REVIEW

Systemic treatment for hepatocellular carcinoma: Still unmet expectations

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Abstract

Many patients with hepatocellular carcinoma (HCC) are diagnosed in an advanced stage, so they cannot be offered the option of curative treatments. The results of systemic chemotherapy are unsatisfactory and this has led to molecular targeted approaches. HCC develops in chronically damaged tissue due to cirrhosis in most patients. Several different cell types and molecules constitute a unique microenvironment in the liver, which has significant implications in tumor development and invasion. This, together with genome instability, contributes to a significant heterogeneity which is further enhanced by the molecular differences of the underlying causes. New classifications based on genetic characteristics of the tissue microenvironment have been proposed and key carcinogenic signaling pathways have been described. Tumor and adjacent tissue profiling seem biologically promising, but have not yet been translated into clinical settings. The encouraging first results with molecular - genetic signatures should be validated and clinically applicable. A more personalized approach to modern management of HCC is urgently needed.

Key words: Systemic; Chemotherapy; Hepatocellular carcinoma; Prognosis

Core tip: The complete failure of chemotherapy in previous years gradually shifted hepatocellular carcinoma (HCC) treatment to the molecular targeted therapies. The initial-albeit limited - effectiveness of the currently approved systemic therapy, sorafenib, is due to the successful combination of targeting cancer cells and their microenvironment. Trials on drugs other than sorafenib, alone or in combination with drugs or transcatheter arterial chemoembolization were disappointing. Recently, genomic based analyses in HCC patients have proposed subclasses, based on molecular characteristics and a proliferative or non-proliferative genotypes. Combined targeted therapies, driven by specific molecular signatures for treatment selection and monitoring, potentially with immunotherapy, could be a future personalized approach.

INTRODUCTION

Hepatocellular carcinoma (HCC) represents globally the fifth most common cancer and is considered the third most frequent cause of cancer related death[1]. In recent years there has been a significant progress in clarifying pathogenesis, etiology, and risk factors for hepatocarcinogenesis. Understanding the importance of underlying cirrhosis in the majority of HCCs led to more integrated approach, as in the majority of cases we have to deal with two diseases, cirrhosis and cancer.

The adoption of the barcelona-clinic liver cancer (BCLC) classification[2,3] offered the opportunity to better categorize HCC patients and select the best treatment option according to tumor stage, degree of liver function impairment and patient characteristics. The outcomes for surgical resection have improved and specific factors, as tumor and liver function characteristics, are being taken into account before the patient is referred for an operation[4]. Moreover, the widespread application of the Milan criteria in the field of transplantation, has changed the transplant procedure from an experimental approach to a standard of care therapy for HCC, which can treat at the same time the tumor and the underlying preneoplastic process (namely cirrhosis)[5].

Despite screening patients at risk[6], adopting regular surveillance rules and the impressive improvements in imaging, still many patients with HCCs are diagnosed in an advanced stage, thus being ineligible for radical treatments [transplantation, resection or Radiofrequency ablation (RFA)] or even for ablative techniques [transcatheter arterial chemoembolization (TACE)] that can also provide survival benefit[7].

Patients with advanced HCC, especially if complicated with advanced cirrhosis, have a dismal prognosis. Several therapeutic efforts on this group of patients gave disappointing results in the past. The complete failure of systemic chemotherapy in previous years gradually shifted HCC treatment to the molecular targeted therapies. The first successful trials of sorafenib[8,9] provided a meaningful survival benefit in patients with advanced HCC, leaving at the same time many unresolved issues. This review attempts to present the effort of the scientific research to address the problem of HCC in multiple levels and to critically evaluate the inadequacies of the current trials of systemic treatments.

THE STORY OF NEAR-FAILED SYSTEMIC TREATMENTS

Initial approaches with systemic therapy were ineffective, as HCC is refractory to conventional chemotherapy and poorly tolerable in the context of liver cirrhosis due to altered drug metabolism and toxicity. Initial evidence for some efficacy of the anti-estrogen agent Tamoxifen in small trials were not confirmed in larger clinical trials and the drug has been abandoned[10].

