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**cfDNA ULP-WGS for prognosis in HCC**

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Prognosis of MASLD



## Editorial

# Exploring the prognostic value of ultra-low-pass whole-genome sequencing of circulating tumor DNA in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and a leading cause of cancer-related mortality worldwide. Many cases of HCC are diagnosed at advanced stages and are accompanied by underlying liver cirrhosis.<sup>1</sup> Despite considerable progress in the management of HCC with both immune checkpoint inhibitors and tyrosine kinase inhibitors over the course of recent decades, the prognosis of patients with HCC remains poor.<sup>2</sup> The identification of reliable biomarkers that can predict the therapeutic response to systemic treatment is expected to improve the prognosis of patients with advanced HCC. Because conventional liver biopsy, which is invasive, collects limited tissue samples, the results cannot reflect intra-tumor genetic heterogeneity, and tracking the evolution of the tumor according to treatment is difficult. Therefore, liquid biopsy, which could obtain tumor genetic information via a less invasive method, has gained substantial attention recently.<sup>3</sup> Because of the progress in molecular methodologies, the detection

of circulating tumor DNA (ctDNA) in advanced HCC has yielded promising and intriguing outcomes.<sup>4-5</sup>

In this issue of *Clinical and Molecular Hepatology*, Sogbe et al.<sup>6</sup> demonstrated the prognostic implications of ultra-low-pass whole-genome sequencing (ULP-WGS) of ctDNA in patients with HCC receiving systemic treatment. The ctDNA detected using ULP-WGS was associated with a worse prognosis for patients with HCC receiving systemic treatment. Among the ctDNA of patients with HCC receiving systemic therapy, losses of 5q and 16q were identified as independent prognostic factors for poor overall survival. Prior studies have focused on detecting targeted DNA aberrations, such as single-nucleotide mutations, copy number variations, and epigenetic modifications, such as methylation changes and somatic mutations, in ctDNA using high-depth genetic analysis technology. This study demonstrated that broad-coverage and low-depth genetic analyses can elucidate genetic differences between HCC and cirrhosis without HCC in terms of HCC prognosis. This finding may lead to a relevant, affordable, and cost-effective approach that can enhance the ability to predict the prognosis of HCC, thus significantly impacting

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clinical research and clinical practice.

Recently, ctDNA in HCC has emerged as a significant research topic. CtDNA fragments are derived from cancer cells and can be found in the bloodstream of patients with cancer. With cancers such as HCC, quantitative and qualitative analyses of ctDNA may reflect the genetic and molecular characteristics of the tumor without invasiveness, thereby providing valuable information regarding the diagnosis, prognosis, and treatment response. Previous studies have suggested that cancer-specific mutations of *TP53*, *ITH*, *HCK*, *CTNNB1*, and *TERT* in ctDNA are commonly observed in peripheral blood samples of patients with HCC.<sup>7-9</sup> A study performed in Korea found that *MLH1* single-nucleotide variants were associated with poor survival in patients with advanced HCC, thus highlighting the importance of quantitative analyses of ctDNA.<sup>10</sup>

CtDNA can be measured using various methods, such as targeted polymerase chain reaction-based technology, next-generation sequencing, whole-exome sequencing, and WGS. In the evolving landscape of genomic research, ULP-WGS has emerged as a notable alternative to traditional WGS. Although traditional WGS offers a detailed and comprehensive overview of the genome with high sequencing coverage (typically more than 30x), it is associated with substantial costs and data processing demands.<sup>11</sup> In contrast, ULP-WGS provides significantly lower coverage of approximately 0.1x to 1x, thus providing a broader, albeit less detailed, view of the genomic landscape. This approach is particularly advantageous for large-scale screening and the identification of major genomic alterations, such as copy number variations, with much lower costs and computational requirements. Although ULP-WGS provides more granular information compared to that provided by WGS, its cost-effectiveness and efficiency for detecting large-scale genomic changes make it a compelling choice for specific research and clinical applications that do not require a detailed genetic map.<sup>12-14</sup> Recently, the prognostic implications of ctDNA evaluated by ULP-WGS for various cancers, including metastatic squamous non-small cell lung cancer, Ewing sarcoma, osteosarcoma, metastatic castration-resistant prostate cancer, cervix cancer, and metastatic triple-negative breast cancer, have been reported.<sup>15-19</sup> Alongside these studies, the study by Sogbe et

al.<sup>6</sup> is noteworthy for its meaningful investigation into the potential use of ctDNA evaluated with ULP-WGS as a prognostic biomarker in patients with advanced HCC.

The detection of ctDNA and exploration of copy number alterations using ULP-WGS have significant implications. Copy number alterations, which refer to gains or losses of parts of chromosomes, play a crucial role in tumor development and progression. By analyzing copy number alterations of ctDNA using ULP-WGS, researchers and clinicians can non-invasively gain insights into the genetic landscape of tumors at a relatively low cost. In this study, the authors investigated the copy number alterations of ctDNA across various chromosomal loci using ULP-WGS and highlighted the loci where losses (8p, 4p, 13q, 16q, and 5q) and gains (1q, 8q, 7q, and 5p) were most prevalent. Notably, they identified the loss or deletion of 5q and 16q as independent biomarkers that predict poor survival in patients undergoing systemic therapy. These findings significantly contribute to the improvement of treatment strategies and can enable personalized approaches for patients with HCC.<sup>6</sup>

Although research on ctDNA using ULP-WGS has presented an economically viable option for clinical applications, thus enabling the identification of genomic features at a cost-effective rate, it is important to acknowledge that this method has certain limitations. First, as Sogbe et al.<sup>6</sup> underscored, its low sensitivity poses a challenge when detecting ctDNA in patients with low tumor burden. Moreover, the low resolution of ULP-WGS limits the detection of detailed genetic variants, and difficulties encountered while interpreting noise-related data further complicate its application. Additionally, the majority of studies that have utilized ULP-WGS, including the current study, were retrospective and included limited sample sizes. Hence, further prospective studies with larger sample sizes are required to validate its clinical effectiveness.<sup>20</sup> Furthermore, the predominance of sorafenib treatment among patients in this study introduced bias. In the context of the shift toward immunotherapy-based first-line treatments for HCC, such as atezolizumab plus bevacizumab or durvalumab plus tremelimumab, further research of the role of ctDNA analyzed using ULP-WGS in the prediction of progression-free survival or the tumor response to immunother-

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#### Abbreviations:

CtDNA, circulating tumor DNA; HCC, hepatocellular carcinoma; ULP-WGS, ultra-low-pass whole-genome sequencing

apy in patients with advanced HCC is expected to have significant implications.

In conclusion, the study by Sogbe et al.<sup>6</sup> represents a significant contribution to the understanding of the role of ctDNA in the prognosis of HCC using ULP-WGS, thus highlighting the need for further research on this topic. Although ULP-WGS is a non-invasive and cost-effective approach to tumor genetic profiling, it is associated with challenges, including low sensitivity and resolution, that need to be addressed. It is anticipated that further investigations of ctDNA using ULP-WGS will more clearly define its potential as a prognostic biomarker for patients with advanced HCC.

### Authors' contribution

All authors were responsible for the conceptualization, interpretation of data, drifting, and critical revision of the manuscript.

### Conflicts of Interest

The authors have no conflicts to disclose.

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