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Systematic Chemotherapy of HCC: Review

Personalized Clinical Trials in Hepatocellular Carcinoma Based on Biomarker Selection

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Key Words
Biomarkers · HCC · Liver cancer · Molecular therapy · Tivantinib

Abstract
Background: Since the approval of sorafenib there have been numerous failures of new agents in Phase III studies for treatment of advanced hepatocellular carcinoma (HCC). These studies have generally ignored the molecular heterogeneity of HCC and they have not enrolled patients based on predictive markers of response. The development of molecular targeted therapeutics in HCC needs to model the approach that has been taken with great success in other solid tumors, to decrease the likelihood of failure in future studies. ***Summary:*** Here we review the paradigm taken with novel targeted agents in other solid tumors and highlight ongoing studies in HCC that are incorporating biomarkers in clinical development. ***Key Messages:*** With the appreciation of the molecular diversity of HCC, clinical development of new agents in HCC will need to be targeted towards those patients who are most likely to benefit. This strategy, based on biomarkers for patient selection, is more likely to yield positive results and mitigate the risk of continued negative Phase III studies.

Introduction

Hepatocellular carcinoma (HCC) is one of the most commonly diagnosed cancers globally and is a leading cause of cancer death [1]. HCC is both clinically and molecularly heterogeneous, resulting from various underlying risk factors that cause liver inflammation, followed by fibrosis, and eventual cirrhosis. Common risk factors include hepatitis B and/

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or hepatitis C virus infection, heavy alcohol use, diabetes mellitus, and non-alcoholic steatohepatitis (NASH) Less common causes aflatoxin B1exposure, hereditary hemochromatosis, α-1 antitrypsin deficiency, and autoimmune hepatitis [2]. Due to the complex etiologies and limited surveillance available, most patients at the time of diagnosis have advanced disease and are not eligible for curative therapies such as surgical resection or liver transplantation [3]. Loco-regional therapies including radiofrequency ablation or transarterial chemoembolization (TACE) are used in patients with early to intermediate stage disease who are not surgical candidates [4]. However, their efficacy is limited and 5-year recurrence rates have been reported as high as 70% in early stages of HCC [5, 6]. The only systemic therapy approved for advanced HCC is the multi-kinase inhibitor sorafenib. This is not for lack of trying, but it is the result of numerous Phase III trial failures that have not been directed towards those patients who are most likely to benefit based on identifiable patient factors. However, in respect to other solid tumors, there have been significant improvements in clinical outcomes when predictive markers of response have been incorporated into clinical trials. As we gain a better insight into the molecular pathways involved in HCC, it is imperative that patient enrichment strategies be incorporated into clinical development. In this article, we will focus on emerging clinical trials that are utilizing biomarkers in clinical trial design.

Sorafenib: The Benchmark

Sorafenib was the first and still only approved therapy for advanced HCC. It is a multikinase inhibitor targeting the vascular endothelial growth factor receptors (VEGFR), platelet-derived growth factor (PDGFR)-β, RAF) kinase, and stem cell factor receptor (c-kit). Two randomized, placebo-controlled Phase III trials (Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) trial and the Asia-Pacific trial) demonstrated a significant improvement in overall survival (OS) in patients with advanced HCC [7, 8]. The SHARP trial was stopped after an interim analysis showed a significant advantage in overall survival (OS) for the patients who had received sorafenib versus placebo (10.7 months vs. 7.9 months, hazard ratio 0.69, $p<0.001$) [7]. Since its Food and Drug Administration (FDA) approval in the United States in 2007, no trial has demonstrated superiority versus sorafenib in the front line setting, or improved survival in the second-line setting. Importantly, no biomarkers have been identified to select patients that could derive the most benefit from this molecular targeted therapy.

Why We Need a New Approach

Since sorafenib, numerous agents have been evaluated in Phase III studies in advanced HCC and have failed. Typically these trials were based on limited pre-clinical observations, and single-arm Phase II data that was felt to be encouraging, only to disappoint in larger, controlled studies. In the front-line setting, phase III trials evaluating sunitinib, brivanib, and linifanib as single agents and the combination of erlotinib and sorafenib all failed to meet their study endpoints of superiority, or in some cases non-inferiority when compared with sorafenib [9–12]. In the second-line setting, brivanib did not meet its endpoint, possibly as a result of clinical imbalances between the treatment and placebo arms [13]. The mammalian target of rapamycin (mTOR) inhibitor everolimus was evaluated in the second-line setting as well based on laboratory and pre-clinical data suggesting that mTOR signaling was important in a subset of liver cancers [14]. EVOLVE-1 was a randomized, Phase III clinical trial test-

ing everolimus versus placebo in patients with advanced HCC who progressed on sorafenib. Unfortunately this study also failed to meet its study endpoint of improving OS [15]. There have nevertheless been some biomarker studies that have come out of these negative studies. There is evidence that loss of function of Tuberous Sclerosis-2 (TSC2), a negative regulator of mTOR, may be associated with greater sensitivity to everolimus treatment; [16] and therefore TSC2 loss may be a predictive biomarker for response to everolimus. This observation requires prospective validation in the context of a controlled clinical trial. Ramucirumab, a monoclonal antibody to the VEGFR has also yielded negative results in a similar population and will be discussed further, as retrospective analysis has identified alpha-fetoprotein (AFP) as a potential marker of benefit [17].