More interesting data came in to light with clinical studies of Somatostatin and its long acting analogues for advanced HCC with very promising initial results[11,12], given the antiproliferative activity of the hormone and the positivity of HCC in somatostatin receptors in roughly 40% of the tumors[13]. Further publications have documented that somatostatin leads to apoptosis and has antineoplastic properties. Nevertheless, randomized trials - mainly from western countries - did not identify a clear survival benefit and this treatment is no longer recommended. There has been criticism for the methodology of these trials and the heterogeneity of selected patient population[14].

Sorafenib, the only currently approved systemic treatment, that demonstrated statistically significant improvement in overall survival and prolonged time to progression in two large randomized controlled trials (Sharp and Asian Pacific)[8,15]. The efficacy of Sorafenib has been attributed to blockade of multiple kinases, most of them involved in the VEGF, PDGF, c-Kit and B-Raf and p38 signaling pathways[16]. Despite the low response rates and the associated toxicity, the drug showed survival benefit in Child's A patients with a good performance status.

The safety and efficacy of this treatment was further investigated in the Gideon trial (global phase IV, ongoing), focusing on patients with Child's B that were under represented in the registration trials. The interim analysis showed better outcomes for patients on the full dose (800 mg) as compared to the reduced (400 mg) dose, without significant differences in safety profile[17,18]. However, the median life expectancy of patients under Sorafenib treatment is generally less than one year, and this clearly needs to be improved. For the time being there are no validated factors to predict effectiveness or the possibility of adverse effects[19].

More issues are still open, as what to do when the patient fails to respond or is intolerant to Sorafenib, or if Sorafenib could have a role as adjuvant treatment to other modalities like TACE. More data are expected and towards this direction is a recent study showed that tumor associated neutrophils (TAN) mediate the intratumoral infiltration of macrophages and Tregs by secreting the chemotactic C-C motif ligands CCL2 and CCL17. Thus neovascularization is being stimulated, and HCC growth and metastasis are promoted, all contributing to resistance to Sorafenib[20]. Thus, TAN infiltration is proposed as a potential biomarker.

Sunitinib, a potent multi-targeted receptor tyrosine kinase inhibitor of VEGFR, PDGFR, and c-KIT, reached to phase III study as compared to Sorafenib. The trial was terminated prematurely due to higher incidence of side effects in the sunitinib arm, besides demonstrating no superiority over sorafenib[21].

Brivanib is a potent and selective inhibitor of VEGFR and FGFR and pre-clinical studies have shown *in vivo* antitumor activity[22]. Three phase Ⅲ studies have been conducted, yielding negative results. The BRISK-FL study tested the efficacy of Brivanib *vs* Sorafenib, in patients with advanced HCC without prior systemic treatment[23]. The BRISK-PS study tested Brivanib *vs* placebo in patients that failed or were intolerant to Sorafenib[24]. In both studies Brivanib failed to improve OS but it did improve time to tumor progression (TTP), indicating some anti-tumor activity. Due to these results, a phase Ⅲ trial in which Brivanib was used as an adjuvant to TACE was terminated prematurely[25].

Linifanib is a multi-targeted receptor tyrosine kinase inhibitor effective on VEGFR and PDGFR. A phase Ⅲ trial with 1035 patients comparing Sorafenib with Linifanib, showed similar overall survival in advanced HCC with a more favorable safety profile for Sorafenib; predefined superiority and non-inferiority overall survival boundaries were not met by Linifanib, which was more toxic than Sorafenib[26].

Erlotinib is an orally active inhibitor of epidermal growth factor receptor (EGFR) tyrosine kinase. A phase Ⅲ randomized trial (SEARCH) with 720 HCC patients (Child A cirrhosis) were assigned to Sorafenib/Erlotinib or Sorafenib/placebo[27]. The median OS and TTP were similar in both groups, thus adding Erlotinib to Sorafenib did not improve survival, but increased toxicity instead.

Dovotinib, a VEGFR, PDGFR, FGFR inhibitor was compared head to head with Sorafenib, in a randomized study in the Asian-Pacific in patients with advanced HCC. Although Dovotinib was well tolerated, it failed to show greater efficacy than sorafenib, and thus there will be no phase Ⅲ trial[28].

In patients who stopped Sorafenib due to disease progression or intolerance, a randomized phase Ⅲ trial assessed Ramucirumab, a recombinant monoclonal IgG1 and VEGFR-2 blocking antibody (REACH). Despite acceptable safety profile, the study drug did not reached statistically significant survival benefit *vs* placebo[29]. However, a sub-population with αFP > 400 ng/mL might have benefited from this 2nd line treatment and this is explored in an ongoing trial. Recently Codrituzumab, a humanized monoclonal antibody against Glypican-3 which is expressed in HCC, was studied *vs* placebo in a phase Ⅱ randomized trial without showing any clinical benefit[30].

