

Mini-Review

Hepatocellular Cancer: New Kids on the Block

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Key Words

Biomarkers · Circulating tumor cells · Hepatocellular cancer · New molecular substances · Systemic and local ablative therapy

Abstract

Background: With over 600,000 newly diagnosed hepatocellular cancer (HCC) patients worldwide every year and ongoing clinical research, it is surprising that many of the new molecular entities have not yet resulted in significant prolongation of progression-free or overall survival. Nevertheless, there are a number of promising agents currently under investigation. Given the unique tumor biology and heterogeneous clinical manifestations of HCC, the application of molecular and cellular markers could also benefit patient selection, disease prognosis and trial design. **Summary:** This paper provides an overview of the current therapeutic strategies for HCC in the curative and palliative settings. Furthermore, we introduce some of the promising small molecules and antibodies that may find their way into clinical practice, with a focus on substances that are currently in phase III testing. Finally, we summarize the role of promising biomarkers, such as circulating tumor or cancer stem cells. **Key Message:** Despite the rising prevalence of HCC and active clinical research, few therapeutic options besides sorafenib have been established. This review discusses the new therapeutic agents in the pipeline. **Practical Implications:** Although many promising preclinical studies have resulted in phase I–II trials on HCC, so far only the tyrosine and Raf kinase inhibitor sorafenib has made its way into the hands of physicians. This multikinase inhibitor is the only approved option for systemic treatment of advanced HCC. Currently, the development of promising approaches for disease management is guided by biomarkers such as molecular markers or cellular characteristics. The use of biomarkers may facilitate early diagnosis in high-risk groups and therefore enhance outcomes by detecting patients whose disease is still curable.

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Introduction

Hepatocellular cancer (HCC) is diagnosed 600,000 times per year worldwide and is among the ten most common causes of cancer-related deaths in Germany, with a significant rise in incidence over the last two decades [1]. In Germany high BMI, diabetes mellitus and non-alcoholic fatty liver disease are the mainstay of the recent upsurge, and therefore liver cancer is related to our changing lifestyle more than many other cancers. The 5-year survival rates of symptomatic patients are 10–20% and rise significantly with early detection, but cost-efficient and commonly available screening tools are rare. Several interdisciplinary choices exist in the curative and palliative setting, but so far there are only few tools to individualize treatment decisions. An optimized management of patients at risk of developing HCC and those with diagnosed liver cancer is therefore urgently needed.

Current Options in Diagnosis and Treatment

Curative therapeutic options in HCC are limited to early stages and include mostly resection or orthotopic liver transplantation. Liver transplantation and tumor resection have proven to be the most effective standard therapies and provide 5-year survival rates of 70% for patients within the Milan criteria, i.e. with a single tumor <5 cm in size or up to three tumors <3 cm in size. If patients qualify for liver transplantation, the strict Milan criteria make neoadjuvant, so-called bridging methods, necessary. Otherwise a tumor size doubling time of approximately 4 months results in high dropout rates [2]. If 2–3 lesions or one single lesions >3 cm are present, dropout rates of 90% after 18 months have been reported [3]. Choosing the adequate bridging method in this patient cohort is therefore inevitable, but reliable guidance tools are missing up to now. The 5-year survival rates reach 50% with radiofrequency ablation and transarterial chemoembolization. Another capable localized approach to treat HCC is represented by selective intraarterial radio-nuclide therapy (SIRT). It has been discussed whether some patients may benefit from concomitant local ablative and systemic therapy, but subgroup identifiers are missing so far [4]. However, these therapeutic procedures most often do not provide a complete cure, as half of the treated patients experience tumor recurrence within 3 years [5]. These high recurrence rates after resection and liver transplantation are most likely due to minimal residual disease and the fact that the majority of patients are diagnosed at an advanced stage. In 2008, results of a phase III study of sorafenib in patients with advanced HCC, the so-called SHARP trial, were published in the *New England Journal of Medicine* [6]. In this trial, patients who had not received prior systemic treatment received either sorafenib at a dose of 400 mg daily or placebo. The resulting benefit in median overall survival was about 3 months, which led to FDA approval of the drug as first-line systemic treatment in advanced HCC. Nearly 7 years after the first results of the SHARP trial were presented at the annual meeting of the American Society of Clinical Oncology (ASCO), there still is no other approved systemic option for advanced HCC. The diagnostic and therapeutic options in HCC rely in many aspects on radiological imaging methods, which therefore play an important role in the management of HCC. As of today, the gold standard in the staging and follow-up of HCC is represented by computed tomography. The sensitivity of detecting and distinguishing tumorous lesions can be increased when employing magnetic resonance imaging, especially when applying emerging techniques such as diffusion-weighted imaging [7]. Adding hepatocyte-specific contrast agents to this process enhances sensitivity and specificity, especially when physicians are forced to differentiate between small lesions [8]. Though alpha-fetoprotein (AFP) combined with imaging techniques is currently the standard in

monitoring of therapeutic outcome, relapse or lack of response to surgical or interventional therapy is still hard to predict. Serum-based markers like AFP, des-gamma-carboxy prothrombin or the lectin 3 fraction of AFP (AFP-L3) are incapable of predicting clinical outcome with high accuracy and reproducibility [9].