There are several possible reasons for Phase III trial failures in advanced HCC, including issues around trial design, efficacy of therapy, toxicity, and the lack of patient selection factors in the context of the molecular diversity of HCC. Most of the Phase III trials were based on the efficacy data from relatively small single-arm Phase II trials, the latter of which are difficult to assess. The decision to move sorafenib into the clinical setting was based on a single-arm Phase II study; however, this study was larger and accrued over 100 patients. It has been proposed that randomized Phase II studies should be considered before moving to expensive, large-scale Phase III studies [18]. In addition, surrogate endpoints such as progression-free survival (PFS) and response rate (RR) do not reliably predict OS [15]. Second, drugs that are effective in patients without cirrhosis might have unacceptable toxicity in patients with cirrhosis, such as with sunitinib and linifanib. Considering the majority of patients with HCC have underlying cirrhosis, this toxicity profile is an important consideration. Lastly, the clinical and molecular heterogeneity of HCC makes identifying "oncogenic drivers" and molecular subclasses challenging. None of the previous trials were designed to identify a selected patient population most likely to benefit based on molecular classification and biomarkers, therefore potential efficacy in a subset of patients may have been missed. A related challenge is that unlike other solid tumors such as breast or lung cancer, it is not routine to obtain tumor tissue in newly diagnosed HCC given the imaging characteristics allow for a non-invasive diagnosis [19].

Biomarkers in Oncology

The concept of biomarker driven cancer therapy (or precision medicine) is not new. The National Cancer Institute defines a biomarker as "a biological molecule found in blood, other body fluids, or tissue that is a sign of a normal or abnormal process, or of a condition or disease. Also called molecular marker and signature molecule" [20]. Biomarkers are by definition objectively measured and reproducible. Cancer biomarkers can be used to indicate the natural course and prognosis of a malignancy, predict response to a given therapy in patients, and assess the pharmacodynamics of a drug [21]. In the last two decades, increasing understanding of tumorigenesis and driver genetic alterations have led to the identification of biomarkers to assess risk, prognosis, and selection of therapy for patients in several cancers. Some examples of this approach in other tumor types are highlighted below:

Breast Cancer

The development of the monoclonal antibody trastuzumab serves as a paradigm in oncology drug development. Human epidermal growth factor receptor 2 (HER2) amplification oc-

curs in 20–25% of breast cancers and it was initially identified as a prognostic factor for poor outcome [22, 23]. Trastuzumab, a monoclonal antibody that targets HER2, was approved in 1998 when a pivotal study demonstrated significant improvements with the addition of trastuzumab to chemotherapy versus chemotherapy alone [24] Critical to its success, was the development of the molecule to only target women that had HER2 overexpression. Since that time, numerous studies in the advanced and early setting have validated HER2 as a predictive marker of response to trastuzumab and other HER2 targeted agents [25, 26].

Lung Cancer

Lung cancer, which has been traditionally identified as small cell or non-small cell lung cancer (NSCLC) has undergone a revolution in how it is approached. What was once approached as a single therapeutic entity, NSCLC is now first analyzed for various molecular alterations that are associated with a response to a given therapy. Erlotinib and gefitinib are small-molecule tyrosine kinase inhibitors (TKI) that competitively block the adenosine triphosphate (ATP) binding site of the tyrosine kinase domain of EGFR, and thus block downstream signaling [27]. Another example of this approach in NSCLC, is the discovery of the echinoderm microtubule-associated protein-like 4 (EML4)-anaplastic lymphoma kinase (ALK) fusion gene, and the development of crizotinib. The ALK gene rearrangement with EML4 leads to a fusion between EML4 and ALK, resulting in constitutive, ligand-independent activation of the rearranged ALK-receptor and downstream signaling pathways (Ras/mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3 K)/protein kinase B (Akt), and janus kinase/signal transducer and activator of transcription 3(JAK/STAT3), which are responsible for cell proliferation and survival [28]. The EML4-ALK fusion gene is present in about 3–7% of all NSCLC patients [29]. It is a potent oncogenic driver, associated with a worse survival and an increased risk of brain and liver metastases [30]. Crizotinib is an oral small molecule TKI originally developed as a mesenchymal-epithelial transition factor (c-MET) inhibitor. In a Phase I trial, two patients with EML4-ALK rearrangement showed dramatic improvement in symptoms [31]. This observation led to large-scale prospective screening of NSCLC patients with ALK rearrangement to be treated with crizotinib, and subsequent Phase 1 and Phase 2 trials showed impressive results of 61% and 53% objective responsive rates, respectively [32, 33]. In August 2011, the FDA granted accelerated approval for crizotinib for the treatment of ALK-positive metastatic NSCLC. More recently, two Phase III trials compared crizotinib to chemotherapy in ALK-positive patients, and both showed significant higher overall response rates (ORR) in the crizotinib arms versus the chemotherapy arms [34, 35].

Melanoma

About 40–60% of cutaneous melanomas have mutations in the BRAF gene that leads to constitutive activation of the downstream MAPK signaling pathway, which affects cell division and differentiation [36]. The most common BRAF mutation is the substitution of glutamic acid for valine at codon 600 (BRAF V600E). Vemurafenib is a potent enzyme inhibitor of the BRAF V600E mutation. In a landmark Phase III trial, patients were prospectively selected for BRAF V600 mutations and randomized to vemurafneib or dacarbazine. In this molecularly selected subset of patients, 6-month survival was improved in the vemurafenib group compared to the control group (84% vs 64%), respectively [37].

