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Biomarker discovery in hepatocellular carcinoma (HCC) for personalized treatment and enhanced prognosis

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ABSTRACT

Hepatocellular carcinoma (HCC) is a leading contributor to cancer-related deaths worldwide and presents significant challenges in diagnosis and treatment due to its heterogeneous nature. The discovery of biomarkers has become crucial in addressing these challenges, promising early detection, precise diagnosis, and personalized treatment plans. Key biomarkers, such as alpha fetoprotein (AFP) glypican 3 (GPC3) and des gamma carboxy prothrombin (DCP) have shown potential in improving clinical results. Progress in proteomic technologies, including next-generation sequencing (NGS), mass spectrometry, and liquid biopsies detecting circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), has deepened our understanding of HCC's molecular landscape. Immunological markers, like PD-L1 expression and tumor-infiltrating lymphocytes (TILs), also play a crucial role in guiding immunotherapy decisions. Despite these advancements, challenges remain in biomarker validation, standardization, integration into clinical practice, and cost-related barriers. Emerging technologies like single-cell sequencing and machine learning offer promising avenues for further exploration. Continued investment in research and collaboration among researchers, healthcare providers, and policymakers is vital to harness the potential of biomarkers fully, ultimately revolutionizing HCC management and improving patient outcomes through personalized treatment approaches.

1. Introduction

Hepatocellular carcinoma (HCC) is the primary malignancy of the liver and a significant global health concern, being the sixth most commonly diagnosed cancer and the fourth leading cause of cancer-related deaths worldwide [1,2]. The burden of HCC is particularly pronounced in regions with high rates of hepatitis B and C infections, such as Asia and sub-Saharan Africa [3–5]. However, the rising incidence of metabolic dysfunction-associated steatotic liver disease (MASLD) and alcohol-related liver disease is contributing to an increasing number of cases in Western countries [6,7].

Despite advancements in treatment, current options for HCC, including surgical resection, liver transplantation, locoregional therapies, and systemic treatments, are often limited by late-stage diagnosis and the heterogeneous nature of the disease [8,9]. Many patients present with advanced disease at the time of diagnosis, significantly reducing the effectiveness of curative treatments. Furthermore, variability in patient responses to therapy, driven by genetic, epigenetic, and environmental factors, adds complexity to HCC management [10].

Biomarkers, defined as biological molecules found in blood, other body fluids, or tissues that indicate normal or abnormal processes or disease conditions, are increasingly recognized as essential tools in cancer diagnosis, prognosis, and treatment [11,12]. In HCC, reliable biomarkers are critical for early detection, disease progression monitoring, therapeutic response prediction, and identifying potential therapeutic targets. The complexity of HCC and the limitations of existing diagnostic markers, such as alpha-fetoprotein (AFP), underscore the need for novel biomarkers that can improve early diagnosis, patient stratification, and the development of personalized treatment regimens [13,14]. Effective biomarker discovery can enhance clinical outcomes and extend survival for HCC patients [15,16].

Recent advances in high-throughput technologies, including genomics, proteomics, and metabolomics, have significantly accelerated biomarker discovery [17,18]. Among these, metabolomics, a compre-

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hensive analysis of metabolites within biological systems, offers a non-invasive approach to monitoring metabolic changes associated with HCC progression [17,19]. Integrating metabolomics with other omics data provides a deeper understanding of the molecular mechanisms driving HCC, thereby identifying promising therapeutic targets[19,20].

Given the pressing challenges in HCC management, this review focuses on the current advancements in biomarker discovery, emphasizing novel technologies like next-generation sequencing (NGS), proteomics, and liquid biopsies. We aim to explore how these developments contribute to personalized medicine, providing a pathway for more effective and individualized treatment strategies. By examining diagnostic, prognostic, and predictive biomarkers, we will highlight their transformative potential in HCC management. Additionally, this review will critically analyze existing literature, address areas of consensus and controversy, and identify research gaps that require further investigation.

2. Current landscape of HCC treatment

Current treatments for HCC encompass surgical options, locoregional therapies, and systemic therapies, each with advantages and limitations [21,22]. Surgical resection and liver transplantation are the primary surgical options often employed in early-stage HCC [23,24]. While resection involves removing the tumor and some surrounding tissue, transplantation replaces the diseased liver with a healthy one [25]. Transplantation is particularly beneficial as it addresses both the tumor and the underlying liver condition but is limited by the availability of donor organs and stringent eligibility criteria [26].

Locoregional therapies, such as radiofrequency ablation (RFA) and transarterial chemoembolization (TACE), are used for intermediate stages or patients not suitable for surgery [27,28]. RFA uses thermal energy to destroy cancer cells and is effective for small tumors, while TACE delivers chemotherapy directly to the tumor's blood supply, minimizing systemic toxicity [29]. However, TACE is not curative and associated with significant adverse effects [30].

