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Hepatocellular Carcinoma: Molecular Biology and Therapy

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Abstract

Advanced and metastatic hepatocellular carcinomas (HCC) are challenging to treat, and no cytotoxic agents have impacted survival. The underlying liver cirrhosis that commonly accompanies HCC provides an additional challenge; indeed, functional scoring of cirrhosis and HCC is a critical component of patient evaluation. Currently, the molecular biology and pathogenesis of HCC are being increasingly investigated, which may lead to better understanding of the evolution of the disease, especially differing etiologies and identification of survival genes that may affect outcome. Early studies of targeted therapies in HCC have shown disease stabilization, and an increased understanding of the mechanism(s) of these novel agents combined with correlative studies may lead to the identification of an active agent or combination of agents that impacts the natural history of HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) remains one of the most common solid tumor malignancies worldwide, with Western Africa and China reporting the highest incidence per capita.[1] The rising concern regarding the incidence of HCC in North America[2] is most likely explained by the increased incidence of hepatitis C on the continent.

Advanced and metastatic HCC remain challenging diseases to treat. The hostile environment of the liver with many drug-resistance genes renders chemotherapy relatively ineffective. Furthermore, the underlying cirrhotic condition of the liver that in most instances accompanies HCC provides an additional treatment challenge.

CIRRHOSIS AND SCORING SYSTEMS

The presence of cirrhosis is an important factor to assess when evaluating any patient with HCC. Historically, medical oncologists have used the Child's-Pugh Scoring System[3,4] which consists of five parameters: albumin, bilirubin, prothrombin time, and the presence or absence of ascites, and encephalopathy. Each of those parameters is given one to three points based on severity, and the addition of those points will lead to a score system, A, B or C, which helps define a patient's outcome (Table 1). A limitation of the Child's-Pugh score is the lack of any parameter that directly pertains to the cancer itself. Dr. Okuda and colleagues subsequently developed a scoring system that includes bilirubin, albumin, and ascites parameters from the Child's-Pugh scoring system, and added to it an assessment of the tumor extent in the liver.

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This concept of adding more parameters pertaining to the tumor itself was further developed by several groups, leading to the Cancer of the Liver Italian Program (CLIP) score,[5,6] which includes the Child's-Pugh score parameters, and an assessment of tumor extent in the liver, the presence or absence of portal vein thrombosis, and the level of alpha-fetoprotein (AFP) (Table 2). Another scoring system was developed by Leung et al from the Chinese University in Hong Kong, and is known as the Chinese University Prognostic Index (CUPI).[7] The CUPI, like the CLIP score, prospectively identifies pertinent prognostic factors. These factors include bilirubin, ascites, alpha-fetoprotein, alkaline phosphatase, the tumor extent as defined by the TNM staging system, and the absence or presence of clinical symptoms on presentation (Table 3). These two scoring systems were assessed in two different populations: patients with predominantly hepatitis C-related HCC in the CLIP population, and hepatitis B-related HCC in the CUPI patient population. An attempt by Leung's group[7] to retrofit the data of their predominantly Asian patients in the CLIP scoring system led to erroneous predictions of outcome, suggesting that different scoring systems may apply to different patient populations.

Ideally, when used in the appropriate patient population, a scoring system should give the medical oncologist an objective prediction of the likely overall survival (Figures 1 and 2). This might assist in deciding the optimal treatment approach.

CHEMOTHERAPY

Many chemotherapeutic agents have been tested in HCC, mainly in phase II studies evaluating response. Response rates ranging from 10% to 20%[8–10] have been reported, but no clear impact on survival has been identified.

The most studied chemotherapeutic agent is doxorubicin (Adriamycin), with a response rate of approximately 11%.[11] The regimen that has demonstrated the highest response in HCC is the combination of cisplatin (Platinol), interferon, doxorubicin, and 5-fluorouracil (PIAF), which demonstrated a 26% response rate and 9-month median survival in a recently reported phase II study.[12] Among 13 patients in that study who had a partial response, 9 underwent surgery, and 4 were found to have a complete pathologic response to the chemotherapy. This led to one of the few randomized phase III studies in advanced HCC, comparing PIAF versus doxorubicin (control arm).[11] The study was negative with regard to the primary end-point (ie, median survival), which was 8.6 months with PIAF versus 6.8 months with doxorubicin ($P = .83$). However, response rate doubled in the PIAF arm (21%) relative to the doxorubicin arm (11%). The higher response rate in the PIAF arm was at the expense of significant toxicity, particularly myelosuppression and neutropenic fever. With the lack of a survival impact from PIAF, this combination should not be routinely administered. Nonetheless, PIAF may be considered for selected fit patients with good liver function who have a potentially resectable tumor, justifying the high toxicity against potential curability.

