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Editorial

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Advancements in hepatocellular carcinoma diagnosis and treatment: liquid biopsy and surgery as precision treatment

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Hepatocellular Carcinoma (HCC) is the most common type of primary liver cancer, posing a substantial influence on worldwide health. HCG develops mainly in the setting of cirrhosis, related to a hepatitis B or C viral infection, alcohol-related liver disease, or non-alcoholic fatty liver disease (NASH)[1]. With its rising incidence and often asymptomatic nature in the early stages, early detection and effective treatment are crucial for improving patient outcomes. The diagnosis of HCC still occurs predominantly in advanced stages, posing challenges to treatment interventions, and the prevision is that there will be more than 1 million people to die of HCC by 2030 from the prediction of the World Health Organization (WHO)[2]. Additionally, the absence of reliable predictive and prognostic biomarkers hinders the customization of therapeutic approaches, creating a dilemma in determining the patients who would gain the greatest advantage from specific treatments. This emphasizes the need for thorough access to HCC molecular mechanisms to improve biomarker discovery for more efficient detection and treatment. The advent of molecular diagnostics in oncology implies a revolutionary phase in precision medicine. These tests, which pinpoint distinct genetic changes in cancer cells, provide a deeper understanding of the molecular underpinnings of the disease. This facilitates early detection and directs targeted treatments, increasing the likelihood of positive treatment outcomes. In addition, molecular diagnostics also assist in monitoring minimal residual disease, recurrence and predicting patient prognosis, thereby enhancing counseling and overall management. Despite these advancements, the full potential of molecular diagnostics in

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hepatocellular carcinoma (HCC) is yet to be fully realized, emphasizing the need for ongoing innovation in this critical area of research. This editorial explores the connection between Liquid Biopsy in HCC and consequent precision treatment.

Traditional methods for diagnosing HCC, such as imaging studies and liver biopsy, have limitations in terms of sensitivity, invasiveness, and the capacity to find cancers in their early stages. Liquid biopsy, a non-invasive diagnostic method, has surfaced as an important tool for the early identification and surveillance of Hepatocellular Carcinoma (HCC). This approach entails scrutinizing diverse biomarkers such as circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and extracellular vesicles (EV) present in peripheral blood. These biomarkers provide valuable information about the genetic and molecular alteration of HCC, to tailor treatment strategies to individual patients. The integration of liquid biopsy into routine clinical practice has the potential to revolutionize HCC diagnosis, offering a less invasive and more comprehensive approach to understanding the molecular landscape of the disease.

Currently, EVs are considered better markers than circulating nucleic acids. Indeed, studies have shown that intercellular communication occurs through various means, including direct cellular contact, soluble factors, and extracellular vesicles (EVs)[3].

Released EVs from the HCC may be an excellent source of analysis for surrogate tumor markers and diagnostic information, joining non-invasive skills in a fast and affordable cost-effective way. The isolation and analysis of blood circulating EVs, therefore, constitutes a "key enabling technology" that is the skill that, shortly, will make precision diagnostics possible. In the therapeutic choices of some advanced tumor forms, the molecular evaluation of the "driver" genomic lesions (e.g., KRAS, NRAS, BRAF, EGFR, ALK fusions, ROS-1, PD-L1, *etc.*), performed via tissue analysis, is currently the "gold standard", i.e., the accurate examination in a clinical setting.

EVs, such as exosomes, are very stable functional transporters that, due to their structure, prevent the degradation of their contents, called "cargo". The molecular study of this cargo, therefore, offers countless potentials, both in terms of nucleic acids and in terms of proteins, including their post-translational modifications such as phosphorylation which would otherwise be too unstable to be detected. Innovative cancer therapies are now increasingly oriented towards protein targets, i.e., networks of activated proteins called "pathways", by blocking which it is possible to act more selectively on tumor cells, minimizing the side effects of the therapies.

Numerous studies have shown that patients with liver cirrhosis can be diagnosed with HCC with high diagnostic performance model scores[4-6].

Specifically, the combined Z-score of 10 EV-mRNA (AFP, AHSG, APOH, FABP1, FGB, FGG, RBP4, and TF) showed outstanding diagnostic performance (sensitivity 93.8%, specificity 74.5%, AUC 0.87) for the diagnosis of HCC in at-risk individuals[4]. Tey *et al.* emphasize the significance of EV-pIgR in the development of hepatocarcinogenesis, as well as its potential use in HCC diagnosis and prognosis[3].

