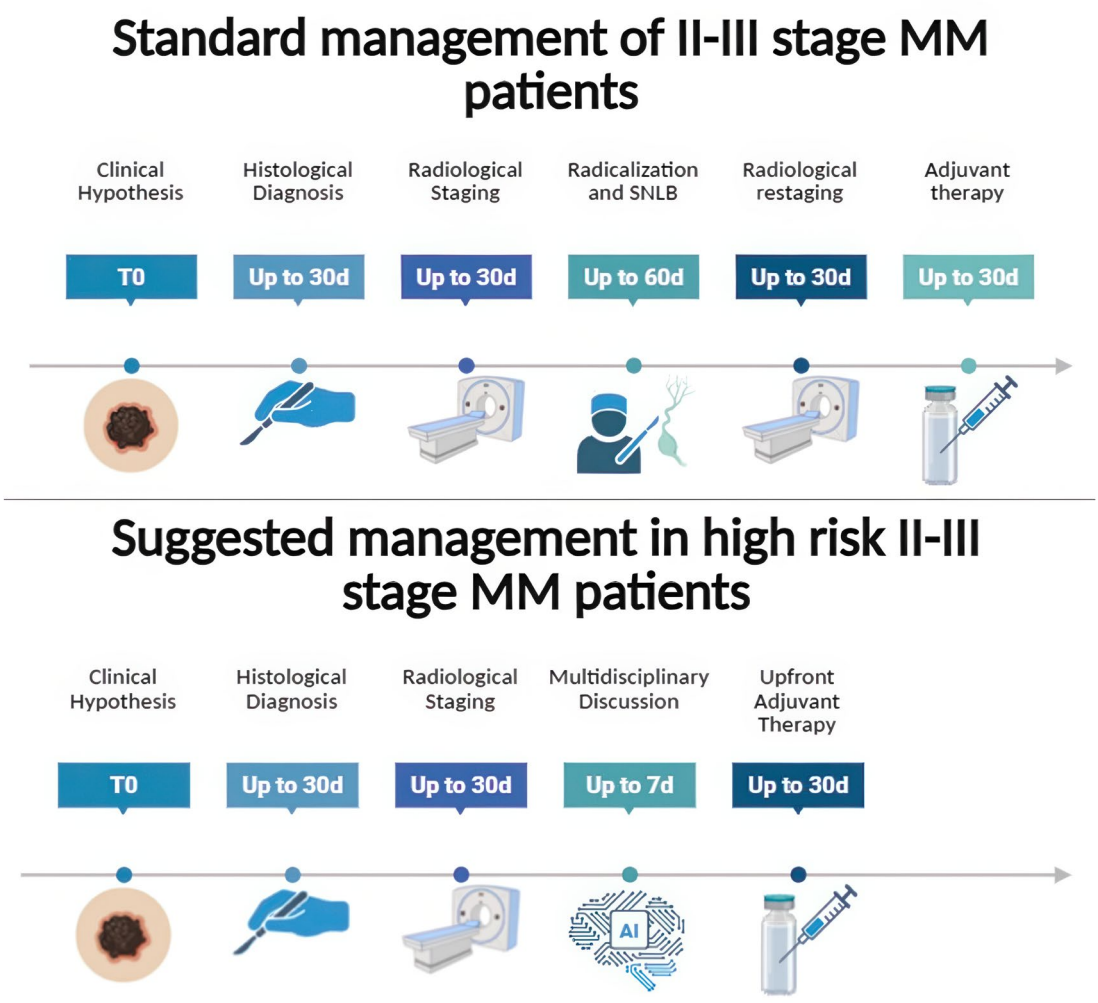

Figure 2. *Timeline of Events in clinical practice and in our proposed schedule.*
In high risk II-III stage MM patients, assessed via multidisciplinary discussion according to CP-GEP characteristics of primary excised lesion, we propose a different schedule of events compared to the Standard of Care. These patients should cut all the time and costs linked to radicalization, SLNB and radiological restaging, harbouring to an upfront adjuvant treatment.

Moreover, there is the necessity of underlining the role of lymphatic drainage pattern that can vary between patients, leading us to a possible false negative SLNB, as enlightened in a study from Cirocchi *et al.* (20), in which there was an important heterogeneity in the localization of the SLNB, in particular in the regions of posterior torso.