Tivantinib is an oral selective small MET tyrosine kinase inhibitor with antitumor activity in MET-high patients. A phase Ⅱ randomized placebo-controlled study in patients with advanced HCC, Child's A score and intolerant or progressing under the first line treatment, showed some promising results on time to progression, but with notable neutropenia in some patients[31]. A phase Ⅲ study in patients with advanced HCC expressing high levels of c-MET after Sorafenib failure is underway.

Mammalian target of rapamycin (mTOR) regulates cell growth, metabolism and aging in response to nutrients, cellular energy state and growth factors[32]. It is frequently up-regulated in cancer, including HCC, and is associated with poor differentiation and bad prognosis. Blocking this pathway appears an attractive option for HCC treatment. It is well known from the research on transplantation - given its immunosuppressive properties - that mTOR inhibitors (Sirolimus) are associated with better clinical outcomes in patients transplanted for HCC[33,34].

Preliminary data in the non-transplant setting with Sirolimus and Everolimus treatment in HCC patients were encouraging. In the EVOLVE-1 phase Ⅲ study, patients with advanced HCC and failure/intolerance to Sorafenib, randomized to Everolimus or placebo[35]. Everolimus did not improve OS with no difference to TTP *vs* placebo. Moreover, Everolimus led to hepatitis B virus (HBV) re-activation in 37% of the cases despite preventive antiviral therapies. A recent phase Ⅱ randomized trial of the combination of Everolimus with Sorafenib *vs* Sorafenib alone, in patients with advanced HCC with Child's score ≤ 7, showed that the combination was not more beneficial; in contrast it was more toxic[36].

TACE is the treatment of choice for intermediate stage HCC. However, following TACE the hypoxic micro-environment promotes up-regulation of proangiogenic factors as VEGF and PDGF. This is the theoretical basis for the combination of TACE with drugs that inhibit angiogenesis, as Sorafenib and Birivanib. A recent review and meta-analysis reported that this combined approach may bring benefit to unresectable HCC in terms of TTP but not OS[37]. Recent studies (START, SOCRATES) that investigated the efficacy and safety of Sorafenib as an adjuvant to TACE displayed good tolerability and interesting response rate[38,39]. Clearly a better defined population of advanced HCC -that might have the maximal benefit from this approach- should be tested in clinical trial. Unfortunately, the recently published SPACE trial[40] showed that despite the combination of DC beads TACE with Sorafenib was feasible, this combination did not actually improve time to tumor progression in intermediate HCC.

Beyond TACE, efficacy and safety of Sorafenib was studied in a randomized phase Ⅲ trial *vs* placebo, in patients with HCC after resection or local ablation (STORM trial)[41]. The recurrence free survival was identical in the two arms, whereas side effects were significantly more frequent in patients receiving Sorafenib in whom dose modification was necessary in 90% of the cases.

The combination of Sorafenib with other cytotoxic agents was tested to improve the disappointing results of conventional chemotherapy. In a phase Ⅱ trial[42] the combination of Sorafenib/Doxorubicin was compared to Doxorubicin alone in Child-A cirrhotic patients with advanced HCC. The trial showed that the combination was better than doxorubicin alone as regards time to progression and overall survival. Whether there is benefit of the combination or this is an effect of sorafenib itself, will be clarified in an on-going phase Ⅲ trial.

The efficacy and safety of GEMOX (Gemcitabin/Oxaliplatin) plus sorafenib, followed by sorafenib mono-

Drug	*n* (patients)	OS (mo) SOR/Exp arm	HR
Table 1 Randomized Phase Ⅲ trials in advanced hepatocellular carcinoma			
First line completed (Sorafenib standard)			
Brivanib	1155	9.9/9.5	1.06
Sunitinib	1074	10.2/7.9	1.3
Sorafenib/Erlotinib	720	8.5/9.5	0.92
Linifanib	1035	9.8/9.1	1.04
Second line completed (placebo standard)			
Brivanib	395	8.2/9.4	0.89
Everolimus	546	7.3/7.6	1.05
Ramucirumab	565	7.6/9.2	0.86