Table 1. Ongoing biomarker driven studies in HCC

Drug	Phase	Biomarker (s)	NCI No.	Setting
Tivantinib	3	Elevated MET	NCT01755767	2nd line
Ramucirumab	3	Elevated AFP	NCT02435433	2nd line
Refametinib	2	RAS mutation	NCT01915602	1st line
LY2157299	2	AFP, TGF-β, E-Cadherin	NCT01246986	2nd line
JNJ-42756493	1/2a	FGF19	NCT02421185	1st or 2nd line
FGF401	1/2	FGFR4/ klothoβ	NCT02325739	1st or 2nd line

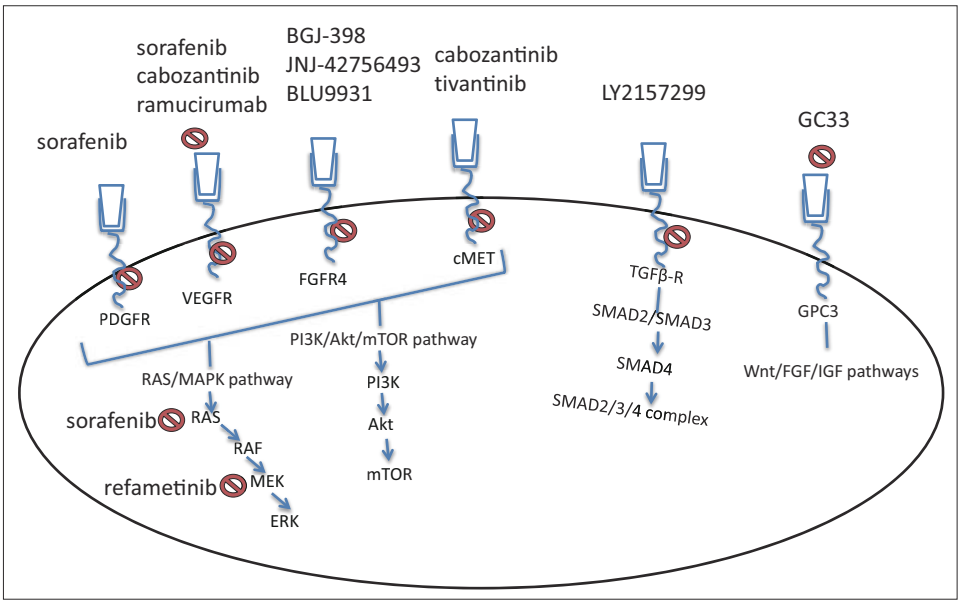

Fig. 1. Targeted agents and molecular pathways in biomarker directed research in hepatocellular carcinoma. PDGFR=platelet-derived growth factor receptor; TGF-βR=transforming growth factor-beta receptor.

Biomarker Backed Trials in HCC: The Time Has Come

The molecular diversity of HCC is well established. Studies have shown that HCC harbors a diverse mutational profile with on average 30–40 mutations per tumor, which may result from the activation of different oncogenic pathways, or from different cells of origin [38, 39]. Five major signaling pathways in HCC have been identified including: 1) Wnt/β-catenin pathway, 2) Tumor protein p53 (TP53) pathway, 3) RAS pathway, 4) oxidative stress, and 5) chromatin remodeling [38]. Several studies have proposed tumor subclasses based on gene expression profiling, including the Wnt subclass (enriched with β- catenin1 (CTNNB1) mutations), a proliferation class with two predominant subclasses: S1/transforming growth factor-β (TGF-β) and S2/epithelial cell adhesion molecule (EpCAM)-positive, and an inflammation class [40–43]. Despite these studies, previous clinical trials have not incorporated these insights. It is important to consider that unless we change approaches in HCC drug development from the traditional "catch all" trials, we are likely to see continued Phase III failures. Recently, new studies are taking lessons from other solid tumor trials by incorporating molecular selection factors into prospective studies (table 1, fig. 1).

Tivantinib and Other c-MET Inhibitors

The hepatocyte growth factor (HGF) binds to its receptor tyrosine kinase, encoded by the MET proto-oncogene and has been implicated in tumor development and progression [44]. MET binding to its ligand HGF and in turn activates downstream signaling cascades, including the RAS-MAPK and PI3K-AKT pathways [44]. Tivantinib is an oral, small molecule MET inhibitor that inhibits proliferation and induces apoptosis in MET-expressing cell lines [45]. A Phase II trial evaluated tivantinib in patients with advanced HCC who failed or were unable to tolerate first-line therapy with sorafenib. In the intent-to-treat (ITT), biomarker unselected population; there was minimal improvement in time to progression (TTP) with tivantinib over placebo (1.6 months versus 1.4 months). However, a retrospective analysis of tumors for c-MET expression by immunohistochemistry (IHC) identified a significant improvement in those patients that had high expression of c-MET (2.7 months versus 1.4 months). In addition, there was no benefit with tivantinib in the low c-MET expression group. While these observations were based on a small number of patients, they serve as a hypothesis for further development. A prospective randomized Phase II study would be one approach, whereas [46] currently a confirmatory Phase III trial METIV-HCC (NCT01755767) evaluating tivantinib versus placebo as second-line therapy in patients with advanced high c-MET expressing HCC is ongoing. This is the first Phase III study in HCC that is requiring a biomarker for inclusion.