Systemic therapies are primarily reserved for advanced HCC [31,32]. Tyrosine kinase inhibitors (TKIs) like sorafenib and lenvatinib have been pivotal in managing advanced HCC [33,34], offering survival benefits albeit with notable side effects such as hypertension and gastrointestinal issues [35]. More recently, immune checkpoint inhibitors (ICIs) such as nivolumab and pembrolizumab have shown promise by targeting the PD-1/PD-L1 pathway, resulting in durable responses in some patients [36,37]. Despite their potential, ICIs are not universally effective and can cause immune-related adverse events [38,39].

The treatment landscape for HCC is fraught with challenges, primarily due to the heterogeneity of patient responses and the development of drug resistance [40,41]. The genetic, epigenetic, and environmental diversity among patients contributes to variable treatment outcomes, necessitating a personalized approach to therapy [42]. Additionally, resistance to systemic therapies such as TKIs and ICIs, driven by genetic mutations and alternative signaling pathways, limits their long-term efficacy [43]. Adverse effects further complicate treatment, affecting the patient's quality of life and adherence to therapy [44].

Integrating reliable biomarkers into clinical practice offers a pathway to overcoming these challenges by enabling more precise patient stratification, predicting treatment responses, and guiding personalized therapy choices. Continued research into novel biomarkers and personalized treatment strategies is essential to overcome these limitations and improve the management of HCC [45,46].

3. Biomarkers in HCC: types and functions

Biomarkers play a critical role in managing HCC by aiding in diagnosis, prognosis, and treatment decisions. The discovery and validation of effective biomarkers can significantly enhance the precision and effectiveness of HCC management [47].

AFP is one of the most widely used biomarkers for HCC diagnosis [48,49]. Elevated levels of AFP are associated with HCC, especially in the presence of liver cirrhosis [50]. However, AFP has limitations in sensitivity and specificity, particularly in early-stage HCC and patients with non-malignant liver diseases. Despite these limitations, AFP remains a valuable tool, often used with other diagnostic methods to improve accuracy [51,52].

Glypican-3 (GPC3) is a cell surface proteoglycan that is significantly overexpressed in HCC, but it is not found in normal liver tissue or benign liver conditions [53,54]. This selective expression makes GPC3 a valuable diagnostic biomarker for HCC. Immunohistochemical staining for GPC3 is increasingly used to distinguish HCC from non- cancerous liver conditions and other types of liver cancer [55]. GPC3's high specificity for HCC enhances its diagnostic utility, particularly in cases where AFP levels are not elevated [14].

Des-gamma-carboxy prothrombin (DCP), a protein induced by vitamin K absence or antagonist-II (PIVKA-II), is another critical biomarker for HCC [56–58]. Elevated DCP levels are associated with tumor aggressiveness and poor prognosis. DCP is particularly useful in diagnosing HCC in patients with normal AFP levels, providing an additional tool for early detection and monitoring of disease progression [59,60].

The following table summarizes the key biomarkers in HCC, their roles, diagnostic utility, prognostic value, and therapeutic implications.Table 1

The following figure provides a visual flowchart to further illustrate the diagnostic process using these biomarkers. Fig. 1

Prognostic biomarkers are essential for predicting disease progression and patient outcomes [11,15]. These biomarkers help stratify patients based on the likely course of the disease, enabling more tailored and effective treatment plans. Prognostic biomarkers in HCC include markers like AFP, DCP, and GPC3, which are useful in diagnosis and provide insights into disease prognosis [13,45,61]. High levels of these biomarkers are often associated with advanced disease stages, larger tu-

Table 1
Key Biomarkers in HCC and Their Roles.

Biomarker	Role	Diagnostic Utility	Prognostic Value	Therapeutic Implications
Alpha-fetoprotein (AFP)	A glycoprotein produced by the liver and yolk sac during fetal development.	Elevated AFP levels can indicate HCC, especially in patients with liver cirrhosis. Used in combination with other markers to improve accuracy.	High AFP levels are associated with larger tumor size, advanced stage, and poorer prognosis.	AFP levels can help monitor treatment response and detect recurrence post-treatment.
Glypican−3 (GPC3)	A cell surface proteoglycan overexpressed in HCC but not in normal liver tissue or benign liver diseases.	GPC3 is used for immunohistochemical staining to differentiate HCC from benign liver conditions.	High GPC3 expression correlates with tumor aggressiveness and poorer prognosis.	Potential target for immunotherapy, GPC3 inhibitors, and vaccines are under investigation.
Des-gamma-carboxy prothrombin (DCP)	An abnormal prothrombin protein induced by vitamin K absence or antagonists.	Elevated DCP levels help diagnose HCC in patients with normal AFP levels, providing an additional diagnostic tool.	High DCP levels are associated with larger tumor size, vascular invasion, and increased risk of recurrence.	DCP levels can guide treatment decisions and monitor disease progression.

