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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.

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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

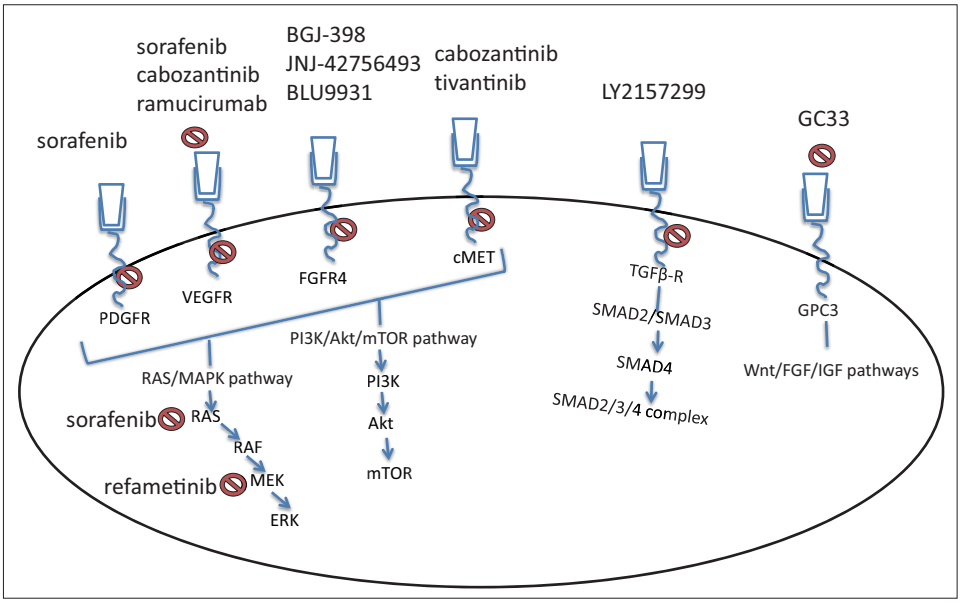

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Fig. 1. Targeted agents and molecular pathways in biomarker directed research in hepatocellular carcinoma. PDGFR=platelet-derived growth factor receptor; TGF-βR=transforming growth factor-beta receptor.

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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