OS: Overall survival; SOR: Sorafenib arm.

therapy was examined in a small trial with 49 patients diagnosed with advanced HCC[43]. This approach was found effective (overall survival 15.7 mo) with manageable toxicity, and these results should be validated in a larger controlled trial. The data of a subsequent phase Ⅱ randomized study on this combination, as well as the results of a single arm phase Ⅱ study combining sorafenib with oxaliplatin/capecitabin, showed modest synergistic effect[44]. Further combinations that were tested, such as sorafenib with EGFR inhibitors or with mTOR inhibitors, both failed to show any meaningful antitumor activity.

Finally, the combination of Sorafenib with Octreotide was tested in a phase Ⅱ study, recruiting 50 patients with advanced HCC and Child-Pugh score A or B[45]. The combination was well tolerated and displayed TTP 7 mo and median overall survival 12 mo. Nevertheless these results have not been confirmed in a larger phase Ⅲ study as yet. We believe that this combination could provide an option for patients with inadequate response or intolerance to sorafenib (Figure 1).

The apparent failure of phase Ⅲ trials beyond sorafenib, was disappointing but not discouraging for the scientific community (Table 1). Factors contributing to this failure and were related to drug toxicity (especially in cirrhotic patients), lack of significant antitumoural potency, lack of our understanding on diverse mechanisms of tumor progression and metastasis or biomarkers predictive of the efficacy of therapy[16]. Study design was another weak point for some trials. Trials in patients with advanced HCC should also pay attention to specific factors as portal vein invasion, the extrahepatic metastases, and the degree of liver impairment.

EXPLORING THE ETIOPATHOGENESIS

Molecular and phenotypic diversity of HCC - Oncogenic pathways

Beyond the success and wide adoption of the BCLC system on staging and prognosis of HCC[46], recently new molecular classifications based on genetic characteristics of the tissue microenvironment have been proposed. However, HCC is a heterogeneous disease and each tumor is a result of unique combination of several genomic defects that lead to a significant diversity in the pathways

of carcinogenesis. It is documented that several differences exist not only amongst different patients, but also between different tumor nodules in the same liver, and even differences in the same nodule.

Cancer cells and stem cells have similar capacity as regards self-renewal, indefinite division, and generation of heterogeneous cell population. The concept of cancer stem cell, referring to a subset of cells bearing stem cell characteristics that is indispensable for tumour development and perpetuation, has been recently adopted[47]. Cancer stem cells are now considered an important target for the eradication of HCC. Furthermore, a 20%-40% of HCC subtypes show progenitor signature suggesting that these tumours derive from liver progenitor cell. These subtypes are highly aggressive and correlate with early recurrence after treatment and metastatic potential, thus correlated to worse prognosis. CD133 antigen (prominin-1) has been identified as a cancer stem cell marker in various cancers, including HCC. Patients with increased CD133 levels have shorter overall survival and higher recurrence rates compared to those with low expression. Recent data showed that IL-6/STAT3 signalling induced CD133 expression, through function co-operation with NF-κB and hypoxia-inducible factor 1 alpha (HIF-1α) during hepatocarcinogensis[48] (Figure 2A).

Recently genomic based analyses in HCC patients have identified subclasses, based on molecular characteristics and proliferative and non-proliferative genotypes have been proposed[49]. The proliferative subclass - which is associated with a poor outcome - has been linked to the activation of RAS, mTOR, and/or IGF signaling. This has been further categorized into two phenotypic groups: The Wnt/TGF-β group (activation of these pathways) and the progenitor cell group. In the former, the activation of the Wnt and the TGF-β was the predominant feature, while the latter was enriched in progenitor cell, epithelial cell adhesion molecule and cytoskeletal markers and was associated with increased α-fetoprotein at early stages[50]. On the other hand, the non-proliferative subclass was a heterogeneous one, with patients sharing only β-katenin in their molecular profiles[51]. The prognostic implications of these subclasses have been studied but there is no consensus on it and there is no translational clinical research has been done yet.

The paradigm from the management of other cancers such as colorectal cancer and non-small lung cell carcinoma, where mutations of K-Ras and EGFR drive the therapeutic choices, supports this new approach[52]. Unfortunately, HCC is still away from this path despite the success of sorafenib, a multi kinase inhibitor, which seems a proof towards the right direction. A key point may be that in HCC an average of 30-40 mutations were estimated per tumor, with 5-8 of them being the driver mutations[49] affecting cellular homeostasis and involved in the development of malignant phenotype.

