Current and Future Considerations in Precision Medicine Oncology

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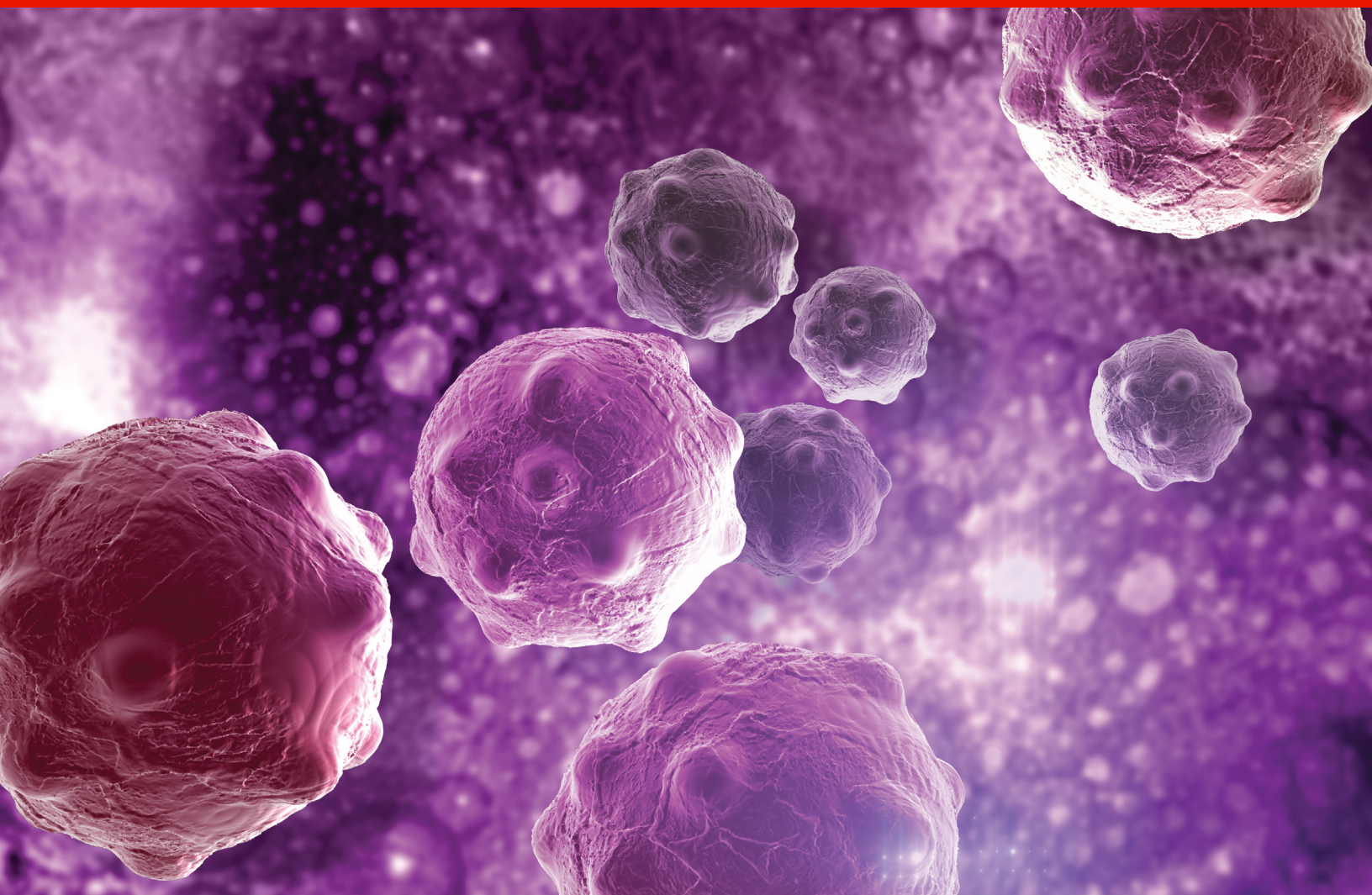


Current and Future Uses of Liquid Biopsies

Liquid biopsies – a less invasive way to identify biomarker profiles[1–3]

Liquid biopsy biomarker testing utilizes body fluids, typically blood,* to detect ctDNA, ctRNA, CTC, and exosomes for genomic testing[1–3]

Most available assays:

Requires a **blood sample** (typically 6–10 mL)[1,3]

Analyze **ctDNA** to assess genomic alterations[1]

Commonly utilize **NGS**, with **PCR** and **ddPCR** as alternative methods[1,3]

