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EDITORIAL

Biomarkers for hepatocellular carcinoma: What's new on the horizon?

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Abstract

Treatment of advanced hepatocellular carcinoma remains unsatisfying and so far only prognostic biomarkers like α-fetoprotein have been established. No clear predictive biomarker is currently available for standard of care therapies, including targeted therapies like sorafenib. Novel therapeutic options like immune checkpoint inhibitors may pose new challenges to identification and validation of such markers. Currently, PD-L1 expression *via* immunohistochemistry and tumor mutational burden *via* next-generation sequencing are explored as predictive biomarkers for these novel treatments. Limited tissue availability due to lack of biopsies still restricts the use of tissue based approaches. Novel methods exploring circulating or cell free nucleic acids (DNA, RNA or miRNA-containing exosomes) could provide a new opportunity to establish predictive biomarkers. Epigenetic profiling and next-generation sequencing approaches from liquid biopsies are under development. Sample size, etiologic and geographical background need to be carefully addressed in such studies to achieve meaningful results that could be translated into clinical practice. Proteomics, metabolomics and molecular imaging are further emerging technologies.

Key words: Hepatocellular carcinoma; Biomarker; Next-generation sequencing; Liquid Biopsy; Functional imaging; Molecular imaging; Circulating free DNA; Circulating tumor cells; Immune checkpoint inhibitors

Core tip: Hepatocellular carcinoma (HCC) is a heterogeneous disease with various underlying etiologies and an overall still poor prognosis. Biomarkers to identify optimal treatment for distinct patients are still lacking for HCC due to limited availability of biopsies. Novel treatment options, esp. immune checkpoint inhibitors, may need novel biomarker approaches and non-tissue based technologies might provide a solution to identify those biomarkers. In this article, the current status of biomarker identification for HCC is discussed.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary tumor of the liver and ranks 3rd in cancer-related deaths worldwide[1,2]. While its incidence continues to be high in Africa and Asia, Western countries also showed increasing incidences rates in the past decades due to chronic hepatitis C virus (HCV) infection, alcohol consumption and high rates of obesity linked to non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and subsequent development of chronic liver disease with cirrhosis[3,4]. Although, a slight improvement in global HCC mortality was recently reported, certain sub-populations and regions esp. in Western countries still have an unfavourable prognosis and risk assessment with continuous high incidence and mortality[5,6]. Curative treatment options like surgical resection or orthotopic liver transplantation are only feasible in a minority of patients at early disease stages and with preserved liver function. Thus, the overall prognosis for patients with HCC remains unsatisfying, its 5 year survival being a dismal 6.9%, an incidence to mortality ratio of 0.95 and a median overall survival of only 11 mo[7]. For advanced stages, treatment is based on multi-kinase inhibitors like sorafenib or regorafenib, while recent data indicate that immune checkpoint inhibitors will lead to increased response rates also in this setting[8,9].

Biomarkers are defined as characteristics that are measured as indicators of physiologic or pathologic processes or in response to various diagnostic or therapeutic procedures. The reader is referred to the recent definitions of the FDA-NIH Biomarker Working group for full definition of different biomarker types[10]. Various prognostic biomarkers, *e.g.*, α-fetoprotein (AFP), AFP-L3 or Des-γ-carboxyprothrombin (DCP), are currently used or under investigation for the early diagnosis and surveillance of HCC patients. Here, newer biomarkers like osteopontin, glypican-3 or high c-met expression have shown additional value, esp. when combining these parameters as was shown for osteopontin and AFP[11-13]. Yet, little progress was achieved in developing predictive biomarkers for targeted and other novel treatment options[14,15]. In this article, the current status of predictive biomarkers for identification and selection of patients for novel therapies will be discussed.

BIOMARKERS FOR TARGETED THERAPIES

Sorafenib is the current standard of care for advanced HCC. Initial phase 2 data indicated that pretreatment levels of phosphorylated ERK (p-ERK) correlated with time to progression (TTP)[16], which was later confirmed by several preclinical and *in vitro* studies[17,18]. Due to limited availability of tissue samples, this finding could not be confirmed in the registrational phase 3 study (SHARP trial)[19]. Instead, an extensive program investigating different biomarkers from plasma samples was initiated. Surprisingly, none of the investigated biomarkers predicted the response to sorafenib while biomarkers related to clinical performance, *e.g.*, vascular invasion or AFP, as well as markers of angiogenesis like Ang2 or VEGF were shown to be independent predictors of survival in patients with advanced HCC[20] and thus need to be seen rather as prognostic biomarkers. In other smaller studies, elevated p-ERK and VEGF2 tissue levels were shown to be predictive for poor response to sorafenib treatment in a cohort of 77 advanced HCC patients[21]. Similarly, high p-ERK correlated to poor overall survival, but not time to progression, in another study with 44 patients[22]. Interestingly, also the multi-kinase inhibitor regorafenib, which was recently approved for second-line therapy of HCC, stratified patients only on clinical parameters and thus does not have a predictive biomarker available so far for HCC patients[23], while promising results on plasma circulating cell free DNA were obtained for patients with colorectal cancer[24].