A recent elegant study performing exome sequencing analysis of 243 surgically resected HCCs revealed mutational signatures associated with specific risk factors, as combined tobacco and alcohol use, or aflatoxin
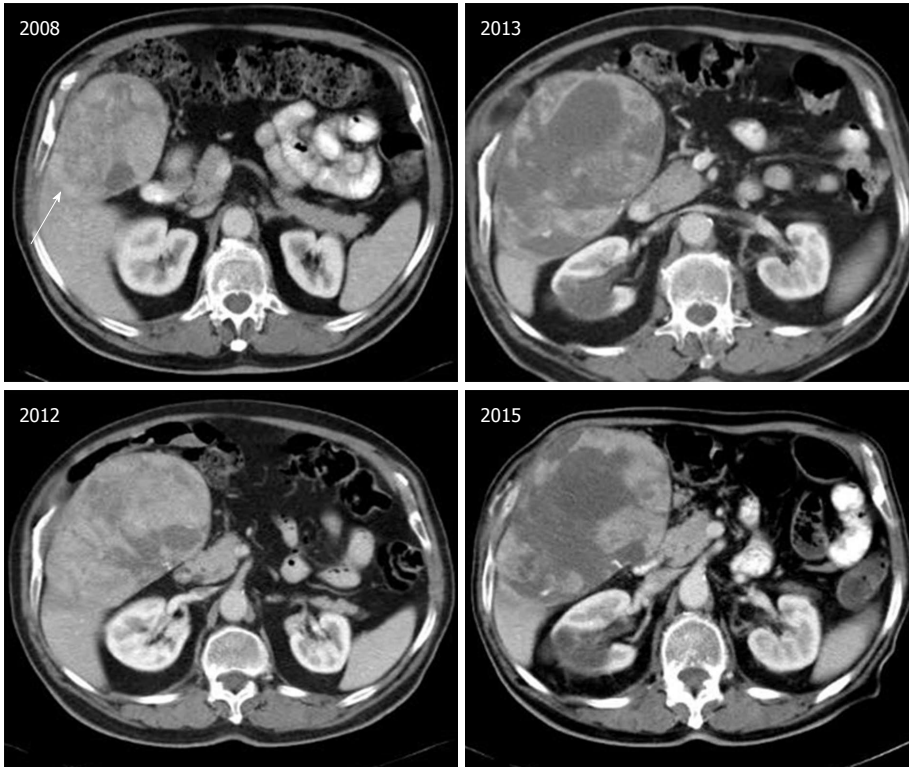
Figure 1 Serial computed tomography scans of a hepatocellular carcinoma patient with multiple co-morbidities precluding radical treatment, surviving 7 years with sequential approach in systemic treatment (Octreotide long acting release, followed by sorafenib). Despite an increase in tumor size, it is evident the central necrosis related to Sorafenib treatment (which was commenced when it became available).
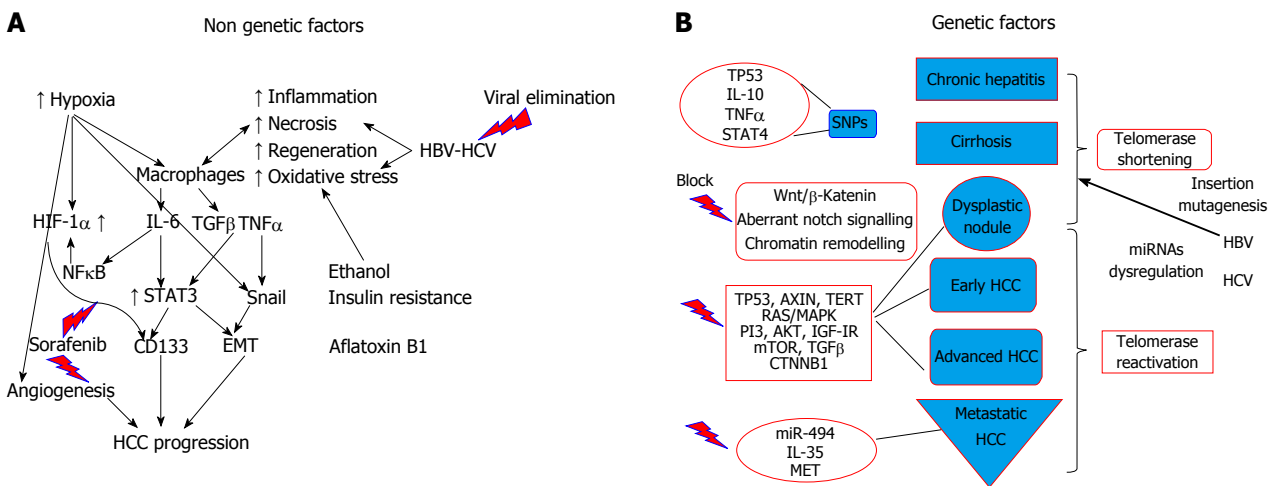