Additionally, cabozantinib is an oral receptor multi-tyrosine kinase inhibitor (TKI) whose targets include MET, RET and VEGFRs. The CELESTIAL trial (NCT01908426) is a Phase III trial evaluating cabozantinib versus placebo in patients with HCC who received prior sorafenib. However, unlike the tivantinib study, this trial is not designed for specific molecular subgroups. Although the analysis of serum biomarkers is included as an additional endpoint, this raises the risk for failure like studies before it.

Additional selective c-MET inhibitors are in earlier stage development in HCC including INC280 (NCT01737827) and MSC2156119 J (NCT02115373).

Ramucirumab

Ramucirumab is a human IgG1 monoclonal antibody that binds specifically to the extracellular domain of the human VEGFR-2 and blocks the interaction of VEGFR-2 and its ligands, inhibiting endothelial proliferation and migration [47]. A single-arm Phase II study in advanced HCC suggested some evidence of its ability to induce disease control [48]. The Phase III REACH trial (NCT01140347) evaluated ramucirumab as second-line therapy in patients with advanced HCC, and like other studies before it, it failed to meets its endpoints. However, in a retrospective subgroup analysis, patients with baseline AFP >400 ng/ml or at least 1.5 times the upper limit of normal did however demonstrate a significant survival benefit compared to the placebo group [17]. This suggests that elevated baseline AFP may be a predictive marker for survival benefit to ramucirumab, and it is now the basis for a biomarker selected Phase III study (REACH-2, NCT02435433) in the same setting.

Refametinib

In the setting of activating mutations, *ras* induces phosphorylation and activation of *raf* kinase, which leads to a cascade of downstream phosphorylations of MEK1/MEK2 and

ERK1/ERK2. Phosphorylated ERK dimerizes and translocates to the nucleus, where it is involved in several important cellular functions that regulate proliferation, survival, differentiation, and apoptosis [49]. RAS and RAF mutations are rare in HCC [50, 51]. However, the RAF/MEK/ERK pathway may play a role in the pathogenesis of HCC [52–54]. MEK inhibitors such as selumetinib and refametinib have been studied. A Phase II study of selumetinib in unselected patients did not show significant activity [55]. Subsequent studies with the combination of refametinib and sorafenib in Asian patients again showed limited treatment benefit partially due to dose reduction secondary to significant adverse events. Interestingly, four patients with RAS mutations had a better clinical response [56]. Based on this insight, a Phase II trial evaluating refametinib plus sorafenib in patients pre-selected for RAS mutations is ongoing (NCT01915602).

LY2157299

TGF-β signaling complex is felt to play a role in the pathogenesis of HCC [57]. An ongoing Phase II trial is evaluating the TGF-β inhibitor LY2157299 in patients who either failed or were ineligible for sorafenib (NCT01246986). The primary endpoints of the study are time to progression (TTP) and changes in serum biomarkers (AFP, TGF-β, E-Cadherin) in relationship with different dose regimens (160 mg/day or 300 mg/day). An interim analysis reported in 2014 demonstrated an AFP decline of >20% from baseline occurred in 24% of patients. Median OS was 93.1 weeks in AFP responders vs 29.6 weeks in non-AFP responders (p=0.0006) [58]. The relationship between AFP and E-cadherin is also being explored in the study to better understand the significance of AFP responses. While not a prospective selection marker, these changes in AFP may identify patients early that benefit from treatment.

FGFR as a Target in HCC

The fibroblast growth factor (FGF) signaling family is involved in liver fibrosis and its progression to cirrhosis [59, 60]. FGF receptors 3 and 4 are the main isoforms expressed in the liver [61]. In particular, studies suggested that the FGF receptor 4 and FGF19 signaling axis may be a predictive and prognostic biomarker for HCC therapy. For example, overexpression of FGF19 is associated with highly proliferative tumors and poorer prognosis in HCC. Inhibition of FGF19 in models with FGF19 amplification stopped the clonal growth of human HCC cells [62, 63]. Several FGFR tyrosine kinase inhibitors are in development. BGJ-398 is a selective inhibitor of FGFRs 1–4, and FGF19 amplification has been identified as a predictive marker of response [64, 65]. Similarly, JNJ-42756493 is a pan-FGFR tyrosine kinase inhibitor in clinical development for HCC (NCT 02421185). Besides pan-FGFR inhibitors, there is now a new generation that are very selective for fibroblast growth factor receptor (FGFR4) specifically. BLU9931 is very selective for FGFR4 versus other FGFR family members [66]. Similarly, FGF401 is a selective FGFR4 inhibitor in early phase clinical studies for patients with FGFR4 and klotho beta expression (NCT02325739). Klotho beta is a single span membrane protein that is a co-factor for FGF19 and FGFR4 binding. A recent study suggests that HCCs harboring FGF3/4 amplifications have increased sensitivity to sorafenib, but this requires further validation [67].