Clinical utility has been established and approved for predictive biomarker testing to inform some available targeted treatments[1–4]

Emerging uses for liquid biopsy-based assays include multi-cancer early detection (MCED), minimal residual disease (MRD) testing, and use of fluids other than blood[4,5]

*Urine, saliva, CSF, and other bodily fluids may also be used for diagnosis, disease monitoring, or assessing acquired resistance.[1,3]

Multiple factors may influence liquid biopsy assays and lead to false negative or false positive results[4]

Liquid biopsy test results may be impacted by

ctDNA concentration
- Refers to the amount of ctDNA relative to the amount of 'normal' DNA[4]
- Impacted by:
  - Tumor burden and vascularity[1]
  - Tumor shedding[3]
  - Specimen handling[6]

Variant allele frequency (VAF)
- Refers to the proportion of tumor cells that have the variant allele or genetic alteration[4]
- Impacted by:
  - Tumor heterogeneity[2]
  - Tumor evolution[7]
  - Tumor size/volume[7]

Limit of detection (LOD)
- Refers to the minimum concentration of an analyte that can be reliably detected[4,8]
- Impacted by:
  - Testing technology[8]
  - Assay design[4]
  - Specimen handling[6]

Clonal hematopoiesis of indeterminate potential (CHIP)
- Refers to the presence of somatic mutations that drive clonal expansion of hematopoietic stem cells, without evidence of hematologic malignancy[9]
- Impacted by:
  - Age[4]
  - Smoking[4]
  - Prior chemotherapy/ radiotherapy[4,10]

Linked with false negatives

- Samples with low tumor shedding, low ctDNA, and/or low VAF are more likely to have a false negative result[4]
- Assays with a high LOD are more likely to have false negatives[5,6]

Linked with false positives

- Advanced prostate, lung, and breast cancers are more likely to have false positives due to overlap between CHIP-related and solid tumor mutations (eg, *TP53, ATM, CHEK2*)[4,9]

Liquid biopsy can be considered[1]:

- As a complement to tissue testing to assess actionable alterations and monitor treatment response
- Where tissue biopsy cannot be performed or is inadequate
- When archival tissue is very old or damaged

Liquid biopsy has demonstrated utility to aid in treatment selection in patients with some advanced disease[1]

Sample processing

- LOD can be impacted by amount of nucleic acids, WBC lysis, and contamination by aerosolized nucleic acids[6]

Testing results

- Liquid biopsy may have more rapid overall TAT compared with tissue-based NGS[1,11]
- It may be associated with false negatives (up to 30%) or may identify variants of uncertain significance (VUS)[12,13]*

Treatment decisions

- Positive test result may direct treatment selection[2,3,15]
- Negative test result requires reflex testing of tissue sample[16]

Opportunity to optimize

Proper and timely sample processing may help ensure integrity of ctDNA anaylsis[6]

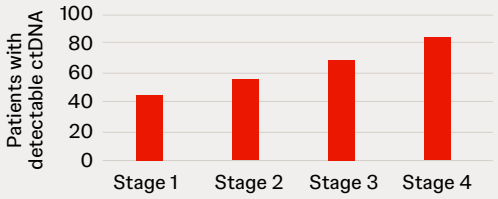
Samples with ctDNA tumor fraction (TF) ≥1% and a negative liquid biopsy are unlikely to have a driver mutation on tissue testing, which means they are true negatives[14]

Guidelines recommend using both tissue and liquid biopsies simultaneously or sequentially to aid in clinical decision-making[12,17]*

Liquid biopsy may be a valuable tool for early cancer detection across multiple tumor types[5]

Detection considerations: ctDNA levels are proportional to tumor burden[18]

- Larger tumors, fast growing malignancies and advanced or metastatic disease are more likely to have detectable levels of circulating markers[1,3,19,20]
- To identify patients with early-stage disease and/or a low tumor burden, more sensitive assays and/or new markers are needed[1,3,19]



Advances in **genomics** and **machine learning** have enabled the development of **new assays** that have **increased sensitivity** and/or **examine methylation patterns**, to help overcome this barrier[3,20]

Proof of concept:
The PATHFINDER study, a prospective multicenter trial, demonstrated the potential of multi-cancer early detection (MCED) testing in clinical practice by analyzing circulating free DNA (cfDNA) and methylation signatures[20]

Assay[20]:
- Uses methylation patterns in cfDNA to detect cancer

Performance[20]:
- Among positive cases, **38%** were confirmed to have cancer
- **98.6%** of the time was able to accurately identify no disease