Figure 2 Interplay between genetic and non-genetic factors in the pathogenesis of hepatocellular carcinoma. Potential treatment targets. Hepatocellular carcinoma (HCC) is a complex entity with multifactorial pathogenesis. Control of non-genetic factors (A) (*e.g.*, viral elimination, inhibition of CD133 positive cancer cell overexpression) may lead to alteration of the progress from cirrhosis to HCC. On the other hand, the various genetic irregularities (B) may lead to different HCC profiles with respect to invasiveness (miR-494) or response to treatment. New targeted treatments are also directed against Wnt/β-katenin. HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIF-1α: Hypoxia-inducible factor 1 alpha; TNF: Tumor necrosis factor; IL-6: Interleukin-6; NFκB: Nuclear factor-kappa B.

B1. The researchers identified 161 putative driver genes associated with 11 recurrent pathways[53]. Moreover, a molecular 5 gene score (based on combined expression level of HN1, RAN, RAMP3, KRT19, and TAF9) was studied in surgical resected samples of 314 HCC, and was found significantly associated with outcomes[54]. Also recent data show that it is possible to modulate gene expression profiles (interfering with histone acetylation)

and thus increase the sensitivity to chemotherapeutic agents[55].

Activation of telomerase is the earliest and most frequent alteration in the process of HCC development (mutations in TERT promoter in 60% - most frequently mutated gene - associated with increased telomerase expression)[56]. Genes as *TP53* and *CTNNB1* are also frequently mutated in HCC, whereas inactivating muta-

tions in TP53 are commonly found (especially with HBV etiology). Recently identified alterations in genes encoding metabolic enzymes, chromatin remodelers and a high rate of mTOR pathway activations could offer potential therapeutic targets[57]. Members of the Wnt pathway (crucial for hepatocarcinogensesis) are involved in the process of cell differentiation, which is frequently altered in cancer cells, whereas failure to control oxidative stress can favor additional DNA mutations and cellular damage.

Key carcinogenic signaling pathways have been described for HCC: Wnt/β-katenin [that can be triggered *via* both catenin β1 (CTNNB1)-dependent and CTNNB1 independent pathways][58], a proliferation and hepatoblastoma-like pathway[59]. Nevertheless, their molecular signature is broad and for the time being this knowledge is unlikely to have clinical application. Notch signaling is important for normal liver development and aberrant Notch signaling is related to hepatocarcinogenesis (Figure 2B). Chromatin remodeling is important for the maintenance of DNA integrity, which is in turn crucial for cellular homeostasis. Aberrant chromatin remodeling has been implicated with HCC pathogenesis[57] as well as genes that are involved in oxidative stress (which induces mutations).

In respect to the receptor signaling pathways, RAS/MAPK pathway is activated in all patients with advanced HCC and in a large proportion of those with an early stage HCC[60]. PI3/AKT/mTOR and MAPK pathways related to proliferation, apoptosis and survival, as well as pro inflammatory cytokines (IL1, TNFα) and growth factors, such as TGFβ (tumor stroma, progression, metastasis), are potential future clinical targets in HCC therapeutics (Figure 2B).