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Glypican 3: Challenges in Biomarker Driven Studies

Glypican 3 (GPC3), a member of the glypican family, is highly expressed in HCC and is used as a marker to differentiate HCC from benign liver tissues [68-70]. GPC expression is associated with poor prognosis as patients with GPC3-positive HCC tend to have shorter disease free survival (DFS) than those with GPC-negative HCC after surgery [71]. GC33 is a humanized monoclonal antibody against GPC3 and it mediates antibody-dependent cell cytotoxicity [72]. A Phase I study demonstrated that GC33 was well tolerated in HCC [73]. In a recent randomized Phase II trial, 185 patients that had advanced HCC and had failed prior systemic therapy were randomized to receive GC33 at 1600 mg intravenously on days 1 and 8 and then every 2 weeks afterwards, or placebo. The primary endpoint was PFS. The results did not show a significant difference in PFS or OS of the GC33 arm versus the placebo arm. A subsequent analysis suggested that increased GC33 exposure was associated with prolonged PFS and OS, leading the authors of the study to conclude that the failure was potentially due to suboptimal dosing [74]. While this may be the case, the failure of the GC33 trial also highlights additional challenges in biomarker driven studies such as the expression of the target (GPC3 in this case) does not necessarily equal tumor dependence. Currently several other antibodies targeting GPC3 are being developed, including YP7, HN3, and MDX-1414 [75]. One potential future direction would be to develop an antibody-drug conjugate to GPC3 to directly deliver effective cytotoxics, similar to trastuzumab emtansine (T-DM1) in HER-2 positive breast cancer treatment.

Conclusion

Systemic treatment options for advanced HCC remain extremely limited. To date, sorafenib is the only approved targeted therapy for advanced HCC. Potential barriers to develop new therapeutic agents include issues around trial design, therapeutic efficacy and toxicity, and the molecular diversity of HCC. In particular, the clinical and molecular heterogeneity of HCC makes identifying oncogenic drivers and molecular subclasses challenging. To date, most of the prospective clinical trials have not been designed for a pre-selected patient population based on molecular classification and biomarkers, which may explain the failures. However, as we begin to incorporate tumor biology into clinical trial design, we are seeing an increase in biomarker directed studies. Given the failures with the “all-comers” approach, hopefully, by enriching for the responsive population, we will see significant improvements in clinical outcomes for our patients as in other malignancies.

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References

1 Stewart B, Wild CP: World Cancer Report 2014. City: International Agency for Research on Cancer, World Health Organization; 2014.
2 El-Serag HB, Rudolph KL: Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132:2557–2576.
3 Bruix J, Sherman M, Practice Guidelines Committee, American Association for the Study of Liver Diseases: Management of hepatocellular carcinoma. Hepatology 2005;42:1208–1236.
4 Saraswat VA, Pandey G, Shetty S: Treatment algorithms for managing hepatocellular carcinoma. J Clin Exp Hepatol 2014;4:S80–89.
5 Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M: Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. J Hepatol 2003;38:200–207.
6 Poon RT, Fan ST, Lo CM, Liu CL, Wong J: Intrahepatic recurrence after curative resection of hepatocellular carcinoma: long-term results of treatment and prognostic factors. Ann Surg 1999;229:216–222.
7 Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J, SHARP Investigators Study Group: Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–390.
8 Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z: 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;10:25–34.
9 Cainap C, Qin S, Huang WT, Chung IJ, Pan H, Cheng Y, Kudo M, Kang YK, Chen PJ, Toh HC, Gorbunova V, Eskens FA, Qian J, McKee MD, Ricker JL, Carlson DM, El-Nowiem S: Linifanib versus Sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. J Clin Oncol 2015;33:172–179.
10 Cheng AL, Kang YK, Lin DY, Park JW, Kudo M, Qin S, Chung HC, Song X, Xu J, Poggi G, Omata M, Pitman Lowenthal S, Lanzalone S, Yang L, Lechuga MJ, Raymond E: Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. J Clin Oncol 2013;31:4067–4075.
11 Johnson PJ, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, Hsu CH, Hu TH, Heo J, Xu J, Lu L, Chao Y, Boucher E, Han KH, Paik SW, Robles-Aviña J, Kudo M, Yan L, Sobhonslidsuk A, Komov D, Decaens T, Tak WY, Jeng LB, Liu D, Ezzeddine R, Walters I, Cheng AL: Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. J Clin Oncol 2013;31:3517–3524.
12 Zhu AX, Rosmorduc O, Evans TR, Ross PJ, Santoro A, Carrilho FJ, Bruix J, Qin S, Thuluvath PJ, Llovet JM, Leberre MA, Jensen M, Meinhardt G, Kang YK: SEARCH: a phase III, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with advanced hepatocellular carcinoma. J Clin Oncol 2015;33:559–566.
13 Llovet JM, Decaens T, Raoul JL, Boucher E, Kudo M, Chang C, Kang YK, Assenat E, Lim HY, Boige V, Mathurin P, Fartoux L, Lin DY, Bruix J, Poon RT, Sherman M, Blanc JF, Finn RS, Tak WY, Chao Y, Ezzeddine R, Liu D, Walters I, Park JW: 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. J Clin Oncol 2013;31:3509–3516.
14 Villanueva A, Chiang DY, Newell P, et al: Pivotal role of mTOR signaling in hepatocellular carcinoma. Gastroenterology 2008;135:1972–1983, 1983 e1–11.
15 Zhu AX, Kudo M, Assenat E, Cattan S, Kang YK, Lim HY, Poon RT, Blanc JF, Vogel A, Chen CL, Dorval E, Peck-Radosavljevic M, Santoro A, Daniele B, Furuse J, Jappe A, Perraud K, Anak O, Sellami DB, Chen LT: Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial. JAMA 2014;312:57–67.
16 Huynh H, Hao HX, Chan SL, Chen D, Ong R, Soo KC, Pochanard P, Yang D, Ruddy D, Liu M, Derti A, Balak MN, Palmer MR, Wang Y, Lee BH, Sellami D, Zhu AX, Schlegel R, Huang A: Loss of Tuberous Sclerosis Complex 2 (TSC2) Is Frequent in Hepatocellular Carcinoma and Predicts Response to mTORC1 Inhibitor Everolimus. Mol Cancer Ther 2015;14:1224–1235.
17 Zhu AX, Ryoo BY, Yen CJ, et al: Ramucirumab (RAM) as second-line treatment in patients (pts) with advanced hepatocellular carcinoma (HCC): Analysis of patients with elevated α-fetoprotein (AFP) from the randomized phase III REACH study. 2015 Gastrointestinal Cancers Symposium, San Francisco, USA;2015.
18 Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J, Gores GJ, Panel of Experts in HCC-Design Clinical Trials: Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698–711.
19 Llovet JM, Hernandez-Gea V: Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design. Clin Cancer Res 2014;20:2072–2079.
20 National Cancer Institute NCI Dictionary of Cancer Terms: Available at: http://www.cancer.gov/dictionary?cdrid=45618. Accessed April 6, 2015.
21 Sawyers CL: The cancer biomarker problem. Nature 2008;452:548–552.
22 Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177–182.
23 Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, et al: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989;244:707–712.