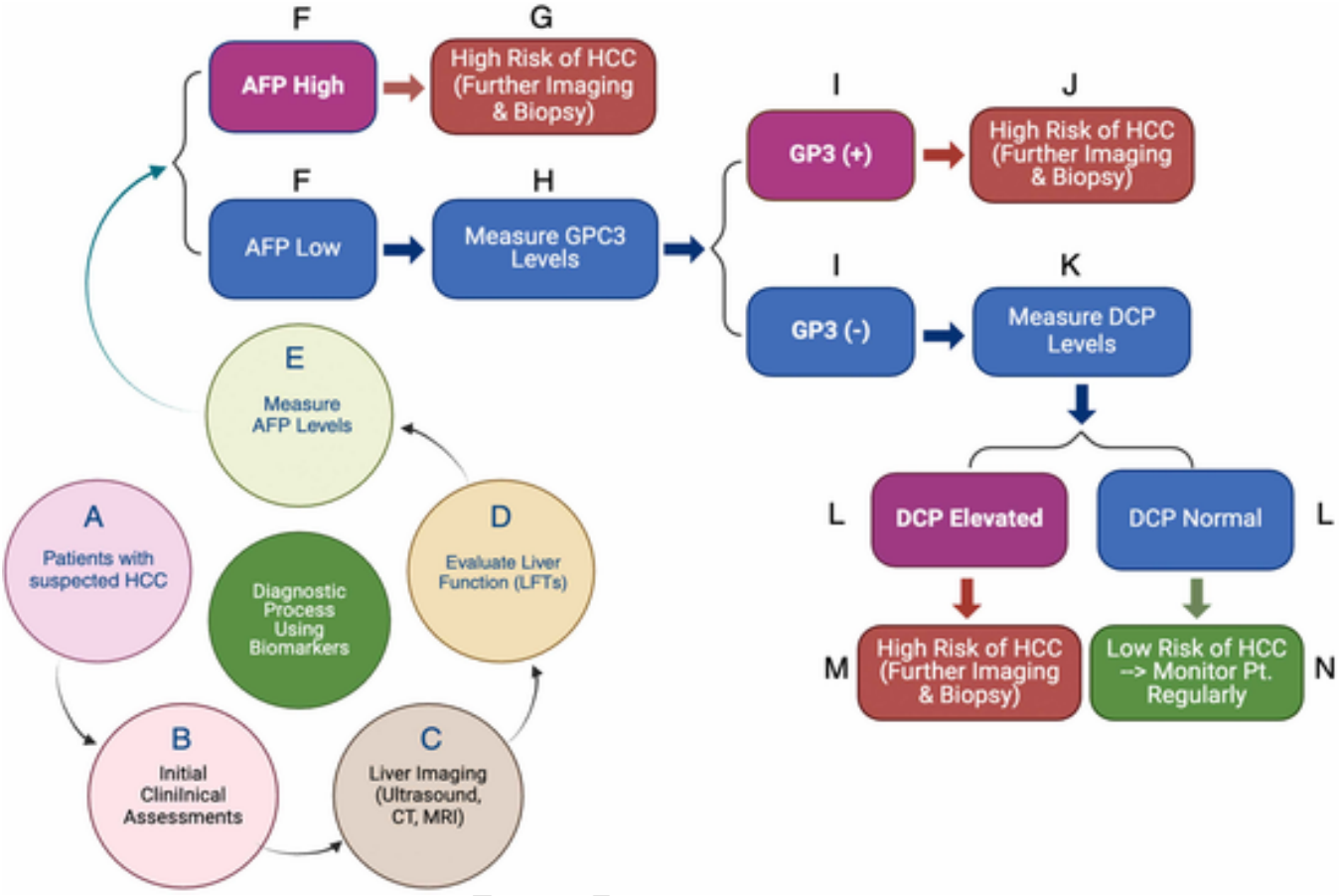

Fig. 1. The diagnostic process using biomarkers. The flowchart uses key biomarkers to illustrate the step-by-step diagnostic process for suspected HCC patients. Starting with the initial clinical assessment (A) and liver imaging (B), liver function tests (LFTs) are evaluated (D). AFP levels are measured (E), with high AFP levels indicating a high risk of HCC and prompting further imaging and biopsy (G). If AFP levels are low, GPC3 levels are measured (H). Positive GPC3 results indicate a high risk of HCC (J), while negative results lead to the measurement of DCP levels (K). Elevated DCP levels suggest a high risk of HCC (M), while normal DCP levels indicate a lower risk, leading to regular patient monitoring (N).

mor sizes, and increased risk of metastasis and recurrence [62,63]. For instance, elevated AFP levels post-treatment can indicate residual disease or recurrence, guiding further therapeutic interventions [64]. Genetic and molecular markers such as mutations in the TERT promoter, CTNNB1, and TP53 genes have also been identified as significant prognostic indicators [65–67]. These genetic alterations can influence tumor behavior and patient survival, offering valuable information for prognosis.

Predictive biomarkers are crucial for guiding treatment decisions and predicting responses to therapy [68]. They help identify which patients will benefit from specific treatments, optimizing therapeutic outcomes and minimizing unnecessary side effects [11,15]. In HCC, predictive biomarkers include markers that indicate likely responses to systemic therapies such as tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs) [11,68]. For example, high expression levels of PD-L1 in tumor tissues may predict better responses to ICIs like nivolumab and pembrolizumab [69,70]. Similarly, genetic mutations and alterations in signaling pathways, such as the Wnt/β-catenin pathway, can influence the effectiveness of targeted therapies and immunotherapies [71,72]. The presence of certain biomarkers can also predict resistance to specific treatments, helping to avoid ineffective therapies and focus on more promising alternatives [11,73]. For instance, alterations in the VEGF pathway may suggest resistance to anti-angiogenic therapies, guiding clinicians to consider other treatment options [74,75].