Implications[20]:
- PATHFINDER provided **early evidence** of the feasibility of MCED assays
- MCED assay detected many cancer types for which screening **tests do not exist**
- Still a need for confirmative diagnostic assessments to **confirm a positive result**

MCED assays using liquid biopsy may facilitate the early detection of multiple cancers in patients with non-specific symptoms, but positive results should be confirmed with diagnostic testing[5,20]

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Evaluating MRD through ctDNA may be a valuable prognostic tool and inform treatment decisions[4]

In patients with solid tumors, **MRD** refers to the presence of residual cancer cells in early-stage disease following treatment with curative intent. ctDNA assays may be used for its detection[4,21]

Types of ctDNA assays for MRD detection[6,21]*

Tumor-informed assay

- Guided by patient-specific alterations previously identified via NGS of tumor tissue[6,21]
- Genomic profile from tumor tissue may not reflect all mutations[21]
- May also be referred to as bespoke assay[6]

Tumor-agnostic assay

- Uses common mutated genes and/or methylated vs unmethylated DNA that has been validated across various cancers[6]
- Preferred choice for efficiently detecting targeted genomic alterations[21]
- May yield a false negative result if the patient's cancer harbors mutations that are not included in the assay's targeted gene panel[6]

ctDNA detection in patients with eBC following treatment with curative intent predicted **early relapse** with a median lead time of 7.9 months[22]

In a study of patients with colorectal cancer pre- and post-surgery, ctDNA status was the **most significant prognostic factor** for relapse-free survival[23]

Detection of MRD via ctDNA in blood, urine, or CSF may help inform adjuvant treatment decisions, but more data are needed to establish clinical utility[4,21]

Current and future uses of liquid biopsies summary

Liquid biopsy has the potential to support the entire cancer care journey by enabling early detection, guiding treatment selection, and monitoring MRD and treatment response across multiple cancer types[3–6]

Initiation and interpretation of liquid biopsy involves assessing the most suitable sample type for patient's cancer and considering aspects of the sample and test that may impact the accuracy of results[3,4]

Liquid biopsy tests have shown clinical utility for predictive biomarking identification in some cancers[3]

Further studies are needed to validate the clinical utility of liquid biopsy for MCED, MRD, and its broader adoption in clinical practice, as well as for the future development of assays[4,6]

*Long-term prospective studies are needed to validate ctDNA-based MRD detection for clinical practice.[4,6]

Artificial Intelligence in Healthcare

Artificial intelligence (AI) technologies have the potential to enhance the quality of healthcare[24]

AI refers to the simulation of human intelligence in computer systems, enabling them to perform tasks like learning and problem-solving[24]

Stages in AI system development

Definition of intended use[25,26]
Identify scope, feasibility, and stakeholders

Model design and training[25,26]
Use prepared data for model training based on appropriate algorithms

Deployment[26]
Integrate into the system and interact with users

Data management[26]
Involves collection and labeling of data

Validation[25]
Test performance of the model retrospectively and prospectively*

Some AI-enabled devices go through regulatory review prior to deployment. However, such a step does not exist for many AI systems[25,27,28†]

Applications in healthcare‡

- Medical imaging, digital pathology, and diagnostics[29,30]
- Virtual patient care and monitoring[29]
- Drug discovery and precision medicine[29,30]
- Patient engagement and compliance[29]
- Rehabilitation and sports medicine[29]
- Administrative applications and pharmacy services[29]

*Retrospective validation tests AI with historical data, while prospective validation evaluates it using data collected in real time according to predefined protocols that reflect actual practice.[25]

†AI/ML-based software, when intended to treat, diagnose, cure, mitigate, or prevent disease or other conditions, are medical devices under the FD&C Act and called "software as a medical device" (SaMD) and are subject to FDA regulation. Non-device software functions intended (1) for administrative support, (2) for maintaining a healthy lifestyle, (3) to serve as electronic patient records, (4) for transferring, storing, converting formats, or displaying data, or (5) to provide certain, limited clinical decision support are not medical devices and are not subject to FDA regulation.[25,27,28]

‡These are only some examples of AI applications in healthcare and not an exhaustive list.