24 Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–792.
25 Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, Pegram M, Oh DY, Diéras V, Guardino E, Fang L, Lu MW, Olsen S, Blackwell K, EMILIA Study Group: Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012;367:1783–1791.
26 Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, Jagiello-Gruszfeld A, Crown J, Chan A, Kaufman B, Skarlos D, Campone M, Davidson N, Berger M, Oliva C, Rubin SD, Stein S, Cameron D: Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 2006;355:2733–2743.
27 Mendelsohn J, Baselga J: The EGF receptor family as targets for cancer therapy. Oncogene 2000;19:6550–6565.
28 Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G: The anaplastic lymphoma kinase in the pathogenesis of cancer. Nat Rev Cancer 2008;8:11–23.
29 Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, Solomon B, Stubbs H, Admane S, McDermott U, Settleman J, Kobayashi S, Mark EJ, Rodig SJ, Chirieac LR, Kwak EL, Lynch TJ, Iafrate AJ: Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009;27:4247–4253.
30 Yang P, Kulig K, Boland JM, Erickson-Johnson MR, Oliveira AM, Wampfler J, Jatoi A, Deschamps C, Marks R, Fortner C, Stoddard S, Nichols F, Molina J, Aubry MC, Tang H, Yi ES: Worse disease-free survival in never-smokers with ALK+ lung adenocarcinoma. J Thorac Oncol 2012;7:90–97.
31 Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Jänne PA, Costa DB, Varella-Garcia M, Kim WH, Lynch TJ, Fidias P, Stubbs H, Engelman JA, Sequist LV, Tan W, Gandhi L, Mino-Kenudson M, Wei GC, Shreeve SM, Ratain MJ, Settleman J, Christensen JG, Haber DA, Wilner K, Salgia R, Shapiro GI, Clark JW, Iafrate AJ: Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693–1703.
32 Camidge DR, Kono SA, Lu X, Okuyama S, Barón AE, Oton AB, Davies AM, Varella-Garcia M, Franklin W, Doebele RC: Anaplastic lymphoma kinase gene rearrangements in non-small cell lung cancer are associated with prolonged progression-free survival on pemetrexed. J Thorac Oncol 2011;6:774–780.
33 Kim DW, Ahn MA, Shi Y, et al: Results of a global phase II study with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC). 2012 ASCO Annual Meeting,New York City, USA;2012.
34 Mok T, Kim DW, Wu YL, et al: First-line crizotinib versus pemetrexed–cisplatin or pemetrexed–carboplatin in patients (pts) with advanced ALK-positive non-squamous non-small cell lung cancer (NSCLC): results of a phase III study (PROFILE 1014). 2014 ASCO Annual Meeting, Chicago, USA;2014.
35 Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, Moro-Sibilot D, Camidge DR, Mok T, Hirsh V, Riely GJ, Iyer S, Tassell V, Polli A, Wilner KD, Jänne PA: Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med 2013;368:2385–2394.
36 Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho KH, Aiba S, Bröcker EB, LeBoit PE, Pinkel D, Bastian BC: Distinct sets of genetic alterations in melanoma. N Engl J Med 2005;353:2135–2147.
37 Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA, BRIM-3 Study Group: Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011;364:2507–2516.
38 Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, Clément B, Balabaud C, Chevet E, Laurent A, Couchy G, Letouzé E, Calvo F, Zucman-Rossi J: Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012;44:694–698.
39 Kan Z, Zheng H, Liu X, Li S, Barber TD, Gong Z, Gao H, Hao K, Willard MD, Xu J, Hauptschein R, Rejto PA, Fernandez J, Wang G, Zhang Q, Wang B, Chen R, Wang J, Lee NP, Zhou W, Lin Z, Peng Z, Yi K, Chen S, Li L, Fan X, Yang J, Ye R, Ju J, Wang K, Estrella H, Deng S, Wei P, Qiu M, Wulur IH, Liu J, Ehsani ME, Zhang C, Loboda A, Sung WK, Aggarwal A, Poon RT, Fan ST, Wang J, Hardwick J, Reinhard C, Dai H, Li Y, Luk JM, Mao M: Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. Genome Res 2013;23:1422–1433.
40 Chiang DY, Villanueva A, Hoshida Y, Peix J, Newell P, Minguez B, LeBlanc AC, Donovan DJ, Thung SN, Solé M, Tovar V, Alsinet C, Ramos AH, Barretina J, Roayaie S, Schwartz M, Waxman S, Bruix J, Mazzaferro V, Ligon AH, Najfeld V, Friedman SL, Sellers WR, Meyerson M, Llovet JM: Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. Cancer Res 2008;68:6779–6788.
41 Yamashita T, Forgues M, Wang W, Kim JW, Ye Q, Jia H, Budhu A, Zanetti KA, Chen Y, Qin LX, Tang ZY, Wang XW: EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. Cancer Res 2008;68:1451–1461.
42 Boyault S, Rickman DS, de Reyniès A, Balabaud C, Rebouissou S, Jeannot E, Hérault A, Saric J, Belghiti J, Franco D, Bioulac-Sage P, Laurent-Puig P, Zucman-Rossi J: Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology 2007;45:42–52.
43 Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR: Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Res 2009;69:7385–7392.