By providing precise diagnostic, prognostic, and predictive information, these biomarkers can help overcome the limitations of current HCC treatment strategies and enable more personalized and effective care for patients. In summary, identifiing and applying diagnostic, prognostic, and predictive biomarkers are transforming the landscape of HCC management [15,45]. These biomarkers enhance early detection and accurate diagnosis and provide critical insights into disease progression and treatment responses, paving the way for more personalized and effective therapeutic strategies [76]. Continued research and validation of these biomarkers are essential to fully realize their potential in improving patient outcomes in HCC.

4. Advances in biomarker discovery for HCC

Recent advances in biomarker discovery for HCC have significantly progressed through genomic, proteomic, and immunological approaches, revolutionizing disease management. These approaches provide deeper insights into the molecular mechanisms driving HCC, enabling more precise and personalized treatment strategies [10,77]. Next-generation sequencing (NGS) facilitates a comprehensive analysis of genetic alterations in HCC, identifying mutations, copy number variations, and gene fusions linked to disease progression and therapy response [78,79]. For instance, frequent mutations in the TERT promoter, CTNNB1, and TP53 genes are associated with poor prognosis and treat-

ment resistance. These findings have led to targeted therapies and personalized treatment plans that improve patient outcomes [42,78].

Proteomics complements genomic approaches by elucidating the functional consequences of genetic alterations through mass spectrometry. This technique allows for detailed analysis of protein expression, posttranslational modifications, and protein-protein interactions in HCC [79]. Proteomic studies have identified novel protein biomarkers in tumor growth, metastasis, and drug resistance [80]. Dysregulations in pathways such as PI3K/AKT/mTOR and Wnt/β-catenin, identified through proteomic analysis, provide potential therapeutic targets [81, 82].

Moreover, liquid biopsies have emerged as a crucial tool in HCC management. Critical components of liquid biopsies include circulating tumor cells (CTCs) and cell-free DNA (cfDNA), mainly circulating tumor DNA (ctDNA) [12,83]. The detection and analysis of CTCs offer valuable information about the presence and characteristics of HCC, including genetic and phenotypic heterogeneity [84]. CTCs can monitor disease progression, assess treatment efficacy, and detect early metastasis or recurrence [85]. Analyzing ctDNA provides real-time insights into tumor dynamics, tracks resistance mutations, and guides treatment adjustments. Additionally, cfDNA analysis shows promise in detecting minimal residual disease and predicting relapse before clinical symptoms appear [86,87].

Immunological biomarkers are also essential for understanding the tumor-immune interaction and guiding immunotherapy [88]. PD-L1 expression on tumor and immune cells in TME is a critical biomarker for predicting response to ICIs [89,90]. High PD-L1 expression is associated with better responses to ICIs like nivolumab and pembrolizumab, which target the PD-1/PD-L1 pathway [91]. Additionally, tumor-infiltrating lymphocytes (TILs) are critical immunological biomarkers that reflect the host immune response against the tumor. Higher levels of TILs are often associated with better prognosis and improved response to immunotherapy [92–96]. Analyzing TILs provides insights into the immune landscape of HCC, aiding in predicting treatment outcomes and guiding the development of combination therapies that enhance anti-tumor immunity [97,98].

These innovative techniques are revolutionizing our understanding of HCC and paving the way for more personalized and effective therapeutic strategies. Continued research and integrating these biomarkers into clinical practice are essential to fully realize their potential in improving patient outcomes. Fig. 2 summarizes these key advancements and their clinical applications, mainly focusing on the role of liquid biopsies in HCC management.

A. Genomic, proteomic, and immunological approaches in HCC biomarker discovery. (1) Genomics: DNA sequencing, gene expression profiling, copy number variation, and mutation analysis identify biomarkers that guide targeted therapy. (2) Proteomics: Mass spectrometry and protein analysis reveal altered pathways, such as PI3K/AKT/mTOR and Wnt/β-catenin, which are involved in tumor aggressiveness and treatment response. (3) Immunology: Immune biomarkers like PD-L1 and TILs guide immunotherapy, with anti-PD-1 (e.g., pembrolizumab) and anti-PD-L1 (e.g., atezolizumab) enhancing T cell activity.

