Published in final edited form as:

J Hepatol. 2016 March ; 64(3): 736–745. doi:10.1016/j.jhep.2015.09.008.

CLINICAL IMPLICATIONS OF BASIC RESEARCH IN HEPATOCELLULAR CARCINOMA

Renumathy Dhanasekaran¹, Sudhakar K. Venkatesh², Michael Torbenson³, and Lewis R. Roberts¹

¹Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, Rochester, MN, USA

²Departments of Radiology, Mayo Clinic College of Medicine, Rochester, MN, USA

³Division of Anatomic Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

Clinical Vignette

A 58-year old Caucasian female has compensated hepatitis C related cirrhosis. Her surveillance ultrasound showed hypodense liver nodules and subsequent triple phase CT scan showed five tumor nodules with diameters ranging from 3-5 cms involving both hepatic lobes. The nodules showed characteristic radiologic findings on the CT scan and she was diagnosed with hepatocellular carcinoma (HCC) based on non-invasive criteria. There was also associated right portal vein tumor thrombosis. Her functional capacity at diagnosis was slightly limited, but she was capable of performing all activities of daily living and self-care. Her laboratory tests at diagnosis were as follows: Na 129, K 3.6, BUN 22, creatinine 1.0, albumin 2.9, bilirubin 1.8, ALT 87, AST 68, Alk Phos 139, WBC 3.5, Hgb 10.4, Plt 73,000, INR 1.9 and AFP 5200 ng/ml. An upper endoscopy was negative for esophageal or gastric varices. Based on the tumor burden, presence of macrovascular invasion, ECOG performance status of 1 and Child Pugh class A she was classified to have BCLC stage C HCC. She was started on sorafenib therapy at 400 mg oral twice daily but unfortunately this had to be discontinued since she experienced severe diarrhea and skin rash. She now returns for follow up and requests information on the available therapeutic options.

This particular case scenario is not uncommon and does raise several clinically relevant questions:

- a. Should her liver lesions have been biopsied for diagnosis?
- b. Are there any serum or tissue biomarkers that could have helped in prognostication?

Correspondence: Lewis R. Roberts, M.B. Ch.B., Ph.D. (Corresponding Author), Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905, roberts.lewis@mayo.edu; Phone: 507-538-4877; Fax: 507-284-0762.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

- c. Was sorafenib the best first option for her and were there any biomarkers that could have predicted the adverse reactions she experienced?
- d. What other potential therapies will be available for her in the near future?

This review provides a comprehensive overview of the current state of HCC management and also examines the clinical implications of recent basic research in HCC.

Introduction

Hepatocellular carcinoma (HCC) is a global problem and the second most common cause of cancer related deaths in the world (1). Its global incidence has been reported to be on the rise and is predicted to exceed a million cases per year by 2025 (1). The overall survival of patients with HCC is dismal with a five-year survival of less than 15%. This is largely due to the fact that a majority of HCCs are diagnosed at advanced stages when patients are not eligible for curative therapies such as resection or transplantation; and advanced HCCs are resistant to most standard chemotherapy regimens. Sorafenib was the first systemic drug to be approved for the management of advanced HCC (2, 3). Although sorafenib therapy is only associated with modest survival benefits, its arrival raised hope for rapid approval of more targeted therapies for HCC. But unfortunately in the past few years several drugs including sunitinib (4), brivanib (5) and everolimus (6) have failed in phase III trials for HCC. There is therefore an urgent need to address several critical challenges faced in the treatment of this cancer in order to improve clinical outcomes. Current research efforts are directed towards discovery of biomarkers for early diagnosis, recognition of molecular subclasses of HCC, correlation of molecular signatures with radiologic/histologic features, characterization of new druggable targets and personalization of therapies based on individual tumor biology. A deep understanding of the molecular pathogenesis of HCC is essential to achieve these goals. The advent of rapid next generation sequencing technology has made it easier to understand tumors from a systems biology perspective and the arrival of newer techniques for rapid genetic manipulation of target genes is enabling us to directly apply the knowledge gained from the 'omics' data. In this review we focus on recent advances in basic research on HCC which have the potential to make the transition from bench to bedside and to eventually have a translational impact on the lives of thousands of patients suffering from this cancer

Diagnosis of HCC- Is there a role for biopsy?

The histological diagnosis of HCC is made by a combination of morphological changes and special studies. The morphological changes of HCC include increased nuclear to cytoplasmic ratios, nuclear hyperchromasia and atypia, and architectural changes. The architectural changes that indicate the tissue is neoplastic are principally the loss of normal portal tracts, thickening or otherwise loss of the normal hepatic plates, and aberrant arterioles in the lobules (in normal livers, the arteries are found only in portal tracts). The morphological findings are then supplemented by additional studies. If the tumor is well differentiated, a panel of markers will be used to distinguish a hepatocellular carcinoma from benign mimics, such as hepatic adenomas, focal nodular hyperplasias and

macroregenerative nodules. If the tumor is clearly cancer, but is poorly differentiated, then a different panel of markers will be used to confirm hepatic origin. Currently, molecular studies are not used routinely for diagnosis. However, in specific variants, molecular studies are helpful in diagnosis, the best example being fibrolamellar carcinoma, where FISH based studies or RT-PCR can be used to detect the PRKACA-DNAJB1 fusion production that is typical of fibrolamellar carcinoma (7).