MOLECULAR CONCEPTS

In recent years, investigators have achieved a better understanding of the molecular events leading to oncogenesis of HCC. In parallel, multiple novel therapeutic agents are being assessed in HCC.

With regard to etiology, as an example, chronic hepatitis can lead to phenotypically altered hepatocytes and ultimately cirrhosis. Occasionally, chronic hepatitis B leads to HCC without the interval development of cirrhosis. Phenotypically altered hepatocytes have high epidermal growth factor receptor (EGFR) expression and non-committal epigenetic changes (Figure 3). These phenotypically altered hepatocytes may become dysplastic and show more committed genetic changes, eg, increased telomerase activity and varied allelic deletions. These

developments may ultimately lead to the evolution of HCC with additional genetic changes, eg, an increase in c-myc and decrease in p16 expression, amongst others.[13]

It is important to note that while many of the genetic changes are generic among differing etiologies that lead to the development of HCC, some are etiology-specific. For example, in the case of hepatitis C, EGFR and Raf expression are expressed to a greater extent, compared with hepatitis B-related HCC. These varied genetic make-ups of the differing etiologies of HCC may one day serve as a molecular fingerprint for a specific etiology.[13] Determining those genetic alterations may not only help define the etiology of the disease, eg, in a patient with hepatitis B who develops hepatocellular carcinoma, but also may contribute to the design of etiology-specific therapy.

Differentiation at the molecular level may explain the reason behind the more aggressive behavior of a poorly differentiated HCC compared to a well-differentiated tumor. This is nicely depicted in a study by Lee and Thorgeirsson,[14] who collected 91 tissue samples of patients with HCC and evaluated them for an array of 4,184 genes. They found them to cluster in two groups: cluster A and cluster B. Cluster A samples showed high expression of survival genes whereas cluster B samples had less of these survival genes. The cluster B group had a better survival. Many of those survival genes have been identified and consist mainly of cell cycle regulation, cell proliferation, and anti-apoptosis genes, as well as histones and HIF-1A regulators. Cluster A was shown to have 46% of the survival genes compared with only 19% in cluster B.

These concepts of differentiation undoubtedly support and partly explain the well described resistance to therapy phenomenon noticed in HCC. HCC is comprised of highly resistant clones of cancer cells.[15] This has been shown not only through clinical experience, but also in laboratory systems such as the rat hepatocyte model studied by Farber et al.[16,17] HCC cells carry a high “genetic mutational load” that makes them less amenable to the destructive actions of chemotherapy. In terms of clinical applicability, these patient-specific profiles may lead to custom-designed therapy for individual patients with HCC who have different etiologies for their disease and express different survival genes.

NOVEL THERAPEUTICS

Many novel therapeutic agents for HCC have been evaluated in phase I and II studies. Sorafenib (Nexavar), erlotinib (Tarceva), bevacizumab (Avastin), and flavopiridol will be discussed herein. Sorafenib is a Raf kinase inhibitor with anti-vascular endothelial growth factor (VEGF) and –platelet-derived growth factor (PDGF) activity.[18] VEGF and PDGF have been implicated in tumor angiogenesis of highly vascular HCC.[19,20] A phase II study evaluating sorafenib in patients with HCC has been completed.[21] The primary endpoint was response. Of 137 patients accrued, 5% showed a partial response. However, 43% of patients had stable disease for at least 4 months. Interestingly, some of these patients who had progression of disease by radiologic criteria had a decrease in alpha-fetoprotein, and on closer inspection of the radiologic evaluations, were shown to have an increase in tumor necrosis. This suggests a response to therapy despite progression by routine radiologic criteria. In addition, patients whose tumors expressed higher baseline staining intensity of pERK (n = 33), downstream from Raf (2–4+; n = 18), had a significantly longer time to progression (TTP) than those with a lower staining intensity (0–1+; n = 15) (P = .00034), suggesting the validity of the target and its response to sorafenib. Sorafenib is being further evaluated in two international randomized studies in HCC; one is a phase III placebo-controlled trial, and the other is a randomized phase II comparison of doxorubicin plus sorafenib versus doxorubicin plus placebo. The latter study emanates from the concept that antiangiogenic agents have limited single-agent activity and are likely more

active in combination with cytotoxic therapy, perhaps by facilitating improved chemotherapy delivery to the tumor.

Erlotinib, an oral tyrosine kinase inhibitor, has also been evaluated in a phase II trial in patients with HCC and those with cholangiocarcinoma. Twelve of 38 (32%) evaluable patients had progression-free survival at 6 months, the primary objective of the study.[22] Similarly, in a phase II trial[23] evaluating the VEGF inhibitor bevacizumab in patients with HCC, stable disease was observed in 18 of 25 (72%) evaluable patients. The latter trial is ongoing.