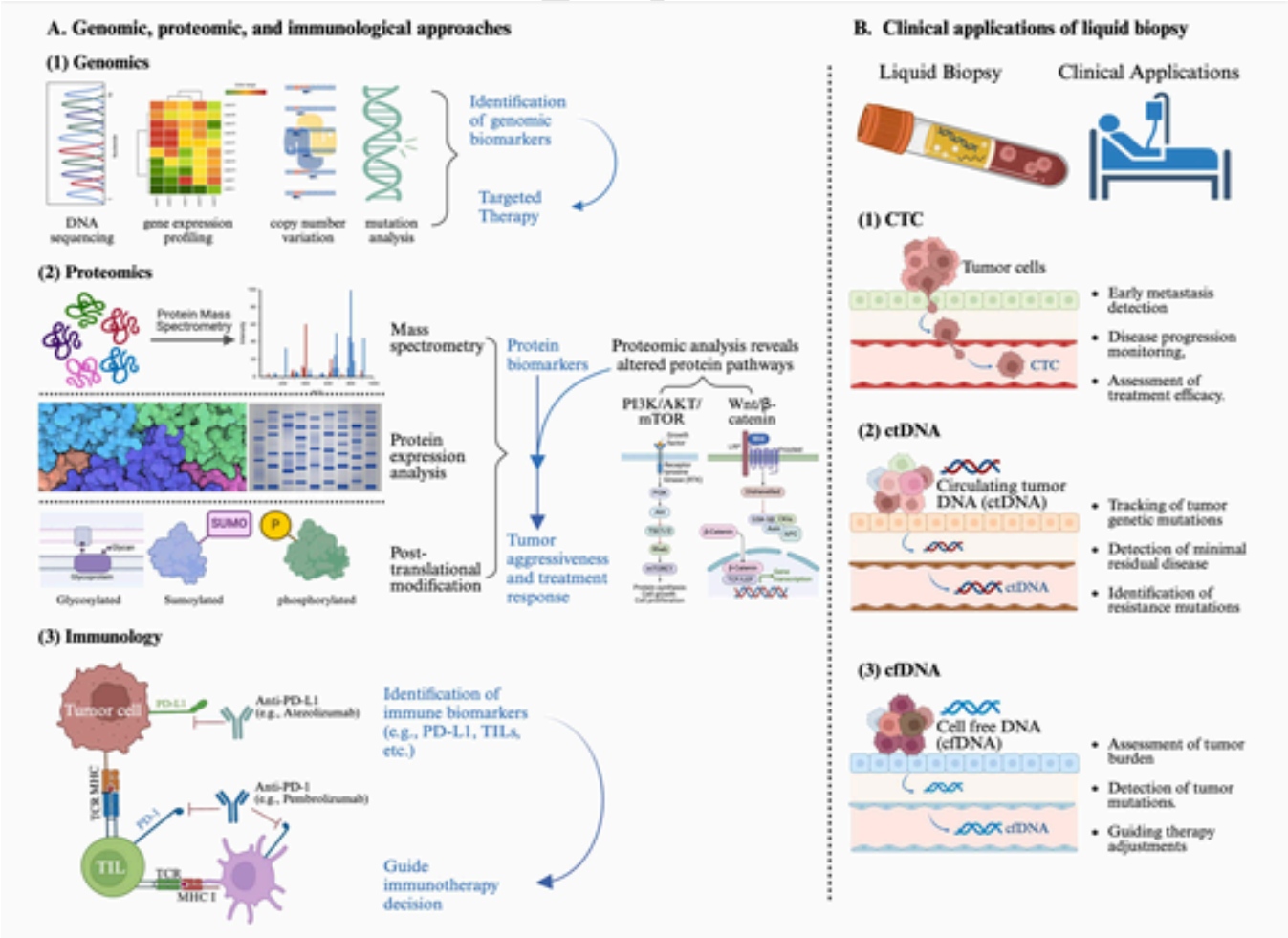

Fig. 2. Approaches in HCC biomarker discovery and applications of liquid biopsies.

B. Clinical applications of liquid biopsy in HCC management. (1) CTCs: Facilitate early metastasis detection, disease monitoring, and treatment assessment. (2) ctDNA: Enables tracking of genetic mutations, detection of minimal residual disease, and identification of resistance mutations. (3) cfDNA: Assesses tumor burden, detects mutations, and guides therapy adjustments.

5. Clinical applications and implications

The advancements in biomarker discovery for HCC are not merely academic, they have significant clinical applications that can transform patient care [14,99,100]. These biomarkers are essential for developing personalized treatment strategies and improving overall prognosis and patient outcomes.

5.1. Personalized treatment strategies

Tailoring therapy based on individual biomarker profiles ensures patients receive the most effective treatments while minimizing unnecessary side effects [10,101]. Biomarkers such as AFP, GPC3, DCP, PD-L1, TILs, and specific genetic mutations like CTNNB1 provide crucial information about the tumor's molecular characteristics. This information guides the selection of appropriate therapies, ranging from targeted treatments like tyrosine kinase inhibitors (TKIs) to immune checkpoint inhibitors (ICIs) and combination therapies [14,63,102].

5.2. Early detection and treatment assessment

Biomarkers also play a pivotal role in the early detection of HCC and continuous monitoring of treatment efficacy. For instance, liquid biopsies detecting circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) offer a noninvasive method for early diagnosis and tracking molecular changes in tumor [103]. Monitoring biomarker levels over time can assess treatment efficacy and detect recurrence, allowing for timely intervention [104].