However HCC is the only cancer which does not require histological confirmation to make a diagnosis. Major society guidelines recommend making a diagnosis of HCC if new masses developing in a cirrhotic liver have characteristic radiological features of arterial hyperenhancement followed by venous washout on cross sectional triphasic imaging (8, 9). Computed tomography (CT) and magnetic resonance imaging (MRI) overall have a high pooled sensitivity (55%–80%) and specificity (70%–91%) in diagnosing HCC (10) but these values are lower when lesions are smaller than 2 cm in size (11) with false negative rates reported to be as high as 20% for small lesions (12). Although guidelines do not distinguish between the use of CT or MRI for diagnosis, a recent large meta-analysis of 40 studies showed that the overall per-lesion sensitivity of MR imaging was higher than that of multidetector CT (80% vs 68%, $P = 0.0023$) (13). Advances in multidetector row CT allow acquisition of multiple arterial phases and use of dual energy CT technique which may improve the sensitivity for detection of HCCs (14). The recent incorporation of hepatocyte specific contrast agents such as gadoxetic acid (Primovist [Bayer Healthcare] in Canada, Europe, and Asia and Eovist [Bayer HealthCare] in the United States) has increased the discriminatory potential of MRIs as the appearance of a hypointense nodule in the hepatobiliary phase is a good predictor of pre-malignancy (15-17). Contrast enhanced ultrasound (CEUS) is another imaging modality used in the diagnosis of HCC which uses echographic agents that generate a map of intralesional vascularization (18). Typical arterial phase enhancement and washout in late phase in CEUS is found in about 97% of HCCs in the background of cirrhosis and the overall the accuracy of CEUS for the diagnosis of HCC is approximately 80% which is comparable to CT imaging (19). CEUS using Kupffer cell agents such as Sonazoid demonstrates HCC as an hypoechoic nodule as HCCs lack Kupffer cells, however clinical utility of this phase is still not well validated (20). A recent study has shown that CEUS may also provide information regarding the degree of differentiation of HCC based on intratumor vascularization (21). Currently, CEUS has not been qualified for the diagnosis of HCC by major societies like AASLD and EASL due to the risk of misclassification of intrahepatic cholangiocarcinomas as HCC. Despite these advances there still remain a small proportion of cases with atypical imaging characteristics in whom the diagnosis cannot be made on imaging alone.

Biopsy is still a part of the diagnostic algorithm for HCC and is currently reserved only for lesions greater than 1 cm that have atypical imaging features. It has been estimated that up to 50% of lesions between 1-2 cm will be indeterminate by imaging (10). One of the main concerns with routine biopsy of all liver lesions is needle track seeding of the tumor, which has been reported at a low but variable frequency, with a large study of more than 1000 patients reporting a frequency of 0.76% (22) and a meta-analysis of 8 studies reporting it to be 2.7% (23). The other risk associated with liver biopsy is bleeding, but this risk is low at around 0.1-0.01% (24). Although the aforementioned risks are small they are not

inconsequential. Based on the current guidelines, the vast majority of HCCs arising in cirrhotic livers are not biopsied, especially given that radiological diagnoses carry a high specificity. Many practitioners elect for close radiological follow up of lesions in the 1-2 cm range, because by the time they reach 2 cm they have often acquired typical features, and particularly because patients are not usually eligible for listing for liver transplantation until their HCC tumors are at least 2 cm in size. In addition, high rates of falsely negative diagnosis occur in biopsies of tumors less than 1 cm HCC owing to the difficulty of targeting a small lesion at a distance from the abdominal surface and the frequent well-differentiated tumor histology.

For successful biomarker discovery in cancer, verification of markers developed in a preclinical setting using a retrospective cohort of stored clinical specimens is critical before final validation in expensive large prospective, randomized, controlled trials can occur. The lack of biological tissue specimens from the majority of patients with HCCs will significantly impede this intermediate phase in the development of biomarkers for HCC and delay the advance towards personalized approaches to HCC management. While some experts argue that lack of known biomarkers and molecular subtypes makes routine biopsies of all HCC patients unethical (25) others argue that increasing the frequency of liver biopsies for HCC especially in clinical trial settings is crucial to identify biomarkers and advance the field of personalized targeted therapy for HCC (24).

In breast cancer clinical trials, it was shown that the employment of a validated biomarker based enrichment, such as HER2, reduced clinical trial failure risk by as much as 50% resulting in cost savings of 27% (26). Similarly, in non-small-cell lung cancer (NSCLC) biomarker-enrichment resulted in a six-fold increase in clinical trial success (27). Given that biopsies and biomarker assessment were not part of the six clinical trials for HCC that have failed, it would seem rational to change clinical trial design in the future to include planned tumor biopsies for assessment of expression of the target against which the drug is directed. A good example favoring this approach is a recent phase II study of the MET inhibitor tivantinib for advanced HCC in which tumor samples were biopsied and immunohistochemistry performed to assess MET expression prior to drug initiation. Subsequently, treatment with tivantinib was shown to be associated with improved survival only in patients whose tumors expressed high levels of c-MET (28). Phase III trials of MET inhibitors are currently ongoing and if the above findings hold true, then biopsy of the tumor to assess MET expression will likely be considered a necessary prerequisite in patients being considered for tivantinib therapy. We envision that a similar approach to future clinical trials has the potential to uncover more actionable biomarkers and advance the clinical practice of tumor biopsy to promote personalized approaches to treatment. A particular challenge in this regard is the known intratumoral molecular heterogeneity of HCCs, which will require that molecular tests are carefully validated to ensure that a single or limited number of tissue cores can provide reliable molecular representation of the entire tumor.

staging of HCC- Present and Future

A universal staging system is important for any cancer not just for prognostication but also for guiding management decisions, facilitating effective communication between treating

physicians and informing clinical trial design. Unfortunately, adopting a universal staging system for HCC has been challenging in part because HCC patients suffer from two conditions simultaneously: the cancer and the underlying cirrhotic liver disease. Thus any staging system for HCC should take into account the various elements of tumor burden, liver function and patient performance status. Over the past few decades several different staging systems have been proposed, including the Okuda staging system, the Cancer of the Liver Italian Program (CLIP), Barcelona Clinic Liver Cancer (BCLC) staging system, Japan Integrated Staging (JIS), the AJCC/UICC surgical TNM staging system, and, most recently, the Hong Kong Staging System. There is significant regional preference in the application of staging systems. The BCLC staging system comprehensively incorporates the tumor size and number, presence of vascular invasion, presence of metastatic disease, liver function and performance status of the patient and has been recommended by both AASLD and EASL for prognostication and treatment allocation (8, 9). This staging system has been validated prospectively (29, 30) and has also been shown in some studies to perform better than other staging systems for prognostication (31, 32).