In addition, we have to consider that the liquid biopsy market is an emerging market that is currently almost entirely divided into the offer of preclinical testing services in Europe and so-called "clinical lab" (LDT) services in the US and Canada. The pioneering diagnostic test, InteliScore (ExosomeDx), based on exosome analysis in urine, was initially approved in 2018 for determining a prognostic index in prostate cancer, securing insurance coverage in the US despite lacking FDA approval. Currently, FDA-approved and

marketed liquid biopsy-based *in vitro* diagnostics (IVD) assays include companion diagnostics tests by Roche Dx for detecting mutations in the EGFR gene (plasma), the QIAGEN kit therascreen® KRAS RGQ PCR (tissue), and Guardant360® CDx (plasma).

While these tests have proven effective in the prostate cancer context, there is a strategic initiative to expand their application to hepatocellular carcinoma (HCC). The successful implementation of these assays in prostate cancer serves as a precedent, and the ongoing project aims to leverage the same diagnostic technologies for HCC. This endeavor is part of an innovative approach to utilize liquid biopsy techniques for precise diagnosis and treatment guidance in HCC, building on the success and regulatory experiences gained in the field of prostate cancer diagnostics. The potential application of these diagnostics in HCC is being explored to enhance the capabilities of early detection and targeted therapy, mirroring the progress achieved in non-small-cell lung cancer with the companion diagnostic to Sotorasib (Lumakras™), approved in August 2021 for patients positive for the KRAS G12C mutation. The future involves not only monitoring patients post-surgery to diagnose potential recurrences but also developing a test to detect early-stage HCC in high-risk populations.

Surgical intervention remains a cornerstone in the management of HCC, especially for patients with localized disease. Over the years, surgical techniques have evolved to enhance the precision and effectiveness of HCC treatment. From traditional resection to mininvasive surgery approaches, the goal is to achieve complete tumor removal while preserving liver function. Advances in imaging techniques, such as intraoperative ultrasound, have improved the surgeon's ability to identify and precisely delineate tumor margins, reducing the likelihood of recurrence. For patients with tumors in challenging locations or those with compromised liver function, innovative approaches such as laparoscopic and robotic-assisted surgery (RAS) have gained prominence. These minimally invasive techniques offer the benefits of reduced blood loss, shorter hospital stays, and quicker recovery times compared to traditional open surgeries. In this context, the diagnostic process is very important to detect the right patient for the right treatment.

Liquid biopsy is gaining significant attention primarily because it has the capability to identify recurrence but also early tumors.

In cases of tumor recurrence, there is an increase of ctDNA as a viable biomarker for monitoring treatment response[7]. Cai *et al.* have studied the postoperative levels of circulating tumor DNA (ctDNA) and protein biomarkers, such as AFP, AFP-L3, and des-gamma-carboxy prothrombin (DCP)[8]. In addition, they correlated these findings with MRI scan images during the follow-up period. The study affirmed that both single-nucleotide variants (SNVs) and copy number variations (CNVs) could effectively track the tumor burden in Hepatocellular Carcinoma (HCC). The combination of ctDNA and DCP demonstrated an enhanced ability to detect minimal/molecular residual disease (MRD) in patients who underwent hepatectomy.

Innovative molecular tests for early detection and management of HCC are emerging. Early detection of HCC is vital due to the limited therapeutic window for curative surgical intervention, particularly in its initial stages. It is well known that the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) presently advocate biannual ultrasound surveillance, either with or without monitoring serum alpha-fetoprotein (AFP) levels, for populations at high risk[9]. These markers play a critical role in identifying MRD and monitoring for recurrence, thereby pinpointing individuals who could potentially benefit from adjuvant therapies or simple surveillance.

It is fundamental to detect early diagnosis in HCC to resect as soon as possible. An early, accurate diagnosis and prompt treatment are essential to enhance the prognosis of HCC. One of the most important approaches for managing HCC involves surgical intervention.