BIOMARKERS FOR IMMUNE CHECKPOINT INHIBITORS

The recent success of immune checkpoint inhibitors in other cancer diseases also triggered various approaches in HCC. As HCC development is commonly based on chronic inflammatory liver diseases (viral hepatitis, NASH), a clear rationale to investigate this new treatment paradigm is clearly given. Initial results using anti-CTLA-4 or anti-PD-1 antibodies are encouraging and lead to accelerated approval of the anti-PD-1 antibody nivolumab for the treatment of advanced HCC[25,26]. Combination of checkpoint inhibitors seem feasible and the use of such drugs together with locoregional procedures in early disease settings might even further improve outcome of patients[27].

Still, a significant number of patients (> 60%) do not respond to these novel therapies. Biomarker-based enrichment was initially based on immunohistochemical expression of the respective checkpoint targets, but recent data from various indications suggest that this is not the strongest predictor for treatment response[28]. This could be due to the still tissue based scoring of target expression with an intrinsic risk for sampling error in heterogenous solid tumors[29,30] or due to the still not completely understood biology of checkpoint inhibition[31] as evidenced by approx. 10% of patients who do not express PD-L1 but respond to treatment[32].

LIQUID BIOPSIES

It is intriguing that many promising and potent drugs,

e.g., sunitinib, everolimus, brivanib or tivantinib, failed in HCC clinical trials. Besides careful selection of patients based on clinical parameters like liver function or vessel invasion, all-comer trials are nowadays not considered appropriate and identification of specific patient subgroups based on distinct molecular subtypes is therefore urgently requested[33-35].

In HCC, lack of biopsies and different etiologic backgrounds hampered the identification and validation of such markers for the currently available treatments. Even today, practice guidelines do not recommend taking biopsies of every patient although risk of bleeding and needle track seeding are infrequent and should not be seen as a reason against taking a diagnostic biopsy[36,37]. The latest EASL practice guidelines strongly recommend liver biopsy and blood sampling from patients participating in clinical and diagnostic trials[37].

Genomic profiling established distinct molecular subclasses of HCC that were also linked to specific gene mutations and clinical and histological features. Two major groups, the proliferative (chromosomal instable) and the non-proliferative (chromosomal stable) group, were defined which comprise approx. 50% of HCC cases each. Further analyses defined a stem cell/hepatoblast like and a TGFβ related subgroup in the proliferative group, as well as a hepatocyte like and a Wnt/β-catenin related subgroup in the non-proliferative group[34,38]. While the overall impact of this classification is still under debate, additional common mutations and genetic alterations were described. Overall, mutations in telomerase signaling, the p53 and cell cycle control pathway as well as in Wnt/β-catenin signaling are commonly observed while rarer events include mutations in the Ras/PI3K/mTOR pathway, JAK/STAT signaling and other pathways[38,39]. Interestingly, no clear individual oncogenic driver has been identified in HCC so far and HCC is considered a cancer with medium to low mutational burden.

Analyzing tumor nucleic acids from other sources than tissue, *i.e.*, from circulating tumor cells, cell free DNA/RNA or exosomes, could help to overcome the above mentioned limitations. Liquid biopsies usually detect expression levels, methylation status or mutations of distinct tumor related nucleic acids, including DNA, RNA and miRNAs originating from circulating tumor cells or being shed into the blood directly from living or dying tumor cells. While this approach is considered to reduce sampling error compared to solid tumor biopsies[40,41], there are still technical limitations to this technology. The success rate of detecting circulating tumor cells is depending on the size of the tumor and results seem highly variable, ranging from approx. 25% to 100% success rate within different populations and with different technical approaches[42]. Similar results were obtained for genetic analyses of circulating DNAs[43,44]. Surprisingly, also these studies seem to be underpowered when considering different etiologic and geographical background. A clear advantage of liquid biopsies is also the option of taking serial samples from a patient to detect changes during disease history and imposed by treatment[45].

PROTEOMICS, GLYCOMICS AND IMAGING

As the liver is the primary secretory and metabolizing organ of the body, the use of proteomics and glycomics (usually by liquid chromatography-mass spectrometry (LC-MS) or matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry analysis) could provide an option to identify novel biomarkers, too, without the current limitations of tissue based analyses. Several proteomic factors, *e.g.*, CD44v9[46] or Hippocalcin-like 1 (HPCAL1)[47], including various multimarker approaches were used as prognostic or diagnostic biomarkers for HCC or to predict recurrence[48-52]. Similarly, glycomics-based tests like GlycoCirrhoTest or analysis of N-glycans were developed as further diagnostic tools for better surveillance of patients at risk of HCC development[14,53-55]. So far, these approaches were not used in a predictive setting in advanced HCC.

Beyond LC-MS or MALDI-TOF analysis, functional and molecular imaging represents a further technology to identify potential biomarkers for HCC. Functional imaging is using dynamic computed tomography, dynamic magnetic resonance imaging and diffusion weighted magnetic resonance imaging approaches and is now well established to detect changes in *e.g.,* fibrosis grade or angiogenesis and for early diagnosis of HCC[56]. The development of novel radiotracers (beyond ^{18}F-FDG) for PET imaging could bridge the findings from proteomics and metabolomics analyses to imaging and thus add useful and important information on tissue distribution to these data. Today, molecular imaging for primary liver tumors is still limited by *e.g.,* lack of specific tracer uptake into malignant cells[57].

CONCLUSION

Predictive biomarkers are considered key for the success of developing new drugs. The further development of emerging technologies that are not dependent on tissue will also increase our knowledge for the better treatment of patients but more homogeneous study design regarding technologies and patient characteristics need to be done to achieve meaningful sample sizes with results that can be robustly translated into clinical applications.

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