An important variable which greatly affects clinical outcome and also impacts therapeutic targeting is individual tumor biology. Currently none of the classification systems incorporate molecular subclasses of HCC but efforts are underway to incorporate molecular characterization into staging classifications. In an early study by Lee et al. global gene expression profiling was used to classify HCCs into two subgroups (A and B) with significant survival correlation; the low survival group was shown to strongly express cell proliferation and antiapoptosis gene expression signatures; the same group also later described a hepatoblast subtype of HCC which was associated with poor prognosis (33, 34). Another molecular subclassification was reported by Hoshida et al. who conducted a meta-analysis of gene expression analysis from eight different datasets and described three subclasses of HCC (S1, S2, and S3): subclass S1 exhibited aberrant activation of the WNT signaling pathway, S2 was characterized by proliferation, MYC and AKT activation, and S3 was associated with hepatocyte differentiation (35). Multiple other prognostic subclasses or signatures have been described including chromosomal instability based subclasses (36), MET regulated expression signature (37), transforming growth factor β (TGF β) gene expression signature (38), seven-gene hypoxia signature (39), biliary phenotype signature (40), and a 5-gene score which is reported to predict survival after resection (41) and which is currently being commercialized. The biggest challenges faced while attempting to classify HCC are the marked genomic heterogeneity of this tumor and the epidemiologic dissimilarities between the populations under study, so that currently no single molecular classification is considered to be universally applicable. Given that the prognosis of HCC depends on multiple clinical and molecular features, it is hoped that eventually a classification system for HCC will evolve which will incorporate both the clinical and molecular characteristics of the tumor, thus improving prognostication and refining targeted therapy. Figure 1 depicts such a hypothetical classification system.

Treatment of HCC – Focus on Molecular targeted therapies

The BCLC staging system not only stages HCC but also guides therapeutic strategy; Figure 2 outlines the treatment algorithm proposed by BCLC staging. Broadly speaking, patients

with early stage HCC without portal hypertension are recommended to have evaluation for surgical resection; patients with portal hypertension and tumor within Milan criteria (single tumor ≤ 5 cms or up to 3 tumors ≤ 3 cms) are recommended to undergo liver transplantation evaluation, or if not candidates for liver transplantation, to be treated with ablation (for limited disease of 1-3 nodules no more than 3-4 cm in size) or chemoembolization (for intermediate stage disease); patients with macrovascular invasion or metastatic disease with preserved performance status are recommended to receive sorafenib; and patients with advanced stage tumors with poor liver synthetic function and/or poor performance status are recommended to receive best supportive care. However, the BCLC system is not universally accepted, particularly in Asia, and a few aspects of the treatment algorithm proposed by the BCLC staging system are considered controversial. Most of the disagreement surrounds the care of patients with intermediate stage HCC, which includes a heterogeneous population of patients with tumor burden ranging from a single large tumor (>5 cms) to diffusely infiltrating tumor and liver synthetic function ranging from Child Pugh class A to B. BCLC staging proposes transarterial chemoembolization (TACE) as the mainstay of therapy in this group, but studies have shown that patients with intermediate stage HCC with localized large tumors can still be eligible for resection (42, 43) and some centers successfully transplant patients with tumor burden beyond Milan criteria (44). Patients with intermediate stage HCC who are not candidates for TACE can still be offered sorafenib and other therapies such as radiofrequency ablation, radioembolization with Y90, and Stereotactic Body Radiation Therapy (SBRT), which are routinely employed in the clinical management of HCC, but are not incorporated into the BCLC staging system due to the lack of high quality evidence supporting their use. It is encouraging that large randomized studies evaluating some of these additional local and regional therapies are currently underway. Several experts have proposed further sub-classification of the BCLC intermediate stage HCC to refine the management recommendations and efforts are currently ongoing (45). Following resolution of HBV and HCV with newer drugs, clinical stage downgrading for HCC is expected. Downgrading may modify current therapeutic paradigms, for instance, promoting a shift from transplantation towards hepatic resection or ablation.

We next address the recent advances in the basic research of genomic alterations and molecular pathway dysregulation in HCC that are identifying novel targets for this tumor.

Antiangiogenesis drugs - Sorafenib and beyond

HCC is a highly vascular tumor and derives most of its blood supply from the hepatic artery in contrast to the normal surrounding liver which is predominantly supplied by the portal venous system. Hence targeting angiogenesis has been a major therapeutic strategy in HCC. Several growth factor related pathways promote angiogenesis in HCC including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), and transforming growth factor (TGF β). As a key proof of concept, sorafenib, which inhibits multiple receptor tyrosine kinases including VEGFR and PDGFR signaling pathways, was shown to improve survival in advanced HCC. However, sorafenib is only moderately effective and a substantial proportion of patients have to discontinue the medication either due to intolerable side effects or drug resistance. Hence the ability to predict response and prevent unnecessary

adverse effects is an important area of active research and a number of biomarkers predictive of response to sorafenib have been identified. Arao et al. have shown that tumors with amplification of the FGF3/FGF4 locus on chromosome 11 have profound sensitivity to treatment with sorafenib (46). Horwitz et al. have recently reported that patients with HCCs harboring VEGF-A amplifications showed markedly improved survival after treatment with sorafenib (47). Single nucleotide polymorphisms in the VEGF-A and VEGF-C genes have also been shown to be associated with tumor progression and overall survival (48). An analysis of 77 patients enrolled prospectively in three sorafenib trials who had pretreatment tumor biopsy available showed that elevated tissue expression of pERK and VEGFR-2 was predictive of poor outcome in advanced HCC treated with sorafenib (49). With further validation of these biomarkers it should eventually be possible to predict response to sorafenib and patients who are nonresponders can be offered other systemic therapies, once available, thus avoiding unnecessary side effects. There are also efforts underway to sensitize HCCs to sorafenib and a recent study has suggested that silencing Mapk14 sensitizes HCCs to sorafenib. Combination of sorafenib with Mapk14 blockade therefore has the potential to overcome sorafenib resistance in human HCC (50).

After the initial success of sorafenib, multiple other multikinase inhibitors of growth factor pathways promoting angiogenesis were studied but unfortunately drugs such as brivanib, sunitinib and linifanib (4, 5, 51) have all failed in subsequent phase III trials. Bevacizumab is a monoclonal antibody which directly binds VEGF-A and it initially showed promise in phase II trials but unfortunately it was associated with high rates of adverse events especially variceal hemorrhage (52). Similarly ramucirumab, a VEGFR2 inhibitor, has also failed to meet endpoints in a recent phase III trial (53). One of the main reasons for failure of these trials is the increased toxicity of these drugs compared to sorafenib. Another additional reason for first line therapy failures could have been the enrolment of BCLC A and B patients unfit or non-responsive to standard of care treatments. Failure of second line treatment trials could have been due to biased selection of patients who had progression of tumor on sorafenib. Despite these disappointing results, efforts involving antiangiogenic therapies are still ongoing and results from phase III trials testing lenvatinib, which inhibits VEGFR, FGFR, RET, KIT, and PDGFR- β , and regorafenib, which inhibits VEGFR, PDGFR, FGFR, RET, KIT, and TIE-2 are awaited.

