

REVIEW ARTICLE

Hepatocellular carcinoma: insights in the biological treatment beyond sorafenib

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Summary

Hepatocellular carcinoma has an increasing incidence and an impressive mortality. At present, the only authorized systemic treatment is the multi-kinase inhibitor sorafenib. A multitude of clinical trials are aimed at improving outcomes, both in first- and in second-line therapy. In this multitude of clinical trials, the purpose of our article was to familiarize physicians with the mechanisms of action of new biological therapies and to offer an algorithm for optimal trial selection for each patient, based on clinical and biological indicators. The available data were structured as

follows: antiangiogenic therapy, c-MET inhibitors, combinations of chemotherapy with sorafenib, immune response modulators, cellular metabolism modulators, mTOR inhibitors, other multi-kinase inhibitors.

Conclusion: Treatment of advanced hepatocellular carcinoma remains a challenge for oncologists. Choosing the "right" trial may be the only chance of prolonging patient survival and improve his/her clinical status.

Key words: hepatocellular carcinoma, molecular targeted therapy

Introduction

Hepatocellular carcinoma is the most common primary liver cancer (90 %), the 5th most common cancer and 3rd cause of death from cancer worldwide [1]. In Romania, hepatocellular carcinoma is the 10th most common neoplasia and the 7th cause of death [2]. Although the etiology of hepatocellular carcinoma is well known, the incidence of this disease is steadily increasing, while its high mortality rate is a consequence of late diagnosis (advanced stages) and of the fact that 80% of cases are developed on an already cirrhotic liver. Only 30% of all patients are diagnosed with a localized, potentially curable disease [3].

In the era of molecular targeted therapies with multiple opportunities to intercept inter- and intracellular signaling in cancer tissues, biological research is done on a particularly fertile ground in oncology. Although many targeted therapies

have proved effective in the treatment of different malignancies, some of them act through a mechanism that is not completely understood - a good example is sorafenib in the treatment of hepatocellular carcinoma.

Currently, there are three main known pathways responsible for the occurrence and progression of hepatocellular carcinoma. These pathways are involved in cellular proliferation (RAS/Raf/MAPK pathway), survival (AKT/mTOR pathway) and differentiation (Wnt and Hedgehog pathways) [4]. In addition, angiogenesis mediated by VEGF (vascular endothelial growth factor) and PDGF (platelet-derived growth factor) is highly expressed in hepatocellular carcinoma. Most indolent cancers are initiated by β -catenin gene mutations. Overexpression of Wnt -1 plays a central role in embryonic development, organogenesis, angiogenesis and proliferation of stem cells. In addition, it is of major importance in the progres-

sion of hepatocellular carcinoma. Wnt proteins (19 known until now) are involved in the carcinogenesis of several cancers. Regarding hepatocellular carcinoma carcinogenesis, recent proteomic studies and mRNA analysis showed an overexpression of Wnt -1 in relation with B and C viral infections. Hepatitis B virus protein-Xa (HBx) increases β -catenin stability, a process which is essential in the activation pathway of Wnt/ β -catenin. Wnt -1 overexpression is associated with hepatocyte proliferation. This proliferation is mediated by HCV “core” protein and nuclear factor κ B-p50. All these data support the central role of Wnt in hepatocellular carcinoma carcinogenesis induced by hepatitis viruses. In addition, overexpression of Wnt -1 appears to be a prognostic marker for a high risk of recurrence in patients with viral infections [5]. Unfortunately, pharmacological blockade of Wnt/ β -catenin pathways is not available at the present time. However, the blockade of tyrosine kinase receptors involved in Ras-Raf-MAPK pathway is possible, and the Akt pathway may be intercepted by inhibiting mTOR.

In addition, the development of invasive cancer is heavily dependent on the presence of a favorable tumor microenvironment. The intercellular “dialogue” is a continuous process and it may influence many features, from carcinogenesis to metastasis and invasion [6]. Many innovative molecular therapies that are presented in the following paper address precisely this relationship between tumor and the tumor microenvironment.

Treatment with sorafenib is indicated as first-line therapy for most patients with Barcelona Clinic Liver Cancer stage C (BCLC-C) and some selected patients with stage BCLC-B (who are not candidates for locoregional therapies).

Sorafenib is a small molecule that inhibits tumor cell proliferation and tumor angiogenesis and increases the rate of apoptosis in different tumor models. It acts by inhibiting the serine-threonine kinases Raf-1 and B-Raf and the receptor tyrosine kinase activity of VEGFR (vascular endothelial growth factor receptors) 1, 2, and 3 and PDGFR- β (platelet-derived growth factor receptor β). Cellular signaling that is mediated by the Raf-1 and VEGF pathways has been implicated in the molecular pathogenesis of hepatocellular carcinoma, thus providing a rationale for investigating sorafenib for hepatocellular carcinoma.