Very recently, IL-35 expression was found to correlate with HCC aggressiveness, conferring the rational for another novel therapeutic target[61]. IFG-1R signalling is activated in a proportion of patients with HCC and its targeting had demonstrated antitumor activity in experimental models; however a phase Ⅱ trial with an anti-IFG-1R monoclonal antibody did not show clinical benefit in unselected patients[62]. Finally, dysregulation of MET receptor and its ligand HGF, are crucial for hepatocyte regeneration after liver injury and are common events in HCC patients[49]. Activation of MET is found in half of advanced HCCs, and this pathway is currently tested in clinical trials. A MET inhibitor, cabozantinib, was found to suppress tumour growth and metastasis in a phase Ⅱ study[63] and is further tested in a phase Ⅲ second line clinical trial (in patients with high MET expression, treated with tivantinib).

Tissue microenvironment and the role of cirrhosis
Scientific basis: Chronic liver injury triggers a sequence of cell death, inflammation, compensatory regeneration and genetic damage, which drives the development of HCC. In the majority of cases, HCC develops in chronically damaged tissue due to cirrhosis-irrespective of etiology - whereas the other malignancies develop on an otherwise healthy tissue. This, together with genome instability, contributes to a significant heterogeneity which is further enhanced by the molecular differences of the underlying causes, *i.e.*, viral, alcohol, metabolic[52]. Moreover, epithelial plasticity is an important parameter in HCC, as strong inducers of epithelial to mesenchymal transition like TGFβ are able to co-ordinate both fibrogenesis and carcinogenesis, showing rising cytokine levels in cirrhosis as well as late stage HCC[64].

Several different cell types and molecules constitute a microenvironment in the liver, which has significant implications in tumor development and invasion. Myeloid cells, including macrophages and neutrophils are the most abundant cells in the tumor microenvironment[65]. Tumor-associated macrophages acquire protumorigenic properties in primary and metastatic sites and support cancer development and progression, by stimulating cell proliferation and survival, angiogenesis, invasive behavior and suppression of cytotoxic T lymphocytes responses[66]. Tumor-associated neutrophils exhibit both antitumoral and protumoral functions. Dendritic cells, the main type of antigen presenting cells, play an important role in T cell priming. The generation and protective antitumour immunity depends on dendritic cell maturation and antigen presentation[67].

It is generally accepted that dysregulated microenvironment affects tumorigenesis, based on the concept that chronic inflammation is associated with cancer[68]. Moreover, the stromal microenvironment has been recognized as a crucial element for cancer metastasis in general. A reasonable hypothesis is that an altered liver microenvironment, through reprogramming of the inflammatory milieu, may contribute to hepatocarcinogenesis, taking in account that HCC is an inflammation-associated cancer[69]. This microenvironment plays a major role in anti-tumor immunity.

Therapeutic implications: The effectiveness of the currently approved systemic therapy, sorafenib, is due to the successful combination of targeting cancer cells and their microenvironment, as a result of multiple kinases inhibition. Between sorafenib targets, an increasing amount of evidence has suggested that HSC are key regulators of hepatocarcinogenesis through a variety of mechanisms, including direct effects on malignant hepatocytes, and indirect *via* modulation of the peritumoral stroma and immune responses[70]. Moreover, activated stellate cells produce extracellular matrix.

Laminin-332 is produced and excreted by these cells in HCC but not in the surrounding non-neoplastic liver; this stimulates chemotaxis and migration of HCC cells in experimental models and promotes proliferation as well[52]. An association between Ln-332 and Keratin -19 has been documented, the latter being a marker of cholangiocytes[71].

VEGF not only regulates tumor angiogenesis but also has important immunomodulatory functions. It inhibits dendritic cell maturation *in vitro* and *in vivo*, through activation of NFκB. Additionally VEGF may regulate T-cell

differentiation and its cytotoxic function and can enhance expression of immune checkpoint molecules[72]. This provides the rational of combining anti-VEGF therapy with checkpoint inhibitors.

Another area of active research is on the effect of mTOR inhibitors on advanced HCC in the non-transplant setting. Recent data showed that mTOR inhibition improves FGFR targeting[73] and reduces the activity level of Golgi protein 73, which is a serum marker for HCC[74]. However, the first results of trials with mTOR inhibitors were less than encouraging. Despite potential applications, the role of the whole tissue micro-environment is difficult to be reduced to the effect of just one molecule or protein.

Immunity and implications

Scientific basis: Inflammation affects every single step of tumourigenesis from initiation, to tumor promotion and metastatic progress. Cancer development and its response to treatment are significantly influenced by innate and adaptive immunity, which either promote or attenuate tumourigenesis[66]. Various types of immune and inflammatory cells are present within tumours; these affect malignant cells through production of cytokines, chemokines, growth factors, prostaglandins and reactive oxygen and nitrogen species[68].