MET inhibitors - First biomarker selected targeted therapy in HCC

There has recently been increased recognition that hepatocyte growth factor (HGF) and its tyrosine kinase receptor MET play a significant role in progression of HCC, thus making it an attractive therapeutic target (54). Expression of MET gene signature was shown to be associated with vascular invasion and poor prognosis in human HCC and high plasma HGF levels were associated with poor survival in patients who received sorafenib in the SHARP trial (55, 56). Tivantinib is a MET inhibitor which has shown promise in phase II trials for advanced HCC. Interestingly the survival advantage was only noted in tumors with high MET expression, thus identifying MET expression as the first potential molecular biomarker for targeted therapy in advanced HCC. Currently a phase III trial involving tivantinib in patients with advanced HCC who have failed sorafenib and whose tumors exhibit high MET levels is underway. Cabozantinib is a dual inhibitor of c-MET and VEGFR2 which has also

shown promise in an early phase trial and is also currently in under study in phase III trials (57).

Immunotherapy in HCC - Bench to bedside

Immunological mechanisms are being increasingly recognized to play an important role in regulating HCC tumor growth and development (58, 59). The immune response elicited by the development of a liver tumor in the background of chronic inflammation is complex and involves simultaneous promotion of tumor tolerance and development of anti-tumor immunity. One of the mechanisms of tumor immune tolerance in HCC includes an increase in the number of regulatory T cells (T-regs), in fact, studies in human HCC have shown that patients who have increased T-regs in their tumors have a poor prognosis (60, 61). Thus it appears that T-regs can be used as prognostic biomarkers and targeting them can lead to an enhanced antitumor immune response. Multiple other mechanisms contribute to the immune tolerant microenvironment in HCC including but not limited to changes in the Programmed Cell Death 1 Ligand 1 (PD-L1) / Programmed Cell Death 1 (PD-1) immune checkpoint pathway (62), expression of immune inhibitory ligands like CTLA-4 (63), and an increase in immune-suppressive cells such as tumor associated macrophages (63) and natural killer (NK) cells (64). On the contrary, some anti-tumor immune response is mounted against tumor-associated antigens (TAAs) in most patients, and detection of HCC specific CD8 T cells was associated with decreased HCC recurrence after surgery (65, 66). Unfortunately, the overall balance between the tumor-tolerant and anti-tumor forces favors tumor immune tolerance and hence the complex interplay of immune regulation in the tumor microenvironment ultimately leads to tumor progression.

Multiple immune based therapeutic approaches are currently under study and several phase I-II clinical trials are underway (58). A few clinical trials with vaccines against TAAs like alpha fetoprotein (AFP) (67), telomerase reverse transcriptase (TERT) (68) and glypican-3 (GPC3) (69) have been completed, but have only shown modest efficacy. Further strategies to improve the outcomes of treatment with anti-tumor vaccines include the development of vaccines that simultaneously target multiple TAAs and the use of novel techniques such as computer-guided epitope-optimization to design the most effective vaccines (70). Another rational therapeutic target is the B7-CD28/CTLA-4 pathway. Recently, immune checkpoint inhibiting antibodies including ipilimumab (anti-CTLA-4), pembrolizumab (anti-PD-1) and nivolumab (anti-PD-1) have been approved by the FDA for the treatment of melanoma (71) and have shown promise for the treatment of other cancers including lung cancer and renal cell carcinoma (72). Tremelimumab, a monoclonal antibody that blocks CTLA-4 was the first checkpoint inhibitor studied in human HCC and was reported to have antitumor efficacy while demonstrating a satisfactory safety profile (73). A recent early report from a phase I/II study suggests that nivolumab has clinical efficacy without significant toxicity in patients with advanced HCC, allaying fears about using immune checkpoint inhibitors in a disease associated with viral infections (74). Given that multiple immune-suppressive mechanisms exist in the liver tumor microenvironment, a combination of these strategies may ultimately be needed to improve clinical outcomes.

mTOR inhibitors

The mammalian target of rapamycin (mTOR) pathway has been shown to regulate inflammation, autophagy and angiogenesis in cancer cells. This pathway is upregulated in 40-50% of HCCs (44, 51). In line with this, upregulation of the mTOR pathway has been associated with vascular invasion, tumor recurrence and poor prognosis in HCC (52). Hence, the mTOR pathway has been seen as an attractive therapeutic target for drug development in HCC. Unfortunately in a recent phase III randomized controlled trial (EVOLVE-1), everolimus did not improve overall survival in patients with advanced hepatocellular carcinoma that progressed during or after receiving sorafenib or who were sorafenib intolerant (53). Despite these negative results, given the strong preclinical data supporting a role of the mTOR pathway in HCC, clinical trials evaluating sirolimus for prevention of post-transplant HCC recurrence and trials of second-generation mTOR inhibitors such as temsirolimus and a dual inhibitor of TORC1/TORC2 (CC-223) are underway.

Other molecular targets

There has been recent recognition that TERT promoter mutations are the most common somatic mutations in HCC, occurring in around 40%-60% of HCCs. Since these mutations are also found in dysplastic nodules and hepatic adenomas they are considered to be potential driver mutations (75). Small molecule inhibitors of TERT are in early phase clinical trials for breast cancer and lung cancer (76, 77). Activating mutations in β -catenin (CTNNB1) have been reported to occur in 30-44% of HCCs, while other Wnt pathway-related genes like AXIN1 and AXIN2 have been found to be mutated in a smaller percentage of HCCs (78). Apart from activating mutations several additional mechanisms exist for Wnt pathway activation in HCC, including TGF β pathway activation, Wnt3a upregulation and overexpression of frizzled receptors. Hence inhibiting the Wnt pathway is an area of active interest and promising results have been reported recently using antibodies directed against GPC3, which inhibit the Wnt pathway (79). Mutations in p53 are also very common in HCC, but so far this has molecule has not proven to be a suitable target for drug development (78). The TGF β pathway is activated in a proportion of HCCs and is reported to promote tumor angiogenesis, the epithelial-mesenchymal transition, and immune effects in the tumor microenvironment, leading to tumor progression and metastases; hence it is a promising therapeutic target for HCC (80). LY2157299, a small molecule inhibitor of the TGF β pathway, has shown promise in phase II trials and is currently in a phase III trial (81, 82). Drugs directed against other targets such as Insulin-like growth factor (IGF) inhibitors, FGF inhibitors, MEK inhibitors and histone deacetylase inhibitors are also in early phase studies. Figure 3 depicts the major pathways involved in HCC pathogenesis and shows the currently available drugs directed against specific targets.