44 Gherardi E, Birchmeier W, Birchmeier C, Vande Woude G: Targeting MET in cancer: rationale and progress. Nat Rev Cancer 2012;12:89–103.
45 Munshi N, Jeay S, Li Y, Chen CR, France DS, Ashwell MA, Hill J, Moussa MM, Leggett DS, Li CJ: ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity. Mol Cancer Ther 2010;9:1544–1553.
46 Santoro A, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, Van Vlierberghe H, Trojan J, Kolligs FT, Weiss A, Miles S, Gasbarrini A, Lencioni M, Cicalese L, Sherman M, Gridelli C, Buggisch P, Gerken G, Schmid RM, Boni C, Personeni N, Hassoun Z, Abbadessa G, Schwartz B, Von Roemeling R, Lamar ME, Chen Y, Porta C: Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. Lancet Oncol 2013;14:55–63.
47 Lu D, Shen J, Vil MD, Zhang H, Jimenez X, Bohlen P, Witte L, Zhu Z: Tailoring in vitro selection for a picomolar affinity human antibody directed against vascular endothelial growth factor receptor 2 for enhanced neutralizing activity. J Biol Chem 2003;278:43496–43507.
48 Zhu AX, Finn RS, Mulcahy M, Gurtler J, Sun W, Schwartz JD, Dalal RP, Joshi A, Hozak RR, Xu Y, Ancukiewicz M, Jain RK, Nugent FW, Duda DG, Stuart K: A phase II and biomarker study of ramucirumab, a human monoclonal antibody targeting the VEGF receptor-2, as first-line monotherapy in patients with advanced hepatocellular cancer. Clin Cancer Res 2013;19:6614–6623.
49 Blum R, Cox AD, Kloog Y: Inhibitors of chronically active ras: potential for treatment of human malignancies. Recent Patents Anticancer Drug Discov 2008;3:31–47.
50 Tannapfel A, Sommerer F, Benicke M, Katalinic A, Uhlmann D, Witzigmann H, Hauss J, Wittekind C: Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. Gut 2003;52:706–712.
51 Tsuda H, Hirohashi S, Shimosato Y, Ino Y, Yoshida T, Terada M: Low incidence of point mutation of c-Ki-ras and N-ras oncogenes in human hepatocellular carcinoma. Jpn J Cancer Res 1989;80:196–199.
52 Schmidt CM, McKillop IH, Cahill PA, Sitzmann JV: Increased MAPK expression and activity in primary human hepatocellular carcinoma. Biochem Biophys Res Commun 1997;236:54–58.
53 Ito Y, Sasaki Y, Horimoto M, Wada S, Tanaka Y, Kasahara A, Ueki T, Hirano T, Yamamoto H, Fujimoto J, Okamoto E, Hayashi N, Hori M: Activation of mitogen-activated protein kinases/extracellular signal-regulated kinases in human hepatocellular carcinoma. Hepatology 1998;27:951–958.
54 Calvisi DF, Ladu S, Gorden A, Farina M, Conner EA, Lee JS, Factor VM, Thorgeirsson SS: Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. Gastroenterology 2006;130:1117–1128.
55 O'Neil BH, Goff LW, Kauh JS, Strosberg JR, Bekaii-Saab TS, Lee RM, Kazi A, Moore DT, Learoyd M, Lush RM, Sebti SM, Sullivan DM: Phase II study of the mitogen-activated protein kinase 1/2 inhibitor selumetinib in patients with advanced hepatocellular carcinoma. J Clin Oncol 2011;29:2350–2356.
56 Lim HY, Heo J, Choi HJ, Lin CY, Yoon JH, Hsu C, Rau KM, Poon RT, Yeo W, Park JW, Tay MH, Hsieh WS, Kappeler C, Rajagopalan P, Krissel H, Jeffers M, Yen CJ, Tak WY: A phase II study of the efficacy and safety of the combination therapy of the MEK inhibitor refametinib (BAY 86-9766) plus sorafenib for Asian patients with unresectable hepatocellular carcinoma. Clin Cancer Res 2014;20:5976–5985.
57 Giannelli G, Villa E, Lahn M: Transforming growth factor-β as a therapeutic target in hepatocellular carcinoma. Cancer Res 2014;74:1890–1894.
58 Faivre SJ, Santoro A, Kelley RK, Merle P, Gane E, et al: A phase 2 study of a novel transforming growth factor-beta (TGF-beta1) receptor 1 kinase inhibitor, LY 2157299 monohydrate (LY), in patients with advanced hepatocellular carcinoma (HCC). 2014 Gastrointestinal Cancers Symposium, San Francisco, USA; 2014.
59 Bataller R, Brenner DA: Liver fibrosis. J Clin Invest 2005;115:209–218.
60 Yu C, Wang F, Jin C, Huang X, Miller DL, Basilico C, McKeehan WL: Role of fibroblast growth factor type 1 and 2 in carbon tetrachloride-induced hepatic injury and fibrogenesis. Am J Pathol 2003;163:1653–1662.
61 Kan M, Wu X, Wang F, McKeehan WL: Specificity for fibroblast growth factors determined by heparan sulfate in a binary complex with the receptor kinase. J Biol Chem 1999;274:15947–15952.
62 Miura S, Mitsuhashi N, Shimizu H, Kimura F, Yoshidome H, Otsuka M, Kato A, Shida T, Okamura D, Miyazaki M: Fibroblast growth factor 19 expression correlates with tumor progression and poorer prognosis of hepatocellular carcinoma. BMC Cancer 2012;12:56.
63 Sawey ET, Chanrion M, Cai C, Wu G, Zhang J, Zender L, Zhao A, Busuttil RW, Yee H, Stein L, French DM, Finn RS, Lowe SW, Powers S: Identification of a therapeutic strategy targeting amplified FGF19 in liver cancer by Oncogenomic screening. Cancer Cell 2011;19:347–358.
64 Finn RS, Aleshin A, Zhao D, Anderson L, Dering J, Shi M, Graus Porta D, Busuttil RW: Slamon DJ: Gains in FGF19 are predictive of response to the Fibroblast Growth Factor Receptor (FGFR) Small Molecule Tyrosine Kinase Inhibitor BGJ 398 *in vitro.* American Society of Cancer Research Annual Meeting 2012, Abstract 3858, Chicago, Illinois.
65 Guagnano V, Kauffmann A, Wöhrle S, Stamm C, Ito M, Barys L, Pornon A, Yao Y, Li F, Zhang Y, Chen Z, Wilson CJ, Bordas V, Le Douget M, Gaither LA, Borawski J, Monahan JE, Venkatesan K, Brümmendorf T, Thomas DM, Garcia-Echeverria C, Hofmann F, Sellers WR, Graus-Porta D: FGFR genetic alterations predict for sensitivity to NVP-BGJ39**8**, a selective pan-FGFR inhibitor. Cancer Discov 2012;2:1118-1133.
66 Hagel M, Miduturu C, Sheets M, et al: First Selective Small Molecule Inhibitor of FGFR4 for the Treatment of Hepatocellular Carcinomas with an Activated FGFR4 Signaling Pathway. Cancer Discov 2015;5:424–437.
67 Arao T, Ueshima K, Matsumoto K, Nagai T, Kimura H, Hagiwara S, Sakurai T, Haji S, Kanazawa A, Hidaka H, Iso Y, Kubota K, Shimada M, Utsunomiya T, Hirooka M, Hiasa Y, Toyoki Y, Hakamada K, Yasui K, Kumada T, Toyoda H, Sato S, Hisai H, Kuzuya T, Tsuchiya K, Izumi N, Arii S, Nishio K, Kudo M: FGF3/FGF4 amplification and multiple lung metastases in responders to sorafenib in hepatocellular carcinoma. Hepatology 2013;57:1407–1415.