SHARP (Sorafenib Hepatocarcinoma Assessment Randomized Protocol) phase III study included 602 patients with well-preserved liver function (>95% Child-Pugh A), randomized to receive ei-

ther sorafenib 400 mg or matching placebo orally b.i.d. on a continuous basis, until progression or unacceptable toxicity. Primary outcomes were overall survival (OS) and the time to symptomatic progression. Secondary outcomes were the time to radiologic progression and safety.

Median OS improvement for sorafenib was 2.8 months (10.7 months in the sorafenib group and 7.9 months in the placebo group, hazard ratio 0.69; 95% confidence interval, 0.55 to 0.87; $p < 0.001$). There was no significant difference for the median time to symptomatic progression (4.1 vs 4.9 months, respectively, $p = 0.77$). The median time to radiologic progression was 5.5 months in the sorafenib group and 2.8 months in the placebo group ($p < 0.001$). The disease-control rate was significantly higher for sorafenib vs placebo (43 vs 32%, $p = 0.002$). Diarrhea, weight loss, hand-foot skin reaction, and hypophosphatemia were more frequent in the sorafenib group [7].

Since the approval of sorafenib as the standard systemic first-line treatment, no other systemic therapy has demonstrated superior efficacy in first-line treatment; moreover, the results of clinical trials could not establish a standard of care for patients progressing under treatment with sorafenib. Given the above data (abundance of eligible patients, the limited results in the first-line treatment, absence of standard second-line therapy), there are a multitude of clinical trials underway, with the purpose to identify systemic therapies with better results, both as comparison/combination with sorafenib and in patients with progression after first-line therapy.

On the following pages we attempt a classification of the clinical trial research in hepatocellular carcinoma according to the mechanism of action of the novel drugs, based on data from published clinical trials or trials still in progress [8].

1. Antiangiogenic therapy : ramucirumab , AMG 386
2. c -MET inhibitors : cabozantinib , tivantinib
3. Combinations of chemotherapy with sorafenib
4. Immune response modulators (nivolumab , Pexa - Vec, tremelimumab)
5. Cellular metabolism modulators
6. mTOR inhibitors : everolimus
7. Promotion of apoptosis
8. Multi-kinase inhibitors: brivanib , linifanib , sunitinib.

Antiangiogenic therapy

Angiogenesis can be seen as a physiologic process involved in wound healing, ovulation, fetal development or as a pathologic process such as diabetic retinopathy, rheumatoid arthritis or cancer development. The main engine of angiogenesis is the interaction of VEGFs and their receptors (VEGFRs), but many other factors may also influence this process and its consequences on tumor progression. We can mention here a few examples: inflammation mediators, metalloproteinases, β -PDGF (platelet-derived growth factor β), TNF- α (tumor necrosis factor- α), TGF (transforming growth factor). Therefore, angiogenesis is a process that results from the interaction of endothelial cells, tumor cells and the tumor microenvironment [9].

Development of hepatocellular carcinoma is dependent on new blood vessels formation through various mechanisms such as sprouting, vascular recruitment, vasculogenesis from precursors or by invagination. In all of these, VEGF plays a central role. VEGF synthesis is dependent on many local processes and it may be independently controlled by the local hypoxia and acidosis. Amplification of VEGF expression is a consequence of gene mutations, hormones, cytokines, nitric oxide and MAP kinases. In addition, VEGF synthesis is dependent on the degree of tumor differentiation, presence of vascular invasion or portal thrombosis and chronic liver disease. An increased level of circulating VEGF is associated with poor prognosis and a high rate of relapse and it can occur after applying a local treatment such as surgery, radiofrequency ablation or TACE (transarterial chemoembolization). The consequence of an increasing gradient of VEGF is vascular hyperpermeability, extracellular matrix remodeling and endothelial cell activation. Antiangiogenic therapy may normalize tumor vasculature and enhance the effect of chemotherapy and radiotherapy [10].

As hepatocellular carcinomas are highly vascularized with an increase in microvascular density and high levels of VEGF, various trials have used monoclonal antibodies such as bevacizumab alone [11] or in combination with other drugs, with good preliminary results. One phase II study used the combination of gemcitabine with oxaliplatin (an active regimen in pancreatic and biliary tumors, but also in hepatocellular carcinoma in phase II studies) together with bevacizumab for the treatment of advanced hepatocellular carcinoma. The objective response rate was 20%, and

27% of the patients had stable disease. The median OS was 9.6 months and the progression-free (PFS) survival 5.3 months [12]. This combination has brought encouraging results and should be further investigated in comparative trials.