Conclusions

One of the main challenges faced by clinicians managing patients with HCC is the heterogeneity of this cancer, both at an epidemiologic and at a molecular level. The expectation that the arrival of sorafenib would be followed by the rapid approval of additional, even more effective systemic therapies has been dampened by the recent failure of multiple drugs in phase III trials. Hopefully, ongoing research on the genomic and

molecular landscape of HCC will facilitate meaningful stratification of HCC tumors and help to personalize targeted therapy for HCC based on individual tumor biology. Multiple biomarkers which predict response to sorafenib therapy are now being described and with further validation, these may serve as useful tools for patient selection. The promising results of tivantinib, which was more effective for tumors which expressed high levels of its target c-*MET* raises hope for improved results from future biomarker-enriched clinical trials. We expect that with the ongoing progress in the understanding of the pathogenesis of HCC, biomarker discovery and the identification of actionable drug targets will proceed at a rapid phase over the next several years, resulting in a successful transition of knowledge from bench to bedside.

Back to the Clinical Vignette

The clinical vignette described at the beginning of the review described a 58 year old female with BCLC stage C HCC who had discontinued sorafenib due to severe side effects. Below is an abbreviated summary of answers to the questions raised in this clinical scenario:

a. Should her liver lesions have been biopsied for diagnosis?

Currently, HCC management guidelines do not call for routine biopsy of all liver lesions in cirrhotic livers, as non-invasive radiologic diagnosis has high specificity. However, even with the reported high specificity, a significant number of individuals without hepatocellular carcinoma will be treated as having hepatocellular carcinoma. In addition, identification of molecular biomarkers which can predict response to therapy in the future will likely make biopsy a prerequisite before initiating targeted therapy. For example HCC tumors expressing high MET expression have better response to MET inhibitor tivantinib. Tumor histopathological heterogeneity reduces the prognostic accuracy of molecular tests based on liver tissue sampling, hence it still needs to be determined whether a single biopsy will suffice in patients with multinodular disease, and if more than one is required what the minimum number will be to assure detection of the most promising actionable targets.

b. Are there any tissue biomarkers that could have helped in prognostication?

Gene expression profiling has led to identification of several molecular signatures which can serve as biomarkers both for prognostication and treatment stratification. But unfortunately none have been clinically validated across different populations or achieved broad acceptance. However a 5-gene signature described by Nault et al. (41) which can predict recurrence after resection appears to be promising and will be commercially available shortly.

c. Was sorafenib the best first option for her and were there any biomarkers that could have predicted the adverse reactions she experienced?

The patient described in the vignette had portal hypertension and had tumor burden beyond Milan criteria; hence she was not a candidate for curative resection or liver transplantation. Since the patient had portal vein thrombosis and BCLC stage C disease she was not a candidate for TACE either. Hence sorafenib, which is currently the only FDA approved drug in the management of HCC, was indeed the best treatment option for her. Recent studies have indicated that focal amplifications in FGF3/4 and VEGFA can predict response to sorafenib. Once these results are more extensively validated, these biomarkers can potentially be used for patient selection. Biomarkers predicting adverse reactions to sorafenib have not been clearly identified as yet. Early onset of skin toxicity has been shown to herald a good response to sorafenib (83), hence one could attempt a trial of lower dose sorafenib rather than upfront withdrawal of targeted therapy in this patient.

d. What other potential therapies will be available for her in the near future?

Several phase III randomized clinical trials for HCC are currently ongoing and we hope that newer therapies will potentially be available in the near future. The drug classes which are currently being investigated include angiogenesis inhibitors, MET inhibitors, mTOR inhibitors and immune modulators.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015; 136(5):E359–86. [PubMed: 25220842]
2. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. *The New England journal of medicine*. 2008; 359(4):378–90. [PubMed: 18650514]
3. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol*. 2009; 10(1):25–34. [PubMed: 19095497]
4. Cheng AL, Kang YK, Lin DY, Park JW, Kudo M, Qin S, et al. Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2013; 31(32):4067–75. [PubMed: 24081937]
5. Llovet JM, Decaens T, Raoul JL, Boucher E, Kudo M, Chang C, et al. Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase III BRISK-PS study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2013; 31(28):3509–16. [PubMed: 23980090]
6. Zhu AX, Kudo M, Assenat E, Cattani S, Kang YK, Lim HY, et al. Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial. *Jama*. 2014; 312(1):57–67. [PubMed: 25058218]
7. Graham RP, Jin L, Knutson DL, Kloft-Nelson SM, Greipp PT, Waldburger N, et al. DNAJB1-PRKACA is specific for fibrolamellar carcinoma. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc*. 2015; 28(6):822–9.
8. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *European journal of cancer*. 2012; 48(5):599–641. [PubMed: 22424278]

9. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology*. 2011; 53(3): 1020–2. [PubMed: 21374666]
10. Müllhaupt B, Durand F, Roskams T, Dutkowski P, Heim M. Is tumor biopsy necessary? *Liver Transplantation*. 2011; 17(S2):S14–S25. [PubMed: 21744470]
11. Forner A, Vilana R, Ayuso C, Bianchi L, Sole M, Ayuso JR, et al. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: Prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. *Hepatology*. 2008; 47(1):97–104. [PubMed: 18069697]
12. Leoni S, Piscaglia F, Golfieri R, Camaggi V, Vidili G, Pini P, et al. The impact of vascular and nonvascular findings on the noninvasive diagnosis of small hepatocellular carcinoma based on the EASL and AASLD criteria. *Am J Gastroenterol*. 2010; 105(3):599–609. [PubMed: 19935786]
13. Lee YJ, Lee JM, Lee JS, Lee HY, Park BH, Kim YH, et al. Hepatocellular Carcinoma: Diagnostic Performance of Multidetector CT and MR Imaging—A Systematic Review and Meta-Analysis. *Radiology*. 2015; 275(1):97–109. [PubMed: 25559230]
14. Schima W, Hammerstingl R, Catalano C, Marti-Bonmati L, Rummeny EJ, Montero FT, et al. Quadruple-phase MDCT of the liver in patients with suspected hepatocellular carcinoma: effect of contrast material flow rate. *AJR American journal of roentgenology*. 2006; 186(6):1571–9. [PubMed: 16714645]
15. Jhaveri K, Cleary S, Audet P, Balaa F, Bhayana D, Burak K, et al. Consensus Statements From a Multidisciplinary Expert Panel on the Utilization and Application of a Liver-Specific MRI Contrast Agent (Gadoxetic Acid). *American Journal of Roentgenology*. 2015; 204(3):498–509. [PubMed: 25714278]
16. Lee JM, Choi BI. Hepatocellular nodules in liver cirrhosis: MR evaluation. *Abdominal imaging*. 2011; 36(3):282–9. [PubMed: 21399975]
17. Choi JW, Lee JM, Kim SJ, Yoon JH, Baek JH, Han JK, et al. Hepatocellular carcinoma: imaging patterns on gadoxetic acid-enhanced MR Images and their value as an imaging biomarker. *Radiology*. 2013; 267(3):776–86. [PubMed: 23401584]
18. Danila M, Sporea I, Sirli R, Popescu A, Sendroiu M, Martie A. The role of contrast enhanced ultrasound (CEUS) in the assessment of liver nodules in patients with cirrhosis. *Medical ultrasonography*. 2010; 12(2):145–9. [PubMed: 21173943]
19. Claudon M, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, Nolsoe CP, et al. Guidelines and good clinical practice recommendations for Contrast Enhanced Ultrasound (CEUS) in the liver – update 2012: A WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultrasound in medicine & biology*. 2013; 39(2):187–210. [PubMed: 23137926]
20. Arita J, Hasegawa K, Takahashi M, Hata S, Shindoh J, Sugawara Y, et al. Correlation between contrast-enhanced intraoperative ultrasound using Sonazoid and histologic grade of resected hepatocellular carcinoma. *AJR American journal of roentgenology*. 2011; 196(6):1314–21. [PubMed: 21606294]
21. Xu JF, Liu HY, Shi Y, Wei ZH, Wu Y. Evaluation of hepatocellular carcinoma by contrast-enhanced sonography: correlation with pathologic differentiation. *Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine*. 2011; 30(5):625–33. [PubMed: 21527610]
22. Chang S, Kim SH, Lim HK, Lee WJ, Choi D, Lim JH. Needle tract implantation after sonographically guided percutaneous biopsy of hepatocellular carcinoma: evaluation of doubling time, frequency, and features on CT. *AJR American journal of roentgenology*. 2005; 185(2):400–5. [PubMed: 16037512]
23. Silva MA, Hegab B, Hyde C, Guo B, Buckels JA, Mirza DF. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. *Gut*. 2008; 57(11):1592–6. [PubMed: 18669577]
24. Torbenson M, Schirmacher P. Liver cancer biopsy – back to the future?! *Hepatology*. 2015; 61(2): 431–3. [PubMed: 25271144]
25. Sherman M, Bruix J. Biopsy for liver cancer: How to balance research needs with evidence-based clinical practice. *Hepatology*. 2015; 61(2):433–6. [PubMed: 25308482]

26. Parker JL, Lushina N, Bal PS, Petrella T, Dent R, Lopes G. Impact of biomarkers on clinical trial risk in breast cancer. *Breast Cancer Res Treat.* 2012; 136(1):179–85. [PubMed: 23007573]
27. Falconi A, Lopes G, Parker JL. Biomarkers and receptor targeted therapies reduce clinical trial risk in non-small-cell lung cancer. *J Thorac Oncol.* 2014; 9(2):163–9. [PubMed: 24419412]
28. Santoro A, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, et al. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. *The Lancet Oncology.* 2013; 14(1):55–63. [PubMed: 23182627]
29. Vitale A, Saracino E, Boccagni P, Brolese A, D'Amico F, Gringeri E, et al. Validation of the BCLC Prognostic System in Surgical Hepatocellular Cancer Patients. *Transplantation Proceedings.* 2009; 41(4):1260–3. [PubMed: 19460533]
30. Cillo U, Vitale A, Grigoletto F, Farinati F, Brolese A, Zanusi G, et al. Prospective validation of the Barcelona Clinic Liver Cancer staging system. *Journal of Hepatology.* 2006; 44(4):723–31. [PubMed: 16488051]
31. Kim BK, Kim SU, Park JY, Kim DY, Ahn SH, Park MS, et al. Applicability of BCLC stage for prognostic stratification in comparison with other staging systems: single centre experience from long-term clinical outcomes of 1717 treatment-naïve patients with hepatocellular carcinoma. *Liver International.* 2012; 32(7):1120–7. [PubMed: 22524688]
32. Marrero JA, Fontana RJ, Barrat A, Askari F, Conjeevaram HS, Su GL, et al. Prognosis of hepatocellular carcinoma: Comparison of 7 staging systems in an American cohort. *Hepatology.* 2005; 41(4):707–15. [PubMed: 15795889]
33. Lee JS, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, et al. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology.* 2004; 40(3):667–76. [PubMed: 15349906]
34. Lee JS, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat Med.* 2006; 12(4):410–6. [PubMed: 16532004]
35. Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res.* 2009; 69(18):7385–92. [PubMed: 19723656]
36. Laurent-Puig P, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F, et al. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. *Gastroenterology.* 2001; 120(7):1763–73. [PubMed: 11375957]
37. Kaposi-Novak P, Lee JS, Gomez-Quiroz L, Coulouarn C, Factor VM, Thorgeirsson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. *J Clin Invest.* 2006; 116(6):1582–95. [PubMed: 16710476]
38. Coulouarn C, Factor VM, Thorgeirsson SS. Transforming growth factor-beta gene expression signature in mouse hepatocytes predicts clinical outcome in human cancer. *Hepatology.* 2008; 47(6):2059–67. [PubMed: 18506891]
39. van Malenstein H, Gevaert O, Libbrecht L, Daemen A, Allemeersch J, Nevens F, et al. A seven-gene set associated with chronic hypoxia of prognostic importance in hepatocellular carcinoma. *Clin Cancer Res.* 2010; 16(16):4278–88. [PubMed: 20592013]
40. Fujimoto A, Furuta M, Shiraishi Y, Gotoh K, Kawakami Y, Arihiro K, et al. Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. *Nat Commun.* 2015; 6
41. Nault JC, De Reynies A, Villanueva A, Calderaro J, Rebouissou S, Couchy G, et al. A hepatocellular carcinoma 5-gene score associated with survival of patients after liver resection. *Gastroenterology.* 2013; 145(1):176–87. [PubMed: 23567350]
42. Lin CT, Hsu KF, Chen TW, Yu JC, Chan DC, Yu CY, et al. Comparing hepatic resection and transarterial chemoembolization for Barcelona Clinic Liver Cancer (BCLC) stage B hepatocellular carcinoma: change for treatment of choice? *World J Surg.* 2010; 34(9):2155–61. [PubMed: 20407768]
43. Yin L, Li H, Li A-J, Lau WY, Pan Z-y, Lai ECH, et al. Partial hepatectomy vs. transcatheter arterial chemoembolization for resectable multiple hepatocellular carcinoma beyond Milan criteria: A RCT. *Journal of Hepatology.* 2014; 61(1):82–8. [PubMed: 24650695]

44. Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, et al. Liver transplantation for hepatocellular carcinoma: Expansion of the tumor size limits does not adversely impact survival. *Hepatology*. 2001; 33(6):1394–403. [PubMed: 11391528]
45. Bolondi L, Burroughs A, Dufour JF, Galle PR, Mazzaferro V, Piscaglia F, et al. Heterogeneity of patients with intermediate (BCLC B) Hepatocellular Carcinoma: proposal for a subclassification to facilitate treatment decisions. *Semin Liver Dis*. 2012; 32(4):348–59. [PubMed: 23397536]
46. Arai T, Ueshima K, Matsumoto K, Nagai T, Kimura H, Hagiwara S, et al. FGF3/FGF4 amplification and multiple lung metastases in responders to sorafenib in hepatocellular carcinoma. *Hepatology*. 2013; 57(4):1407–15. [PubMed: 22890726]
47. Horwitz E, Stein I, Andreozzi M, Nemeth J, Shoham A, Pappo O, et al. Human and mouse VEGFA-amplified hepatocellular carcinomas are highly sensitive to sorafenib treatment. *Cancer Discov*. 2014; 4(6):730–43. [PubMed: 24687604]
48. Scartozzi M, Faloppi L, Svegliati Baroni G, Loretelli C, Piscaglia F, Iavarone M, et al. VEGF and VEGFR genotyping in the prediction of clinical outcome for HCC patients receiving sorafenib: The ALICE-1 study. *International Journal of Cancer*. 2014; 135(5):1247–56. [PubMed: 24510746]
49. Negri FV, Dal Bello B, Porta C, Campanini N, Rossi S, Tinelli C, et al. Expression of pERK and VEGFR-2 in advanced hepatocellular carcinoma and resistance to sorafenib treatment. *Liver International*. 2015:n/a–n/a.
50. Avila M, Berasain C. Making Sorafenib Irresistible: In Vivo Screening for Mechanisms of Therapy Resistance in Hepatocellular Carcinoma Hits on Mapk14. *Hepatology*. 2015:n/a–n/a.
51. Cainap C, Qin S, Huang WT, Chung IJ, Pan H, Cheng Y, et al. Linifanib versus Sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2015; 33(2): 172–9. [PubMed: 25488963]
52. Thomas MB, Morris JS, Chadha R, Iwasaki M, Kaur H, Lin E, et al. Phase II trial of the combination of bevacizumab and erlotinib in patients who have advanced hepatocellular carcinoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009; 27(6):843–50. [PubMed: 19139433]
53. Zhu AX, R B, Yen CJ, Kudo M, Poon RTP, Pastorelli D, Blanc JF, Chung HC, Baron AD. LBA16 - Ramucirumab (RAM) as second-line treatment in patients (pts) with advanced hepatocellular carcinoma (HCC) following first-line therapy with sorafenib: Results from the randomized phase III REACH study. *ESMO*. 2014
54. Venepalli NK, Goff L. Targeting the HGF-cMET Axis in Hepatocellular Carcinoma. *International journal of hepatology*. 2013; 2013:341636. [PubMed: 23606971]
55. Kaposi-Novak P, Lee J-S, Gómez-Quiroz L, Coulouarn C, et al. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. *The Journal of Clinical Investigation*. 2006; 116(6):1582–95. [PubMed: 16710476]
56. Llovet JM, Pena CE, Lathia CD, Shan M, Meinhardt G, Bruix J. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin Cancer Res*. 2012; 18(8): 2290–300. [PubMed: 22374331]
57. Verslype C, C AL, Kelley RK, Yang T-S, Su W-C, Ramies DA, et al. Activity of cabozantinib (XL184) in hepatocellular carcinoma: results from a phase II randomized discontinuation trial (RDT). *ASCO Meeting Abstracts*. 2012; 30(15_suppl):4007.
58. Makarova-Rusher OV, Medina-Echeverez J, Duffy AG, Greten TF. The yin and yang of evasion and immune activation in HCC. *Journal of Hepatology*.
59. Miamen AG, Dong H, Roberts LR. Immunotherapeutic Approaches to Hepatocellular Carcinoma Treatment. *Liver Cancer*. 2012; 1(3-4):226–37. [PubMed: 24159587]
60. Ormandy LA, Hillemann T, Wedemeyer H, Manns MP, Greten TF, Korangy F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Res*. 2005; 65(6):2457–64. [PubMed: 15781662]
61. Fu J, Xu D, Liu Z, Shi M, Zhao P, Fu B, et al. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology*. 2007; 132(7):2328–39. [PubMed: 17570208]