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68 Capurro M, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, Filmus J: Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology 2003;125:89–97.
69 Wang FH, Yip YC, Zhang M, Vong HT, Chan KI, Wai KC, Wen JM: Diagnostic utility of glypican-3 for hepatocellular carcinoma on liver needle biopsy. J Clin Pathol 2010;63:599–603.
70 Zhu ZW, Friess H, Wang L, Abou-Shady M, Zimmermann A, Lander AD, Korc M, Kleeff J, Büchler MW: Enhanced glypican-3 expression differentiates the majority of hepatocellular carcinomas from benign hepatic disorders. Gut 2001;48:558–564.
71 Yorita K, Takahashi N, Takai H, Kato A, Suzuki M, Ishiguro T, Ohtomo T, Nagaike K, Kondo K, Chijiiwa K, Kataoka H: Prognostic significance of circumferential cell surface immunoreactivity of glypican-3 in hepatocellular carcinoma. Liver Int 2011;31:120–131.
72 Ishiguro T, Sugimoto M, Kinoshita Y, Miyazaki Y, Nakano K, Tsunoda H, Sugo I, Ohizumi I, Aburatani H, Hamakubo T, Kodama T, Tsuchiya M, Yamada-Okabe H: Anti-glypican 3 antibody as a potential antitumor agent for human liver cancer. Cancer Res 2008;68:9832–9838.
73 Zhu AX, Gold PJ, El-Khoueiry AB, Abrams TA, Morikawa H, Ohishi N, Ohtomo T, Philip PA: First-in-man phase I study of GC33, a novel recombinant humanized antibody against glypican-3, in patients with advanced hepatocellular carcinoma. Clin Cancer Res 2013;19:920–928.
74 Yen CJ, Daniele B, Kudo M, et al: Randomized phase II trial of intravenous ROS137382/GC33 at 1600mg every other week and placebo in previously treated patients with unresectable advanced hepatocellular carcinoma (HCC; NCT01507168). 2014 ASCO Annual Meeting, Chicago, USA;2014.
75 Feng M, Ho M: Glypican-3 antibodies: a new therapeutic target for liver cancer. FEBS Lett 2014;588:377–382.