In patients with cirrhosis regular screening for HCC is suggested, but currently there are no cost-efficient and commonly available screening tools for other high-risk groups. The current standard for screening in risk groups is ultrasound combined with AFP measurement [10]. Ultrasound, though effective and non-invasive, is very much dependent on user and hardware. In Germany and especially in North Rhine-Westphalia, ultrasound performed worse than any other imaging tool [11]. Recent studies indicate that only very few patients actually underwent screening in the last 3 years before they were diagnosed with HCC [12]. Moreover, only some of the patients were actually referred to a specified physician. Though increasing education and demands for quality control may lead to better results, the clinical reality shows that many patients with risk factors will not be referred to those physicians with high experience in gastroenterology or hepatology in time and therefore most likely be diagnosed at advanced stages. Minimally invasive, yet highly specific serum markers are therefore urgently needed.

New Therapeutic Approaches and Biomarkers

Though the multikinase inhibitor sorafenib is currently the only approved systemic treatment in HCC and has been in use for more than 7 years, there still is no reliable biomarker to define patients who would benefit most while others may only suffer from an increased toxicity in comparison to localized approaches. A recent report by Peng et al. [13] supported the usability of vascular endothelial growth factor receptors (VEGFRs), tyrosine kinase receptors that are a target for the small molecule, as potential pre-therapeutic markers. They used immunohistochemistry to stain for active, phosphorylated VEGFR1 (pVEGFR1) and phosphorylated VEGFR2 (pVEGFR2). They also showed that autocrine VEGF promoted phosphorylation of VEGFR1 and VEGFR2 and internalization of pVEGFR2 in HCC cells, which was both pro-proliferative through a protein lipase C-extracellular kinase pathway and self-sustaining through increasing VEGF, VEGFR1 and VEGFR2 mRNA expressions. In high VEGFR1/2-expressing HepG2 cells, sorafenib treatment inhibited cell proliferation, reduced VEGFR2 mRNA expression in vitro and delayed xenograft tumor growth in vivo. The authors were also able to show that in an advanced HCC population on sorafenib treatment for postoperative recurrence, the absence of VEGFR1 or VEGFR2 expression in resected tumor tissues before sorafenib treatment was associated with poorer overall survival. Therefore, VEGFRs could be a biomarker for defining subgroups of patients who would benefit most from sorafenib. Screening of serum biomarkers for sorafenib sensitivity by the SHARP investigators yielded no conclusive results, with VEGF and angiopoietin-2 being prognostic but not predictive [14]. Since HCC seems to be a tumor that is vascularized more than other malignancies, it may be especially detainable by anti-angiogenic treatment [15]. While the small-molecule inhibitors sunitinib, brivanib, erlotinib and linifanib were not successful [16], two of the more promising substances also targeting VEGFRs are currently in phase III trial testing, the recombinant monoclonal anti-VEGFR2 antibody ramucirumab and the small molecule lenvatinib (E7080), like sorafenib a multikinase inhibitor. The latter is currently being tested in a multicenter, randomized, open-label, phase III trial comparing the efficacy and safety versus sorafenib in first-line treatment in unresectable HCC. Another approach is the combination of cytotoxic agents like doxorubicin with anti-angiogenesis. The CALGB 80802 phase III trial with sorafenib

with or without doxorubicin hydrochloride in patients with locally advanced or metastatic liver cancer is still active and results are awaited. The results of the prodromal phase II trial were encouraging with a median overall survival of 13.7 months in the combination arm [17]. Sorafenib in an adjuvant setting after resection or radiofrequency ablation did not deliver beneficial results as presented at the ASCO annual meeting 2014 and cannot be advised currently. Other studies examined the use of radioembolization with yttrium-90 microspheres on overall survival in advanced HCC with or without portal venous obstruction and no extrahepatic extension versus sorafenib, while the combination of both yielded higher peri-transplant biliary complications and potentially tended towards more acute rejections [18].

The inhibition of the mammalian target of rapamycin delivered promising preclinical and phase I data. Zhu et al. [19] recently reported negative outcomes on this pathway in HCC. They reported on the results of the EVOLVE-1 trial, a randomized, double-blind, phase III study conducted among 546 adults with Barcelona Clinic Liver Cancer stage B or C HCC and Child-Pugh A liver function whose disease progressed during or after sorafenib or who were intolerant of sorafenib. No significant difference in overall survival was seen between treatment groups, with a median overall survival of 7.6 months with everolimus and 7.3 months with placebo. Also the median time to progression did not differ significantly between everolimus and placebo (3.0 vs. 2.6 months). The disease control rate was 56.1 vs. 45.1% and also not significant. Nonetheless there are still promising substances being tested right now like the dual orally available inhibitor of TORC1/TORC2 called CC-223 or the disubstituted aminothiazole HBF 079.