The following figure illustrates how different biomarker profiles guide personalized treatment strategies and how regular monitoring of these biomarkers supports early detection and ongoing treatment assessment in HCC.Fig. 3

One notable example of personalized treatment in HCC is the use of ICIs like nivolumab and pembrolizumab for patients with high PD-L1 expression [36,105]. High PD-L1 levels, detected through immunohistochemical staining, can predict better responses to these ICIs, making them suitable candidates for immunotherapy [69,106,107]. Another example is targeted therapies such as sorafenib and lenvatinib in patients with specific genetic mutations identified through next-generation sequencing (NGS) [77,108,109]. These therapies inhibit tyrosine kinase pathways often dysregulated in HCC, providing a more targeted and effective treatment [34,110,111].

Improving prognosis and patient outcomes in HCC heavily depend on early detection and continuous monitoring [112]. For instance, liquid biopsies offer a noninvasive method for detecting molecular changes in the tumor long before they are visible through imaging, allowing for earlier intervention and improved outcomes [15,76,104]. Monitoring biomarker levels over time can also help assess treatment efficacy and detect recurrence, prompting further diagnostic evaluation and potential therapeutic intervention [11,15,104]. Additionally, high-risk biomarker profiles, such as elevated DCP levels, necessitate closer monitoring and may benefit from more aggressive treatment strategies [113,114].

Moreover, integrating multi-omics approaches combining genomics, proteomics, and metabolomics provides a comprehensive understanding of HCC and uncovers new biomarkers for more precise patient stratification and treatment [115,116]. This holistic approach enhances the ability to predict patient outcomes and tailor treatments more effectively [117].

In conclusion, the clinical applications and implications of biomarker discovery in HCC are profound. By enabling personalized treatment strategies and improving early detection and monitoring, biomarkers significantly enhance prognosis and patient outcomes [13,15,76]. Continued research and integrating these biomarkers into clinical practice are essential for advancing HCC treatment and providing patients with the best possible care [21,61].

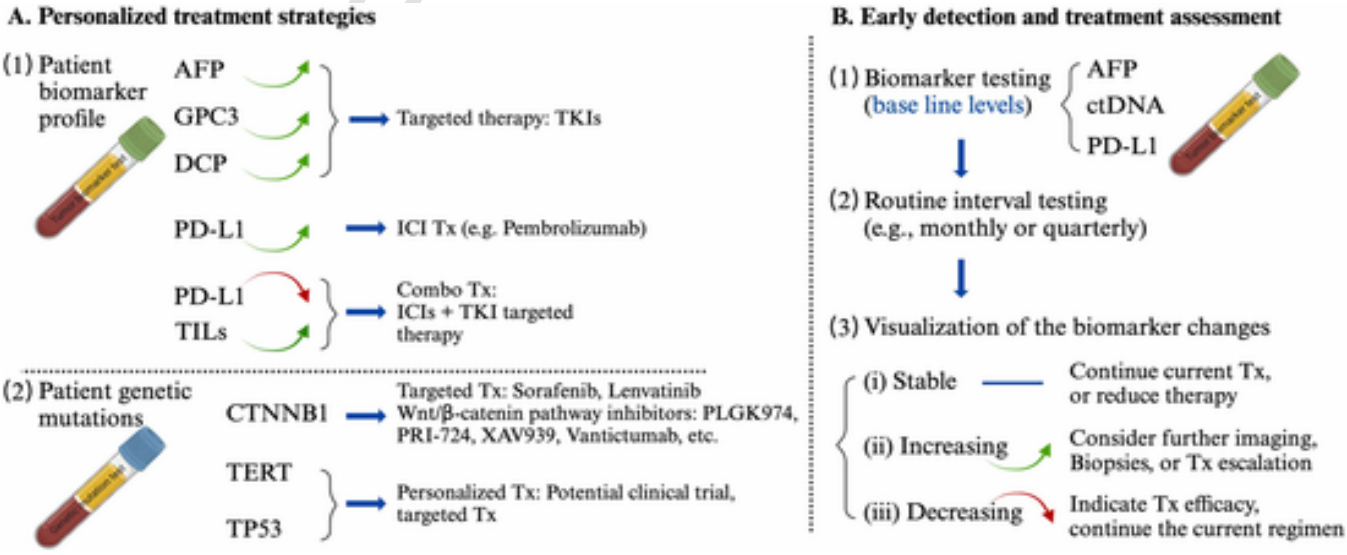

Fig. 3. Personalized treatment strategies and biomarker monitoring in HCC. **A.** Personalized treatment strategies based on patient biomarker profiles, including AFP, GPC3, DCP, PD-L1, and TILs, as well as genetic mutations such as CTNNB1, TERT, and TP53. Treatment options range from targeted therapies (e.g., TKIs) to ICIs like pembrolizumab, and combination therapies that include ICIs with targeted therapies. B. Early detection and treatment assessment involves regular biomarker testing (e.g., AFP, ctDNA, PD-L1) at scheduled intervals (e.g., monthly or quarterly). Visualization of biomarker changes informs clinical decisions, such as continuing current treatment, considering further imaging or biopsies, or adjusting therapy to enhance efficacy.