The liver has been considered as an immunologically advantaged organ. A profound clinical paradigm is the development of tolerance in the context of transplantation. It is equipped with several myeloid and non-myeloid cell populations which affect both innate and adaptive responses in physiological conditions as well as in the context of defense against tumors[75].

Kupffer cells represent the largest macrophage population in the human body and together with sinusoidal endothelial and hepatic stellate cells, play a critical role in physiology and disease. Local immunosuppression by these cells is induced by pro-inflammatory cytokines[69] whereas different immune cell subtypes have been related to antitumor immunity in HCC. Kupffer cells in analogy to the two subtypes of macrophages are now characterized as M1 and M2 types. In the case of HCC M2 cells are detrimental and M1 demonstrate anti-tumoral activity, contrary to the opposite effects of those cell subpopulations have in inflammation.

Among immunosuppressive cell populations, myeloid derived suppressor cells and T regulatory cells have the key role in cancer immunosurveillance[76]. A prominent humoral cytokine profile occurs in metastatic liver milieu and a shift towards anti-inflammatory/immunosuppressive responses is significant for HCC metastases[77].

Therapeutic implications: The liver is a privileged organ with respect to immune function and possesses a unique form of immune regulation: Tolerance is induced to avoid chronic inflammation caused by antigens coming from the portal vein blood. This may hampers an effective immune response against cancer cells[78]. Moreover this is a challenge on the use of conventional immunotherapy is challenged. Immunotherapy trials have so far given suboptimal results. On the other hand, spontaneous immune responses as well as tumor regression have been reported in relation to systemic inflammatory responses[79]. This could as well be a result of M1 effect as previously mentioned.

Adaptive immune responses are well described in various conventional HCC treatments and are related to their effects. This has been extensively investigated in patients undergoing ablative therapies (TACE, RFA), and provide the theoretical basis for combined approaches. This applies to cytotoxic agents as well, and experience with sorafenib in experimental and clinical level is a paradigm.

While growing tumors acquire mutations, some of which create neoantigens that influence the response of patients to immune checkpoint inhibitors[80]. There are other studies supporting that cancers with high rate of somatic mutations respond best to immune check point blockade by triggering tumor rejection *via* activation of cytotoxic T-lymphocytes, a recent approach with acknowledged success in recent years in melanoma and non-small cell lung cancer[81].

Preclinical and clinical studies have shown potential benefit of modulating immunogenicity of HCC and relevant approaches are currently being tested[82]. The rational to target immune-checkpoints is based on data that HCCs may evade the immune system by expressing molecules as PD-1, CTLA-4, TIM-3, LAG-3 and many more. Despite the fact that the blockade of PD-1 and CTLA-4 is already providing encouraging results in initial trials, overall the therapeutic relevance of blocking these agents is unclear[72].

CONCLUSION

HCC is one of the most lethal cancers and management still deems ineffective. Apart from the problems in prevention or early diagnosis, there are no persuasive answers for those (many) patients with advanced neoplasms. Systemic treatment was disappointing in the past, somehow improved with Sorafenib but with many weaknesses and grey zones, whereas the trials of new compounds beyond Sorafenib provided suboptimal results.

The complexity and heterogeneity of HCC pathogenesis is disregarded in treatment decisions. Is a personalized approach feasible with the limitations of current knowledge? Tumor and adjacent tissue profiling seems biologically significant, but not yet translated into the clinical setting. The role of liquid biopsy, *i.e.,* detection of circulating tumor cells, a hot topic in tumor biology is also inadequately explored in the case of HCC.

Nevertheless, encouraging first results with molecular - genetic signatures are promising towards -at least- prognosis. Additionally, miRNAs which are important regulators of gene expression, have been associated

with the occurrence of HCC. In addition, miRNAs are of potential value not only in diagnosis but also in the management of HCC.

Clinical scoring systems incorporating molecular profile characteristics, may better stratify patients at risk for HCC but further prospective validation is needed. The ideal future approach would be combined targeted therapies - driven by specific molecular signatures for the selection and the monitoring during treatment- potentially incorporating immunotherapeutic modalities, such as vaccination and/or check-point blockade.

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