Radiofrequency ablation (RFA) stands as an alternative treatment option for HCC, demonstrating a comparable prognostic impact, particularly for small (< 3 cm) nodular tumors in cirrhotic livers[10,11]. Another curative option for HCC that offers the greatest survival rate for some individuals is liver transplantation[12,13]. By the way, due to the scarcity of liver grafts, this option is not always available because there is an age limit (the age limit for the recipient is usually set at 65 years), or because the patient's comorbidities such as heart diseases, respiratory diseases, or other neoplasms may contraindicate the transplant. Consequently, our focus is on "precision liver surgery" for improving the prognosis of HCC. Liver parenchyma-sparing surgery can be performed by minimally invasive and open surgical techniques. There is presently no agreement on the most effective treatments, such as radiofrequency ablation (RFA) and minimally invasive surgery (MIS), in patients with solitary small peripheral Hepatocellular Carcinoma (HCC) and compensated cirrhosis. A study conducted regarding HCC < 2 cm comparing RFA and MIS showed that the 7-year Disease Free survival (DFS) rates were 86.1% and 35.9% ($P < 0.001$), respectively, and the 7-year recurrence beyond the Milan criteria (RBM) rates were 88.9% and 66.7% ($P = 0.014$), respectively, and the 7-year OS rates were 97.2% and 82.1% ($P = 0.008$), respectively. In addition, RFA was associated with more ipsilateral lobe recurrence (20% *vs.* 83.4%, $P = 0.004$)[14].

In an early diagnosis setting, it is evidently easier to guide a patient towards surgery as the tumor is less extensive, and the amount of parenchyma is less compromised. In the realm of surgical innovations, the refinement of MIS techniques and the development of novel technologies, such as image-guided navigation systems and 3D reconstruction, hold promise for further improving patient outcomes.

Currently, liquid biopsy also represents a way to diagnose other hepatic diseases as well as intrahepatic cholangiocarcinoma (iCCA). Despite the apparent heterogeneity in risk factors associated with iCCA, they are all related to cholestasis and chronic inflammation, which damages the biliary epithelium and suggests that these pathological processes are important to the biology of this cancer. In this setting, the liquid biopsy can analyze ct DNA and ct RNA, exosomes, or cytokines. The most common alterations are isocitrate dehydrogenase 1/2 (IDH1/2) mutations, fibroblast growth factor receptor 2 (FGFR2) fusions, B-Raf (BRAF), and AT-rich interaction domain 1A (ARID1A) mutations[15].

In this setting, there is an ongoing study: The EVEarlyPannel project, supported by funding from the Ministry of Business and Made in Italy (MIMIT) - ISTITUTO SUPERIORE DI SANITA' - Program number F/180032/02/X43 - CUP: B89J24000090005. This project is part of the advancements in technology related to the development of liquid biopsy assays for precision medicine in oncology. Specifically, it focuses on diagnostic and predictive indications of therapeutic benefit for solid tumors, particularly Hepatocellular Carcinoma (HCC). The project involves the identification of surface antigens and those contained in exosomes of tumor origin, encompassing proteins, DNA, and RNA candidates associated with pathways frequently adopted in tumors.

The overarching goal of the project, with a detailed emphasis on personalized preventive medicine, is to qualify and implement a comprehensive set of tools for early/preventive diagnosis and personalization/prediction of response/monitoring approaches to oncological therapies, with a specific focus on HCC. As part of this initiative, one of the project partners has successfully developed a humanized antibody for treating selected antigen-positive HCC. Preclinical studies will incorporate liquid biopsy monitoring in

conjunction with therapeutic antibodies to enhance the drug's effectiveness. This approach enables the use of reduced doses, broadening the pool of eligible patients, including those deemed unsuitable for standard therapy.

The landscape of HCC diagnosis and treatment is evolving rapidly, fueled by advancements in liquid biopsy and surgical innovations. The integration of non-invasive liquid biopsy techniques into routine clinical practice offers a paradigm shift in the early detection and monitoring of HCC, while surgical innovations enhance the precision and efficacy of treatment options. The cost-effectiveness, accessibility, and standardization of liquid biopsy assays need further consideration to facilitate widespread adoption. Additionally, ongoing research is essential to validate the clinical utility of liquid biopsy in predicting treatment response and guiding therapeutic choices.

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Authors' contributions

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Both of authors declared that there are no conflicts of interest.

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