The combination of erlotinib (EGFR tyrosine kinase inhibitor) and bevacizumab produced a median OS of 15.7 months in phase II studies [13-15] and because of these promising results, it is presently studied in comparison with sorafenib. The combination of bevacizumab with capecitabine showed only modest results (median PFS) and OS was 2.7 and 5.9 months, respectively [16].

The treatment with sorafenib was the first systemic treatment that has shown to improve survival in patients with advanced hepatocellular carcinoma [7,17]. Sorafenib continues to be studied with the intention of optimizing the antitumor effect: as an adjuvant therapy or in combination with other methods such as chemoembolisation (DEB-TACE) [18] or in combination with radiation therapy [19]. *In vivo* and *in vitro* tests showed that radiation therapy applied sequentially with sorafenib induced a reduction in tumor blood supply and the mitotic index, with a better effect than concomitant administration, or than radiation therapy alone. These data may have significance in the design of future trials and clinical decisions.

Although multiple anti-angiogenic therapies have shown promising results in phase II trials, not many have been evaluated in phase III. A positive example toward this direction is ramucirumab, a recombinant monoclonal antibody which binds to the extracellular domain of VEGFR-2 (Vascular endothelial growth factor receptor-2), recently approved by the Food and Drug Administration for advanced gastric cancer. In hepatocellular carcinoma, ramucirumab demonstrated satisfactory efficacy in a phase II trial in sorafenib-naïve patients with a disease control rate of 50 % [20]. Ramucirumab is now studied in second-line hepatocellular carcinoma treatment after sorafenib in a phase III trial, called REACH [21], whose results are expected soon. REACH is a double-blind, placebo-controlled study (given that no medication has shown satisfactory efficacy in second-line treatment in order to establish a standard treatment). However, only patients with preserved liver function (Child-Pugh under 7) were included, a relatively rare clinical situation in patients with BCLC-C stage who had progressed after treatment with sorafenib.

Because the majority of patients treated with anti-VEGF therapy develop resistance after a var-

iable period of time, other ways of inhibiting tumor angiogenesis are explored. The Ang/Tie signaling pathway is composed of two receptor tyrosine kinases (Tie1 and Tie2), preferentially expressed by vascular endothelium and three ligands (Ang1, Ang2 and Ang4). As Ang2 functions generally as an antagonist of Ang1, the balance of the two proteins has an important role in defining the vascular phenotype: stabilized or destabilized. Ang1/Tie2 interaction promotes vascular stabilization and reduces vascular permeability. Ang2/Tie2 interaction may induce tumor angiogenesis suppression, but since Ang 2 may act as an Ang1 antagonist, it may promote the destabilization of local vasculature and will allow new vessels to sprout [22]. To tackle these processes, AMG 386 (trebananib) was developed - a peptidobody that binds to and inhibits antiangiopoietin 1 and 2, thus blocking their interaction with the receptor Tie2. This may inhibit angiogenesis and may eventually lead to an inhibition of tumor cell proliferation. AMG 386 is currently investigated in the treatment of hepatocellular carcinoma in a phase II study [23]. Safety data regarding the administration of AMG 386 are available [24]. Taking into account the above data, we must draw attention to the possibility of action as a double-edged sword for dual blockade of both Ang and VEGF. Blocking both molecules appears to be the best therapeutic approach, since VEGF may increase the proangiogenic effect of Ang2 [22].

c-Met inhibitors

c-Met is a tyrosine kinase receptor associated significantly with the progression of hepatocellular carcinoma. Stimulation of this pathway (e.g. by means of des- γ -carboxyprothrombin or DCP) [25] is responsible for promoting tumor growth, angiogenesis and metastasis [26,27]. DCP overexpression is associated with the presence of vascular invasion and metastasis. Thus, inhibiting the function of c-Met represents a goal in the treatment of hepatocellular carcinoma, especially in patients with enhanced expression of the DCP. The main ligand of c-Met is the hepatocyte growth factor (HGF) and their interaction will stimulate tissue regeneration, cell proliferation and local invasion. In addition, the c-Met receptor is a functional partner of VEGF signaling, and c-Met overexpression is associated with resistance to anti-VEGF therapy. There are currently studied molecules targeting c-Met pathway. Cabozantinib (inhibitor of c-Met, VEGFR2, RET, KIT, AXL, and

FLT3, already registered for the medullary carcinoma of the thyroid gland) has demonstrated efficacy in phase II hepatocellular carcinoma trials [28,29]. Tivantinib (that binds to the c-Met protein and disrupts c-Met signal transduction pathways) is studied as single agent or in combination with sorafenib [30-32].

