Clinical Application of Basic Science

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Rethinking future development of molecular therapies in hepatocellular carcinoma: A bottom-up approach

Augusto Villanueva*

HCC Translational Research Laboratory, Barcelona-Clínic Liver Cancer Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Liver Unit, Hospital Clínic, Barcelona, Catalonia, Spain; Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas, Instituto Carlos III, Madrid, Spain

Summary

The high failure rate of phase 3 trials in oncology is forcing the scientific community to rethink drug development strategies and optimize trial design. The current paradigm of systemic therapies is progressively favoring molecular-based patient selection. In hepatocellular carcinoma, four out of the five phase 3 trials that tested molecular therapies in the last 5 years have been negative. None of them included enriched populations using predicted biomarkers of response. Hence, there is an increasing need to provide new targets and refine selection criteria in HCC clinical trials using molecular readouts of tumor biology.

Lessons from negative trials in hepatocellular carcinoma

Disease burden due to hepatocellular carcinoma (HCC) continues to grow. Mortality related to this cancer has increased by more than 50% in the last 20 years, worldwide [1]. In fact, among the leading cancer causes of disability-adjusted life years (i.e., sum of years lost due to premature mortality and years lived with disability), liver cancer shows one of the highest increases [1]. Despite these dismal data, there have been major achievements in the clinical management of HCC. For example, the application of curative therapies such as surgical resection or transplantation provides good survival rates, beyond 65% at 5 years. Unfortunately, most patients are diagnosed at intermediate or advanced stages were curative options are not recommended [2]. This emphasizes the need to direct resources to improve HCC early detection by fully deploying surveillance programs in patients at high risk [2]. When available, effective HCC chemoprevention in cirrhotic patients will also have a positive impact on liver cancer worldwide. A recent phase 3 randomized controlled trial (RCT) demonstrated that a molecular targeted agent, sorafenib, was able to significantly improve survival from 7.9 to 10.7 months in patients with advanced HCC [2], mainly by delaying tumor progression [3]. These positive results obtained with a so-called molecular therapy prompted the proliferation of clinical trials testing different targeted therapies in HCC, both in first and second line [4]. However, initial results of these follow-up trials have been discouraging and suggest that HCC treatment is far more complex that initially anticipated. These recent failures also highlight the need for novel targets and new strategies for drug evaluation in the molecular therapy era.

By the end of 2012, four drugs (i.e., sunitinib, erlotinib, linifanib, and brivanib) have been unable to improve or parallel sorafenib's results in large phase 3 randomized controlled trials (RCT), despite some of them were reported to have some efficacy signals in phase 2. Some of these drugs share molecular targets with sorafenib like VEGFR or PDGFR, while others add additional blockade activity against CKIT, EGFR, and FGFR (Table 1). Results of these trials follow the overall low success rate of phase 3 RCT in other malignancies, usually with a positive rate of less than 30%, and an estimated cost of $2.5 billion lost per year [5]. Different strategies have been suggested to revert this negative trend, including (a) improvements in trial design (e.g., encouraging interim analyses, adaptive trial design, etc.), (b) a thorough re-evaluation of the criteria utilized to identify efficacy signals in phase 2 trials, (c) identification of new molecular targets, and (d) improvements in patient selection. For the case of HCC, we will briefly discuss the latter two.

A number of phase 3 RCT are currently evaluating molecular therapies in advanced HCC, most of them in second line (Table 1). They are testing drugs with blockade activity against mTOR (everolimus), VEGFR (ramucirumab, regorafenib), TIE2 (regorafenib), MET (tivantinib), and PDGFR (regorafenib). Only one of them includes patients based on tumor activation of its predicted target (i.e., MET receptor and tivantinib), which indicates that there is still a long way to go for personalized medicine in trial design for HCC. Nevertheless, positive results of trial enrichment based on oncogene addiction loops in other malignancies are starting to dissipate the reasonable concerns about this new approach.

High-throughput era: Novel targets

The last decade has witnessed a revolution in how scientists study cancer genomes. The emergence and rapid development of sequencing technologies have dramatically impacted

Keywords: Liver cancer; Sorafenib; Personalized medicine; Clinical trials.
Received 17 February 2013; received in revised form 19 March 2013; accepted 21 March 2013
* Address: HCC Translational Research Laboratory, BCLC-Group, CIBEREHD, IDIBAPS, Center Esther Koplowtiz, 3-Floor, 08038 Barcelona, Spain.
E-mail address: augusto.villanueva@ciberehd.org.

Table 1. Phase 3 clinical trials testing molecular targeted therapies in advanced HCC.