6. Challenges and future directions

The discovery and implementation of biomarkers in HCC are promising for advancing personalized medicine and improving patient outcomes [15,41,118]. However, significant challenges remain in validating these biomarkers, integrating them into clinical practice, and leveraging emerging technologies for further advancements [11,98,119].

6.1. Validation and standardization

Ensuring the reliability and reproducibility of biomarkers in clinical settings is a critical challenge. Biomarkers must undergo rigorous validation processes to confirm their sensitivity, specificity, and overall efficacy across diverse patient populations [120]. Achieving this involves large-scale, multicenter studies to ensure that biomarkers can be consistently detected and measured accurately [104,120,121]. Additionally, standardized protocols for biomarker testing and interpretation are necessary to avoid variability in results between different laboratories and clinical settings [122]. For example, while biomarkers like AFP, GPC3, and DCP show promise, their utility can be limited by variations in assay techniques and cut-off values used in different studies [83,123]. Developing universally accepted standards and guidelines is crucial for successfully implementing biomarkers in routine clinical practice [11,14,47,124,125].

6.2. Barriers to clinical integration

Despite the potential benefits, integrating biomarkers into clinical practice faces several barriers. One significant barrier is the cost associated with advanced diagnostic tests and technologies. High costs can limit accessibility, particularly in resource-limited settings [11,126]. Additionally, there may be resistance to change among healthcare providers accustomed to traditional diagnostic and treatment methods. Educating clinicians about the benefits and applications of biomarker-driven approaches is essential for fostering acceptance and adoption. Moreover, integrating biomarker testing into existing clinical workflows requires coordination between various stakeholders, including clinicians, laboratory personnel, and policymakers, to ensure seamless implementation and reimbursement policies [127].

6.3. Future directions

Advances in bioinformatics and machine learning are paving the way for new opportunities in biomarker discovery and application. These technologies can analyze large datasets to identify patterns and correlations that might not be apparent through traditional methods. For instance, machine learning algorithms can integrate genomic, proteomic, and clinical data to predict patient outcomes and treatment responses more accurately [128,129]. Additionally, bioinformatics tools can help identify novel biomarkers by analyzing complex biological data and generating hypotheses for experimental validation [130].

6.4. Combination biomarker strategies

Another promising area of research is the development of combination biomarker strategies. By combining multiple biomarkers, it is possible to improve diagnostic accuracy and predictive power [15,131]. For example, integrating genetic, proteomic, and metabolomic data can provide a more comprehensive understanding of HCC biology and help identify patients likely to benefit from specific treatments [116,132]. This multi-omics approach can also uncover new therapeutic targets and inform the development of combination therapies [133].

6.5. Emerging technologies

Emerging technologies such as single-cell sequencing and spatial transcriptomics also contribute to our understanding of tumor heterogeneity and the tumor microenvironment [134,135]. These technologies can reveal tumor's cellular composition and the spatial distribution of different cell types, providing insights into how tumors evolve and respond to treatment [136–138]. Understanding these dynamics can lead to identifying new biomarkers and therapeutic targets [139,140].

The following figure provides a visual summary of the critical challenges in biomarker research for HCC, alongside emerging technologies and strategies that hold promise for overcoming these barriers and advancing personalized medicine.Fig. 4

In conclusion, while significant progress has been made in the discovery and application of biomarkers for HCC, several challenges must be addressed to fully realize their potential [14]. Ensuring the validation and standardization of biomarkers, overcoming barriers to their integration into clinical practice, and leveraging emerging technologies and research areas are critical steps toward advancing personalized medicine for HCC [10,112]. Continued research and collaboration among scientists, clinicians, and policymakers are essential to overcome these challenges and improve patient outcomes in HCC [141].

7. Conclusion

Biomarker discovery in HCC has significantly progressed, enabling more personalized and effective treatment strategies. Key biomarkers like AFP, GPC3, and DCP have enhanced HCC diagnosis, prognosis, and management. Additionally, advancements in genomic and proteomic technologies, and the introduction of liquid biopsies and immunological biomarkers have expanded our understanding of HCC, paving the way for targeted therapies and improved patient outcomes.

The future of HCC treatment is promising with integrating multi-omics approaches and emerging technologies such as single-cell sequencing and machine learning. These innovations are expected to further refine treatment strategies, tailoring them to individual patient needs and improving clinical outcomes. However, to fully realize the potential of these biomarkers, continued research, large-scale validation studies, and efforts to overcome barriers to clinical integration are essential.

In conclusion, biomarkers are key to transforming HCC management by enabling early detection, precise diagnosis, and personalized therapy. Continued collaboration and investment in research are crucial to fully harnessing their potential and advancing precision medicine in HCC.