Combinations of chemotherapy with sorafenib

Many trials have studied systemic chemotherapy in hepatocellular carcinoma, with limited success. The most used agent was doxorubicin. A study on 60 patients, published in 1988, comparing doxorubicin with supportive therapy demonstrated a statistically significant ($p=0.036$), but clinically very modest benefit in terms of survival, from 7.5 weeks to 10.6 weeks [33]. Doxorubicin has traditionally been used to treat hepatocellular carcinoma despite low response rates and marginal impact on survival. In a phase III study, PIAF regimen (cisplatin, interferon, adriamycin, 5-fluorouracil) was compared with single-agent doxorubicin. Although the response rate favoured the combination (20.9 vs 10.5%), the differences in survival were not statistically significant (8.7 vs 6.8 months, $p=0.83$), and toxicity was increased in PIAF [34]. The GEMOX regimen appeared better tolerated but it has been tested only in a phase II study. Other conventional chemotherapy regimens or even hormones were ineffective and cannot be recommended based on current data [35-38].

However, sorafenib is studied in combination with chemotherapy, like doxorubicin [39] or gemcitabine and oxaliplatin [40] or oxaliplatin and capecitabine [41].

The combinations between biologic therapies and chemotherapy have the disadvantage of increased side effects. Complications such as diarrhea and neutropenia may even be fatal in patients with decompensated chronic liver disease. As a result, administration of such combination may have side effects that outweigh the benefits. To improve the therapeutic index, patients enrolled in such studies must be carefully selected (preserved liver function, good performance status). However, most patients have advanced-stage disease and might be unable to tolerate a combination treatment due to complications caused by the presence of the underlying malignancy, especially long-suffering chronic hepatitis (viral hepatitis, toxic liver cirrhosis).

Immune response modulators

The immune system can play a dual role in the development of liver tumors. In most cases, following interactions in the tumor microenvironment, immune system suppression may be induced. For example, the interaction between PD-1 protein (programmed death-1) and its ligand (PD-L1) induces inactivation of T cell lymphocytes, a major mechanism for local immune suppression. Current studies show a favorable toxicity profile in the context of a good quality response in hepatocellular carcinoma after treatment with monoclonal anti-PD 1 antibody nivolumab [42]. Preliminary data suggest a correlation between the level of PD-L1 expression and response rates [43].

Creating antitumor vaccines remains an important line of research, so the idea of increasing antitumor immune response was investigated in the case of hepatocellular carcinoma. The oncolytic immunotherapy JX-594, also known as Pexastimogene devacirepvec (Pexa-Vec) has three mechanisms of action: viral oncolysis, acute reduction in tumor perfusion and antitumor immune response amplification. Phase I and II studies have shown good tolerability and rapid response regardless of the mode of administration: intratumoral injection or i.v., alone, or sequential with sorafenib. Taking into consideration the fact that efficacy seems to be dose-dependent, the optimal mode of administration remains to be defined [44,45].

Tremelimumab (CP-675206) is a monoclonal anti-CTLA-4 (Cytotoxic T-Lymphocyte Antigen-4) antibody, with similar mechanism of action to ipilimumab (the latter is already authorized for clinical use in malignant melanoma). The expression of CTLA-4 on activated T lymphocytes inhibits the antitumor immune response. Blocking the CTLA-4 mediated inhibition allows antitumor response in multiple malignancies such as melanoma, prostate or bladder. Data from studies conducted so far seem to be promising [46,47].

Cellular metabolism modulators

Hepatocellular carcinoma cells require an increased intake of exogenous arginine for growth and are also deficient in the expression of arginin-succinate synthase, which makes them auxotrophic for arginine. Given these data, ADI-PEG20 (pegylated arginine deiminase) was studied. ADI-PEG20 is an enzyme involved in the degradation of arginine. After analyzing the results from phase II studies for potential efficacy and safety, ADI-PEG20 is currently investigated in

combinations with cytotoxic agents as first-line treatment [48-51], or alone, after progression to sorafenib [52].

mTOR inhibitors

One of the ways to stimulate tumor growth in hepatocellular carcinoma cells is the PI3K-Akt-mTOR (mammalian target of rapamycin) pathway. Stimulation of this pathway is important in the synthesis of VEGF which, in turn, may stimulate the tumor proliferation, progression and metastasis. This process is accomplished by means of EGFR and IGF-1 receptor. Considerable efforts are made to block the Akt pathway, at any level [53,54]. Some new medications, like everolimus (a m-TOR inhibitor approved for clinical use in breast cancer, renal cell cancer and neuroendocrine tumors) seems to have a minor antitumor activity in the hepatocellular carcinoma patients even if they have already been exposed to another therapy [55].