Drug	Main targets	Indication	Biomarker-trial enrichment	Status
Sorafenib	BRAF, VEGFR, PDGFR	First line	None	Approved
Sunitinib	VEGFR, KIT, PDGFR	First line	None	Negative
Brivanib	FGFR, VEGFR	First and second line	None	Negative
Linifanib	VEGFR, PDGFR	First line	None	Negative
Erlotinib	EGFR	First line	None	Negative
Regorafenib	VEGFR, TIE2, PDGFR	Second line	None	Ongoing
Everolimus	MTOR	Second line	None	Ongoing
Ramucirumab	VEGFR	Second line	None	Ongoing
Tivantinib	MET	Second line	High MET (immunohistochemistry)	Ongoing

biomedical research, particularly in the field of target discovery in oncology [6]. Nowadays, it is possible to obtain the complete nucleotide sequence of a given tumor, which enables the identification of new mutations, copy number changes, and fusion events. The addition of data from epigenetic alterations provides new opportunities to dissect the molecular determinants of tumor development and progression. This vast amount of raw data makes functional data integration a major challenge, and sets a new ground for the deployment of personalized medicine approaches in cancer care.

These new techniques have been applied to study HCC. Different studies corroborate that mutations in *TP53* and *CTNNB1* are the most prevalent in HCC, being in fact mutually exclusive [7]. They also underscore the role of chromatin remodeling aberrations in a subgroup of HCC, mostly through mutations in *ARID1A* and *ARID2* [8]. Additional genes with frequent mutations in HCC include *NFE2L2* and the MLL family of histone methyltransferases. Genomic sequencing also confirmed HBV integrations at cancer-related genes such as *TERT*, *MLL4*, and *CCNE1*. Exome sequencing also enabled the identification of tumor suppressor properties for *IRF2*, whose inactivation led to impaired *TP53* function [7]. A major bottleneck of these studies relates to a limited power for functional validation. Unfortunately, the large number of low frequency mutations determines that only a handful of these candidates will be further tested in animal models, despite recent improvements in strategies for cancer modeling *in vivo* [9]. Considering that some of these low prevalence mutations may harbor oncogene addiction loops (discussed below), it is obvious that more extensive validation approaches will be required in the future. Besides mutations, deep sequencing will potential unleash chromosomal rearrangements and fusion proteins. Although fusion events have been mostly described in hematological malignancies, some reports found them in solid tumors, and they may potentially behave like oncogenic addiction loops [10].

Besides sequencing data, numerous studies implicate epigenetic aberrations in HCC development and progression. The epigenome refers to those chemical changes unrelated to DNA nucleotide sequence but with an impact on cellular phenotype through modulation of gene expression. The epigenetic machinery involves DNA methylation, histone modifications, and nucleosome positioning [11]. Potential DNA methylation sites are mostly cytosines when followed by a guanine (i.e., CpG dinucleotides). CpG sites tend to cluster, forming CpG islands, and locate in gene promoters where they control transcription initiation. Human cancers are characterized by a global DNA hypomethylation, which correlates with increased genomic instability. Also, malignant tumors have selective hypermethylation of CpG islands in promoter regions of tumor suppressor genes. Besides DNA methylation, histone modifications can also regulate gene expression. Numerous reagents able to modify DNA methylation and histone conformation have been tested in human cancer, and four of them have received FDA approval, for myelodysplastic syndromes and cutaneous T-cell lymphoma. In HCC, preclinical evidence suggests antitumor activity for a histone deacetylase inhibitor (panobinostat [12]), but clinical studies are still in early developmental phases. Novel treatment modalities currently under early clinical phases include the oncolytic and immunotherapeutic vaccine virus (JX-594). An uncontrolled small phase 2 trial has recently shown 15% objective responses and median survival of 9 months (14.1 in patients receiving higher doses of JX-594 [13]) in patients with advanced HCC. Despite being early phase and taking results with caution, the study opens new therapeutic paths for immunotherapy and viral mediated drug delivery in HCC. Other emerging sources of HCC therapeutics involve underexplored pathways in HCC, such as autophagy, the role of lymphotoxins, gut microbiota, etc. [14].

Molecular-based patient selection

In 2008, a panel of experts reported a set of recommendations for HCC trial design using evidence-based principles. They covered different aspects of HCC clinical research such as end points, assessment of response, stratification strategies and unmet research needs [15]. Regarding patient selection, recommendations emphasized the need for a homogenization of inclusion criteria based on BCLC stage and liver function. Enrichment strategies using molecular biomarkers were barely mentioned, despite the fact that they forecast a future role of molecular-based tumor classification in trial design.

The search for molecular predictors of response is becoming a standard practice in clinical oncology research. This model of patient selection relies on the concept of oncogenic addiction, which basically requires *a priori* knowledge of the specific molecular alterations responsible for tumor progression on an individual basis [16]. There are different successful examples of this approach, but it is remarkable the recent introduction of crizotinib in ALK+ lung cancer. Briefly, between 2007 and 2009 a series of publications demonstrated the presence of an aberrant fusion