A closer look at AI terminology and applications

AI terminology

Artificial intelligence
An umbrella term that covers multiple approaches, including distinct algorithms that each have unique assumptions[27,31,32]

Machine learning
A type of AI where relationships derived from data can be used to make predictions or decisions[27,31,32]

Deep learning
A type of machine learning that utilizes artificial neural networks (a type of software architecture)[31,32]

Applications

Traditional

- Used to predict categorical labels, continuous values, or binary responses[31]
- Concerns include accuracy, generalizability, and biases in training data[33]

Example: Computational pathology to discover novel biomarkers[32]

Generative

- Used to create a statistically probable output (text, images, or other content) that is similar but not identical to training data[34]
- Concerns include accuracy, variability, sycophancy (answer mirrors prompt too closely), hallucinations, and biases in training data[35]

Example: Large language models like ChatGPT[34,35]

Large language models in clinical practice

Large language models (LLMs), like ChatGPT, LLaMA, and Bloom, are AI models that can generate human language and perform natural language processing (NLP) tasks[36]

Potential uses

- **Pathology diagnosis/screening**[37,38]
  - Potentially imitate advanced clinical reasoning processes to arrive at an accurate diagnosis[38]
- **Summarization systems**[39]
  - Simplifying documentation tasks, such as patient visit notes[40]
  - Analyze and distill essential findings from clinical trial reports similar to systematic reviews[39]
- **Treatment decisions**[37]
  - Matching treatment options with genetic alterations[37]

Concerns

- Potential **bias in training** data[40]
- Data privacy[40]
- **Not FDA-approved** or regulated[27]
- Misinformation[40]
  - Propensity to generate **hallucinations**, which are outputs that, while seemingly believable, are factually incorrect[36,38,40]
- Lack of accountability[40]
- Unclear legal liability in the potential case that an LLM recommendation results in patient harm[41]

LLMs' potential utility to support clinical decision-making **does not yet have the quality and credibility required** to safely provide accurate treatment recommendations[37]

Computational pathology uses machine learning to improve diagnostics[24,33,42]

Traditional pathology

Pathologist manually examines histological samples and/or digital images of patient specimens[42]

Computational pathology

Computational analysis using AI methods, such as machine learning, is used to analyze patient specimens and data[42]

Machine learning-based approaches might use[33,42]:

Supervised learning, which uses human-managed workflow with labeled data to provide explicit feedback to guide the learning process

Semi-supervised learning, which uses a combination of labeled and unlabeled data for model training, while also involving a human user

Unsupervised learning, which trains a model based on inherent patterns of the data without the use of labels to guide the learning process

Machine learning can be used to build clinical decision support (CDS) systems to assist diagnosis, identify novel features, and correlate images to patient outcomes[33,42]

Image-based CDS tools have emerged for a variety of uses in oncology[24,30,33,42]

- Detect the presence of tumors on H&E-stained tissue[42]*
- Assess PD-L1 expression via IHC[24,30]
- Differentiate between primary and metastatic tumors[30]
- Predict ICI response in NSCLC[30]

CDS systems may also include the **analysis of non-imaging data[24,30,33]**

- Biomics data (genomics, transcriptomics, circulating immune profiling, single-cell analysis, metabolomics, microbiome)[30]
- EHRs and patient data[24,33]

Computational pathology aims to reduce errors in diagnosis and classification, improve diagnostic accuracy, optimize patient care, and improve operational efficiencies by bringing global collaboration[24,33,42]

*Whole-slide imaging (WSI) is often used, which allows for the digitalization of an entire slide image and allows researchers to identify features not easily analyzed by visual evaluation alone.[42]

Barriers to adoption of computational pathology algorithms

Considerations for developing and implementing machine learning-based CDS systems

AI development needs

IT infrastructure

- Requires a significant investment in IT infrastructure, from processing speeds and memory requirements to data storage and network speeds[24,42]

Data privacy and security

- Storing large amounts of medical data in cloud-based systems requires cybersecurity measures to protect sensitive patient data[42]

Acquiring training data

- Large datasets, from a wide variety of data sources, are critical for developing and training an AI system that can handle variation[24,30,42]
- For supervised algorithms, this includes using sufficient and suitable "ground truth" data, which provide appropriate diagnostic context[33,42]

Generalizability

Variability between AIs

- AIs developed for the same use (eg, assess PD-L1 expression in lung cancer) may not perform equally well based on differences in the training and validation datasets[42]

Validation in clinical practice

- AI systems need to be clinically validated prior to being integrated into clinical workflows[24,33]

Sample variability

- Even in validated AIs, differences in sample preparation (eg, staining variation, air bubbles, tissue thickness) can lead to inaccurate results[24,33]

Human user engagement

Computational pathology team

- Efficient algorithms will require the engagement of a multidisciplinary team that includes pathologists, data scientists, and engineers[24,30]

Role of the pathologist

- Approve the results from the algorithm and support its training[24,42]
- Widen their expertise to include AI technology[24]
- Ensure the algorithm is optimized to work as it was designed[24,42]