Promotion of apoptosis

Mapatumumab is a human agonistic monoclonal antibody that targets one of the TRAIL death receptors, TRAIL-R1 (TRAIL-Receptor 1), and may promote apoptosis of cancer cells. Soon will be available the results of a randomized phase II study, where the Institute of Oncology Ion Chiricuta, the Regional Institute for Oncology Iasi and Oncolab Craiova were actively involved. The study compared the efficacy and safety of sorafenib with or without mapatumumab in advanced hepatocellular carcinoma.

Multi-kinase inhibitors

So far, sorafenib is the only agent that has demonstrated therapeutic benefit, increasing OS in advanced hepatocellular carcinoma [7]. Although its complex mechanism of action is not completely understood, the anti-angiogenic pathway is of therapeutic importance, leading to the conclusion that other tyrosine-kinase inhibitors with anti-angiogenic effects may also be effective.

Brivanib, a dual inhibitor of VEGF and FGF (fibroblast growth factor) was tested in the first-line treatment vs sorafenib [56], and second-line treatment in patients who progressed on sorafenib or were intolerant to sorafenib [57]. Brivanib showed no benefit over sorafenib and no better tolerability profile. In addition, brivanib did not bring any survival benefit over placebo in the second-line

Table 1. Adaptive trial design and selection of patients for hepatocellular carcinoma trials

<i>Mechanism of action/ Decision criteria</i>	<i>Pro</i>	<i>Against</i>
Antiangiogenic therapy	Early recurrence after local treatment	c-Met overexpression (or anti-VEGF failure)
	High level of VEGF	Active bleeding or increased bleeding risk (Esophageal varices, hepatic failure)
	Decompensated liver disease	Future surgery
	Vascular, portal invasion	
c-Met inhibitors	No criteria in favor of VEGF	Unknown sensitivity to anti-VEGF
	DCP overexpression	Low level of DCP
	Liver metastases	
	Anti-VEGF failure	
Combinations of sorafenib and chemotherapy	Treatment with sorafenib has clear benefits. The association of chemotherapy may improve survival without unacceptable toxicity	
	Compensated liver disease	Decompensated liver disease
	Good performance status	Low performance status
Immune response modulators	Novel mechanism of action that may overcome resistance to sorafenib	Incomplete clinical data Lack of predictors of treatment response
Cellular metabolism modulators	The enzymatic degradation of arginine has possible antiviral and antitumor effects	Incomplete clinical data Lack of predictors of treatment response
mTOR inhibitors	The mTOR pathway has been extensively studied and has a clear role in the pathogenesis of hepatocellular carcinoma	Incomplete clinical data
		Lack of predictors of treatment response
Promotion of apoptosis	Combination with sorafenib feasible	Lack of predictors of treatment response
Multi-kinase inhibitors	The only approved systemic therapy for the treatment of hepatocellular carcinoma acts by inhibition of the same mechanism. The likelihood that other inhibitor to be effective is high.	There is no effective multi-kinase inhibitor after sorafenib failure

treatment.

Linifanib, a potent inhibitor of VEGF and PDGF, has demonstrated no superior efficacy compared to sorafenib and no better safety profile [59].

Regorafenib, a dual targeted VEGFR2-TIE2 tyrosine kinase inhibitor (approved for clinical use in colorectal carcinomas and GIST) demonstrated acceptable tolerability and tumor activity in phase II studies in hepatocellular carcinoma patients who have progressed on sorafenib [59] and is currently investigated in a phase III study [60].

Although initial studies had promising results [61-63], sunitinib achieved inferior results compared to sorafenib in a phase III study, both

in terms of OS and toxicity [64].

As a general observation, most of the older studies have recruited “consecutive” patients, regardless of their biological profile and then tried to characterize the responders and to retrospectively identify the predictive biomarkers in those cases. Although this approach is practical, it may be useless for the vast majority of patients. The so-called “adaptive design” of clinical trials (randomization according to genetic / biological / clinical markers, with the purpose to “enrich” the active treatment arm with patients that, in theory, will benefit most) is gaining more and more ground (Table 1).

Conclusions

With the purpose of improving outcomes, there is great interest in defining the new molecular classification in hepatocellular carcinoma [65]. This will allow a better strategy in research and treatment decision [66]. Until then, beside the

routine use of the clinically approved sorafenib, active participation in clinical trials (using the right staging and based on clinical and biologic features) will help the patients and will bring a benefit both to the science and to the clinical practice.

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