REAL-WORLD CLINICAL CHALLENGES

Therefore, the central question remains: to biopsy or not to biopsy? As the studies cited above demonstrate, the exact characteristics of the population that requires this locoregional treatment are not yet fully known. In the future, we will not blindly select all patients based on the characteristics mentioned in the guidelines. Instead, the focus should be on the characteristics appropriate for the individual patient, which will provide clearer information about the likelihood of locoregional or distant metastasis during active oncologic surveillance over 5 to 10 years.

The emerging role of precision medicine has led to studies investigating the use of personalized tests such as Signatera (21). This involves whole-exome sequencing of both tissue and peripheral blood to target patient-specific single nucleotide variants (SNVs), which can then be used to track circulating tumour DNA (ctDNA) in plasma (21). This tool shows promise in identifying high-risk primary melanoma patients under surveillance after resection to detect disease recurrence (21). Of course, other important data, such as the patient's working conditions, medical history, and clinicopathologic features like the Breslow index, must not be overlooked. All of these features are incorporated into predictive algorithms, such as the CP-GEP test Merlin or the GEP test MelaGenix, which will soon help us better identify the high-risk population for recurrence that should be selected for surgical intervention (21).

Another issue to consider is the integration of neoadjuvant or perioperative immune checkpoint inhibitors ICI treatments, as seen in the NADINA trial (7). Neoadjuvant ICIs have been shown to provide superior outcomes compared to approved adjuvant treatments, with a 2-year RFS of around 70-80% after two cycles of neoadjuvant Ipilimumab plus Nivolumab followed by surgery. In these trials, only patients who were non-responders or had a partial response received adjuvant treatment (7). When upfront systemic therapy leads to resectability, trials for advanced unresectable melanoma demonstrate better survival compared to ultimate systemic treatment (1). Therefore, ICIs for preoperative melanoma treatment have the potential to enhance patient outcomes and are likely to reshape the principles of treatment for both advanced and localized melanoma.

CONCLUSIONS

Currently, SLNB remains a crucial procedure for identifying individuals who can benefit from adjuvant therapy by providing precise staging with less invasive surgery. In this work, we have shed light on the clinical needs encountered in everyday practice. With LDH as the only established biomarker, melanoma prognosis remains difficult to assess. The curative role of SLNB must be re-evaluated. Even with potentially perfect timing, the inconsistency in predicting the usefulness of single lymph node excision is becoming evident, and it can also be seen as a hurdle between the patient and the start of adjuvant therapy. The application of precision medicine technologies, such as ctDNA assays, CP-GEP assessment, and the emerging role of neoadjuvant ICIs) is poised to redefine clinical node management.

What emerges from this work is the urgent need to find a new role for node sampling. Patients who would undergo adjuvant treatment with or without SLNB should be assessed with the aforementioned precision medicine tools in multidisciplinary discussions at high-volume centres, ensuring the best clinical practice for every single patient. In this way (**Figure 2**), we could reduce costs and time for national healthcare systems, avoiding surgical overtreatment for patients who would be treated regardless, and in other cases, avoiding unnecessary medications for patients with a low risk of recurrence for whom SLNB alone might be sufficient.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of interest

TT has been advisor for Amgen and Novartis, BMS for Roche, Servier, Pierre Fabre, M.S.D.

Availability of data and materials

The data supporting the findings of this study are available upon reasonable request to the corresponding author.

Authors' contributions

Conception and design: FC, TT. Administrative support: AC, MF. Provision of study materials or patients: AE, MCG. Collection and assembly of data: VDF, FC, AC. Data analysis and interpretation: VDF, FC, AC, TT. All authors have contributed to the writing and the final approval of the manuscript.

Ethical approval

N/A.

Human studies and subjects

N/A.

Animal studies

N/A.

Publications ethics

The publication ethics followed by this study align with those outlined by the International Committee of Medical Journal Editors (ICMJE), regarding publishing and editorial issues in medical journals.

Plagiarism

The article provides a comprehensive review of the latest studies in the field, with accurate citations.

Data falsification and fabrication

The writing and contents of the article are entirely original and were developed entirely by the authors.

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