62. Wu K, Kryczek I, Chen L, Zou W, Welling TH. Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. *Cancer Res.* 2009; 69(20):8067–75. [PubMed: 19826049]
63. Kalathil S, Lugade AA, Miller A, Iyer R, Thanavala Y. Higher frequencies of GARP(+)/CTLA-4(+)/Foxp3(+) T regulatory cells and myeloid-derived suppressor cells in hepatocellular carcinoma patients are associated with impaired T-cell functionality. *Cancer Res.* 2013; 73(8):2435–44. [PubMed: 23423978]
64. Cariani E, Pilli M, Zerbini A, Rota C, Olivani A, Pelosi G, et al. Immunological and molecular correlates of disease recurrence after liver resection for hepatocellular carcinoma. *PLoS One.* 2012; 7(3):e32493. [PubMed: 22396772]
65. Mizukoshi E, Nakamoto Y, Arai K, Yamashita T, Sakai A, Sakai Y, et al. Comparative analysis of various tumor-associated antigen-specific t-cell responses in patients with hepatocellular carcinoma. *Hepatology.* 2011; 53(4):1206–16. [PubMed: 21480325]
66. Hiroishi K, Eguchi J, Baba T, Shimazaki T, Ishii S, Hiraide A, et al. Strong CD8(+) T-cell responses against tumor-associated antigens prolong the recurrence-free interval after tumor treatment in patients with hepatocellular carcinoma. *J Gastroenterol.* 2010; 45(4):451–8. [PubMed: 19936602]
67. Butterfield LH, Ribas A, Disette VB, Lee Y, Yang JQ, De la Rocha P, et al. A phase I/II trial testing immunization of hepatocellular carcinoma patients with dendritic cells pulsed with four alpha-fetoprotein peptides. *Clin Cancer Res.* 2006; 12(9):2817–25. [PubMed: 16675576]
68. Greten TF, Forner A, Korangy F, N'Kontchou G, Barget N, Ayuso C, et al. A phase II open label trial evaluating safety and efficacy of a telomerase peptide vaccination in patients with advanced hepatocellular carcinoma. *BMC Cancer.* 2010; 10:209. [PubMed: 20478057]
69. Sawada Y, Yoshikawa T, Nobuoka D, Shirakawa H, Kuronuma T, Motomura Y, et al. Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival. *Clin Cancer Res.* 2012; 18(13):3686–96. [PubMed: 22577059]
70. Hong Y, Peng Y, Guo ZS, Guevara-Patino J, Pang J, Butterfield LH, et al. Epitope-optimized alpha-fetoprotein genetic vaccines prevent carcinogen-induced murine autochthonous hepatocellular carcinoma. *Hepatology.* 2014; 59(4):1448–58. [PubMed: 24122861]
71. Mahoney KM, Freeman GJ, McDermott DF. The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma. *Clin Ther.* 2015
72. Brahmer JR, Hammers H, Lipson EJ. Nivolumab: targeting PD-1 to bolster antitumor immunity. *Future Oncol.* 2015:0.
73. Sangro B, Gomez-Martin C, de la Mata M, Inarrairaegui M, Garraleta E, Barrera P, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol.* 2013; 59(1):81–8. [PubMed: 23466307]
74. El-Khoueiry AB, Melero I, Crocenzi TS, et al. Phase I/II safety and antitumor activity of nivolumab in patients with advanced hepatocellular carcinoma (HCC): CA209-040. *J Clin Oncol.* 2015; (suppl) (abstr LBA101).
75. Nault JC, Mallet M, Pilati C, Calderaro J, Bioulac-Sage P, Laurent C, et al. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat Commun.* 2013; 4
76. Chiappori AA, Kolevska T, Spigel DR, Hager S, Rarick M, Gadgeel S, et al. A randomized phase II study of the telomerase inhibitor imetelstat as maintenance therapy for advanced non-small-cell lung cancer. *Ann Oncol.* 2015; 26(2):354–62. [PubMed: 25467017]
77. Koziel JE, Herbert BS. The telomerase inhibitor imetelstat alone, and in combination with trastuzumab, decreases the cancer stem cell population and self-renewal of HER2+ breast cancer cells. *Breast Cancer Res Treat.* 2015; 149(3):607–18. [PubMed: 25627551]
78. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat Genet.* 2012; 44(6):694–8. [PubMed: 22561517]

79. Gao W, Tang Z, Zhang Y-F, Feng M, Qian M, Dimitrov DS, et al. Immunotoxin targeting glypican-3 regresses liver cancer via dual inhibition of Wnt signalling and protein synthesis. *Nat Commun.* 2015; 6
80. Giannelli G, Villa E, Lahn M. Transforming growth factor-beta as a therapeutic target in hepatocellular carcinoma. *Cancer Res.* 2014; 74(7):1890–4. [PubMed: 24638984]
81. Mazzocca A, Fransvea E, Lavezzari G, Antonaci S, Giannelli G. Inhibition of transforming growth factor β receptor I kinase blocks hepatocellular carcinoma growth through neo-angiogenesis regulation. *Hepatology.* 2009; 50(4):1140–51. [PubMed: 19711426]
82. Faivre, S.; S, A.; Kelley, RK., et al. A Phase 2 Study of Novel Transforming Growth Factor-beta (TGF- β) Receptor 1 Kinase Inhibitor, LY2157299 Monohydrate in Patients With Advanced Hepatocellular Carcinoma (HCC). Presented at: 2014 Gastrointestinal Cancers Symposium; January 16-18, 2014; San Francisco, CA. See more at: <http://www.onclive.com/conference-coverage/gcs-2014/Novel-Targeted-Agent-Leads-to-Survival-Benefit-in-HCC-sthash.WYz42iCN.dpuf>
83. Brose MS, Frenette CT, Keefe SM, Stein SM. Management of sorafenib-related adverse events: a clinician's perspective. *Semin Oncol.* 2014; 41(Suppl 2):S1–S16. [PubMed: 24576654]

Figure 1. Figure 1 depicts a hypothetical classification system of hepatocellular carcinoma (HCC) that incorporates tumor characteristics, liver function, patient factors and molecular characteristics of tumors. With better characterization of the molecular pathogenesis of HCC, individual tumor biology can hopefully be used to decide optimal molecular directed therapy for tumors in the future.

Figure 2.

The BCLC classification is based on tumor extent, Child-Pugh (CP) score, vascular invasion and ECOG performance status. This algorithm can be used to decide treatment allocation based on tumor stage

Abbreviations: BCLC Barcelona Clinic Liver Cancer, CP Child Pugh score, HTN Hypertension, PS Performance status, ECOG Eastern Cooperative Oncology Group, HCC hepatocellular carcinoma, TACE Transarterial chemoembolization, RFA Radiofrequency ablation.

Figure 3.

Figure 3 depicts the major pathways involved in HCC pathogenesis and shows the currently available drugs (hexagons) directed against specific targets.

Abbreviations: EGFR Epidermal growth factor receptor, VEGFR Vascular endothelial growth factor receptor, PDGFR Platelet derived growth factor receptor, FGFR Fibroblast growth factor receptor, IGF1 Insulin growth factor, HGF Hepatocyte growth factor, TGFBR Transforming growth factor beta receptor, GSK3B Glycogen synthase 3 beta, TERT Telomerase reverse transcriptase.