Ethics approval and consent to participate

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Consent for publication

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CRediT authorship contribution statement

BY and WM collectively conceived and designed this comprehensive review. Both authors contributed to the initial draft of the manuscript. WM provided supervision, graphics support, editing, and finalized the manuscript. Both authors actively participated in the revision of the

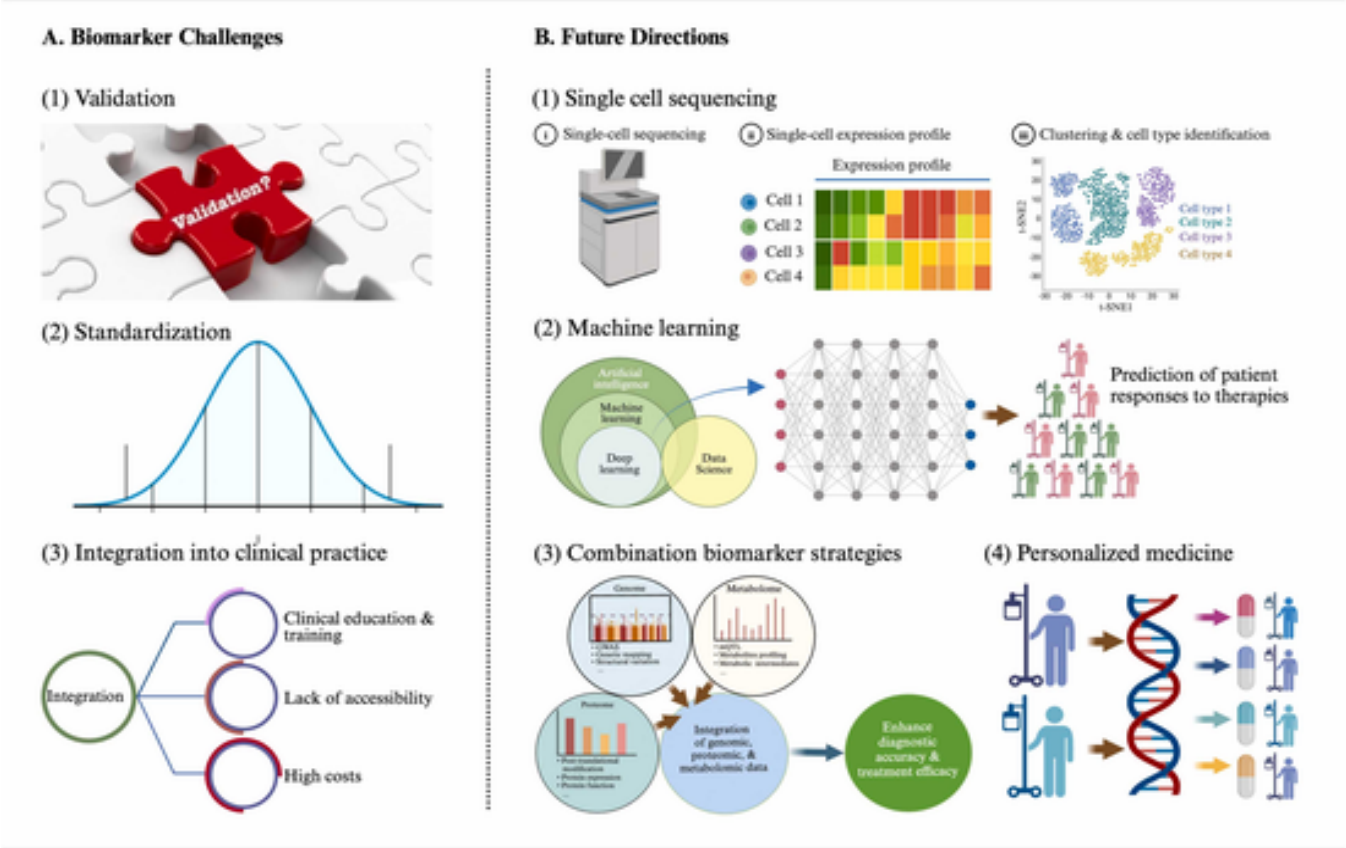

Fig. 4. Challenges in Biomarker Validation and Future Directions in HCC Research. A. Biomarker Challenges summarizes the challenges in biomarker validation, standardization, and integration into clinical practice. Key barriers include ensuring reliable validation across diverse populations, developing standardized protocols to reduce variability, and overcoming high costs and lack of accessibility in clinical settings. B. Future Directions illustrates emerging directions in biomarker research, focusing on advancements such as single-cell sequencing, machine learning, and combination biomarker strategies. These innovations aim to improve diagnostic accuracy, predict patient responses to therapies, and enable more personalized treatment approaches in HCC.

manuscript, carefully reviewed it, and approved the final version for submission.

Abbreviations

AFP	Alpha-fetoprotein
GPC3	Glypican−3
DCP	Des-gamma-carboxy prothrombin
HCC	Hepatocellular carcinoma
NGS	Next-generation sequencing
CTCs	Circulating tumor cells
ctDNA	Circulating tumor DNA
cfDNA	Cell-free DNA
PD-L1	Programmed death-ligand 1
TILs	Tumor-infiltrating lymphocytes
ICIs	Immune checkpoint inhibitors
RFA	Radiofrequency ablation
TACE	Transarterial chemoembolization
TKIs	Tyrosine kinase inhibitors
MASLD	Metabolic dysfunction-associated steatotic liver disease

Declaration of Competing Interest

The authors declare no potential conflicts of interest.

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