Computational pathology may help facilitate a more efficient pathology workflow by increasing the speed and accuracy of diagnosis[24,42]

Artificial intelligence in healthcare summary

AI algorithms are trained and validated on specific datasets and include limitations and biases within that dataset, which can impact the output[31–33]

A validated computational pathology AI algorithm may not be generalizable to samples at a separate lab due to differences in sample preparation[33]

While the application of large language models for clinical support is promising, problems with accuracy, hallucinations, and sycophancy may occur[35–38,40]

New regulatory frameworks are needed to evaluate the risks and benefits of AI clinical decision support tools before they are integrated into clinical practice[27,41]

AI, artificial intelligence; ATM, ataxia-telangiectasia mutated; CDS, clinical decision support; cfDNA, circulating free deoxyribonucleic acid; CHEK2, checkpoint kinase 2; CHIP, clonal hematopoiesis of indeterminate potential; CSF, cerebrospinal fluid; CTC, circulating tumor cell; ctDNA, circulating tumor deoxyribonucleic acid; ctRNA, circulating tumor ribonucleic acid; ddPCR, droplet digital polymerase chain reaction; DNA, deoxyribonucleic acid; eBC, early breast cancer; EHR, electronic health record; FDA, Food and Drug Administration; FD&C, Federal Food, Drug, and Cosmetic Act; H&E, hematoxylin and eosin; ICI, immune checkpoint inhibitor; IHC, immunohistochemistry; IT, information technology; LOD, limit of detection; LLM, large language model; MCED, multi-cancer early detection; ML, machine learning; MRD, minimal residual disease; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NLP, natural language processing; NSCLC, non–small cell lung cancer; PCR, polymerase chain reaction; PD-L1, programmed death-ligand 1; SaMD, software as a medical device; TAT, turnaround time; TF, tumor fraction; TP53, tumor protein 53; VAF, variant allele frequency; VUS, variant of uncertain significance; WBC, white blood cell; WSI, whole-slide imaging.

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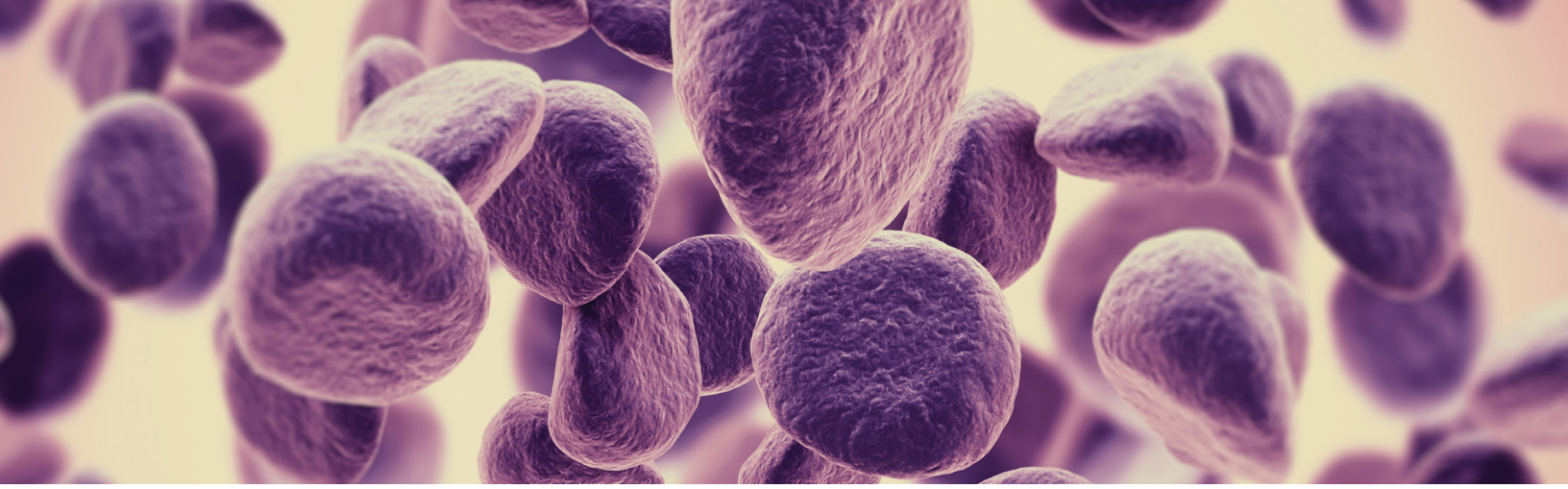

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