Targeting the hepatocyte growth factor (HGF)/c-Met pathway may yield alternatives for sorafenib treatment and substances are currently tested in the first- and second-line setting. HGF stimulation of the MET receptor leads to cell survival, cell proliferation and cytoskeletal changes with enhanced cell mobility. Therefore MET is essential to a process with high plasticity and increasing attention by scientists, the so-called epithelial-mesenchymal transition (EMT), being the switch from an epithelial to a mesenchymal phenotype. Recently Yu et al. [20] showed that in breast cancer these switches are highly associated with response respectively resistance to therapy. The substances that are currently in phase III trial testing are tivantinib (ARQ197) and cabozantinib (XL184). Cabozantinib blocked the HGF-stimulated MET pathway and inhibited the migration and invasion of HCC cells in a study published by Xiang et al. [21]. The small-molecule inhibitor of the tyrosine kinases c-Met and VEGFR2 reduced the number of metastatic lesions in the lung and liver in an experimental metastatic mouse model. Tivantinib, an orally administered, selective inhibitor of MET with a still unclear mode of action, showed promising phase II results versus placebo for second-line treatment of advanced HCC [22]. Patients with advanced HCC and Child-Pugh A cirrhosis who had progressed on or were unable to tolerate first-line systemic therapy were enrolled, and after randomization a tivantinib dose of 240 mg twice daily was administered. For patients with MET-high tumors, median time to progression was longer with tivantinib than for those on placebo.

Other promising phase III trials employ new technologies to deliver drugs for local ablative treatment, like injectable doxorubicin (Livtag[®]) or a delivery system for high-dose cytotoxic substances (e.g. melphalan, Delcath Hepatic Chemostat[®] Delivery System). Furthermore immune-based approaches like the PD-1 inhibitor nivolumab or the CTLA-4 inhibitor tremilimumab in HCV-related HCC may result in changed treatment, but these substances are not yet in phase III testing in HCC. Cancer stem cells (CSCs) in HCC may be another promising target.

Circulating Tumor or Cancer Stem Cells

Tissue-derived molecular markers lack the possibility of monitoring the patient during or after treatment, since this would require repeated biopsies and hence increased risks for the patient. Therefore, the development of minimally invasive diagnostic methods is necessary. Especially in the setting of liver transplantation it is of great importance to identify those patients who will benefit most. Currently, there is a compelling lack of risk prediction strategies that would enable the physician to provide a tailored treatment in terms of primary intervention as well as adjuvant/neoadjuvant therapy. In addition, it is apparent that one of the limitations in risk prediction we face today is the limitation to obtain HCC tissue prior neoadjuvant therapy. Frequently, histological diagnosis (i.e. liver biopsy) is not required, and patients will receive bridging therapies such as SIRT. Furthermore, there is compelling evidence that a fraction of tumor cells might harbor the genetic setup that will allow for implantation and tumor cell dissemination. These cells present with an EMT phenotype and obtain stem-like cell properties that foster migration and metastatic ability of epithelial HCC cells. For these reasons, it is important to expand prognostic profiling from tumor tissue-derived biomarkers to circulating biomarkers. Circulating tumor cells (CTCs) detected in the peripheral blood of HCC patients may represent a possible solution for this diagnostic dilemma [23]. The main obstacle to the broad clinical application of available automated CTC detection methods is the high plasticity and variability of these cells, particularly due to the EMT, a process closely related to the MET pathway as described earlier. EMT inevitably leads to decreased detection of CTCs with techniques based mostly on assumed epithelial characteristics of these cells. Remarkably, changes from epithelial to mesenchymal cell characteristics are significantly correlated to treatment response [20]. Furthermore the intratumoral heterogeneity of HCC is believed to be caused by subpopulations of cells that are genetically identical but display distinct phenotypic states, such as CSCs and non-CSCs. The ability of self-renewal and tumor initiation define CSCs and are relevant to metastasis. CSCs are described as non-equivalent to CTCs. Only CTCs that have the ability to form ectopic metastasis have CSC characteristics and are known as circulating CSCs. An innovative methodology in CSC-targeted therapy is the siRNA-mediated downregulation of signaling pathways involved in carcinogenesis. Though CSC and CTC analysis may not be usable for treatment decisions at the moment, identifying and characterizing the individual tumor cell composition and the constitution of circulating non-hematopoietic cells in the blood of patients before therapy may add important information to the process of personalizing therapy [24].

Conclusions

There are several phase III trials currently recruiting that may change the treatment landscape in HCC. Still the heterogeneous and unique tumor biology of HCC often results in negative studies. Therefore individualized approaches already in the phase I setting are urgently needed, employing panels including molecular and cellular tissue-based as well as circulating markers.

Disclosure Statement

None of the authors has any conflict of interest relevant to this paper.

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