Several other targets further along the signal transduction pathway have been explored, including the cyclin-dependant kinase inhibitor, flavopiridol.[24] In the laboratory, flavopiridol, when given seven hours after SN38 (the active metabolite of irinotecan [Camptosar, CPT-11]), caused cleavage of the poly (ADP-ribose) polymerase (PARP) and caspase-3 cleavage (Figure 4), resulting in enhanced apoptosis.[25] Additional data suggest that patients with wild-type p53 and stable post-treatment p21 levels are most likely to respond to an irinotecan and flavopiridol combination.[26]

This concept has evolved from the laboratory to the bedside in a phase II trial of irinotecan followed by flavopiridol in patients with advanced HCC.[27] Of 16 patients on this study, one patient had stable disease for more than 1 year. This patient had a wild-type p53 tumor; however, p21 expression before and after therapy was not known, as its evaluation was not part of the study design.

Thus far, none of the novel therapeutics discussed above has shown any tangible disease regression against HCC, however, they are good illustrations of the targets that are currently being studied in this disease. An understanding of how these novel therapeutic agents may work will hopefully lead to a better understanding of the molecular pathogenesis of hepatocellular carcinoma.

CONCLUSION

Management of advanced and metastatic HCC continues to be challenging because of high expression of drug resistance genes, underlying cirrhosis, and poor liver function in many patients. Functional scoring of the cirrhosis and the HCC is a critical part of evaluating a patient with HCC. The CLIP scoring system should be used for patients with a hepatitis C etiology, and the CUPI scoring system is optimal for patients with hepatitis B-related HCC.

Thus far, no cytotoxic agent has been shown to have a survival impact in HCC. However, default chemotherapeutic standards, although possibly controversial, include doxorubicin with a response rate of approximately 11%, and possibly single-agent fluoropyrimidine therapy. The molecular biology and pathogenesis of HCC are being increasingly understood, which may lead to a better understanding of the evolution of the disease with regard to differing etiologies and identification of survival genes that may impact outcome. Early studies of targeted therapies in HCC have shown disease stabilization. A better understanding of the mechanism of novel therapeutics with the help of correlative studies may lead to the identification of an active agent or combination of agents with a true impact on the natural history of HCC.

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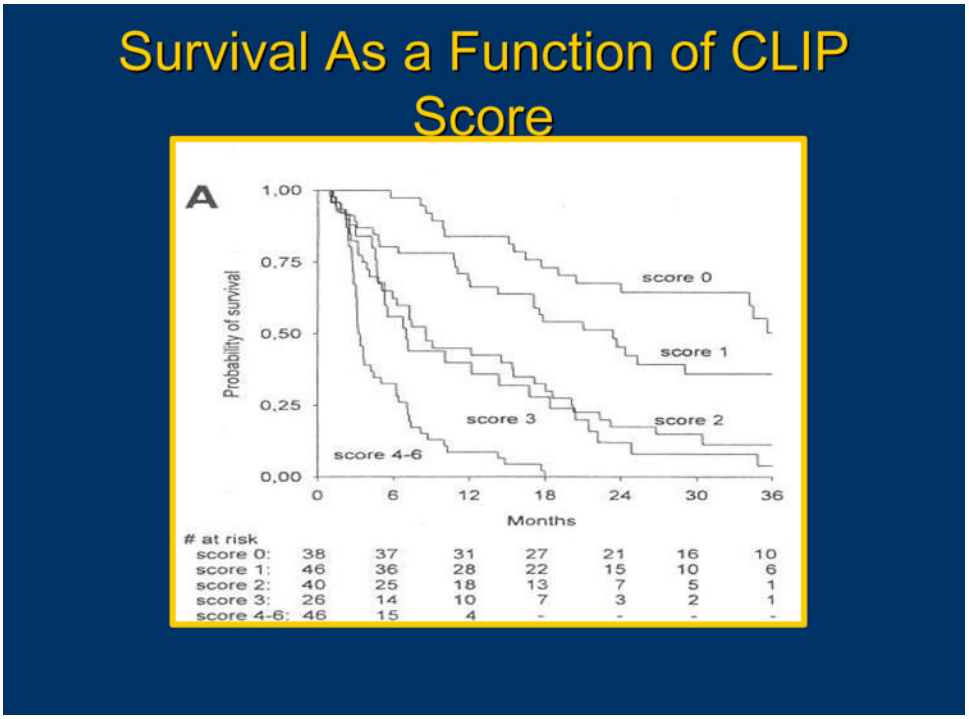

Figure 1.
Survival as a function of CLIP score. Abbreviations: CLIP, The Cancer of the Liver Italian Program.

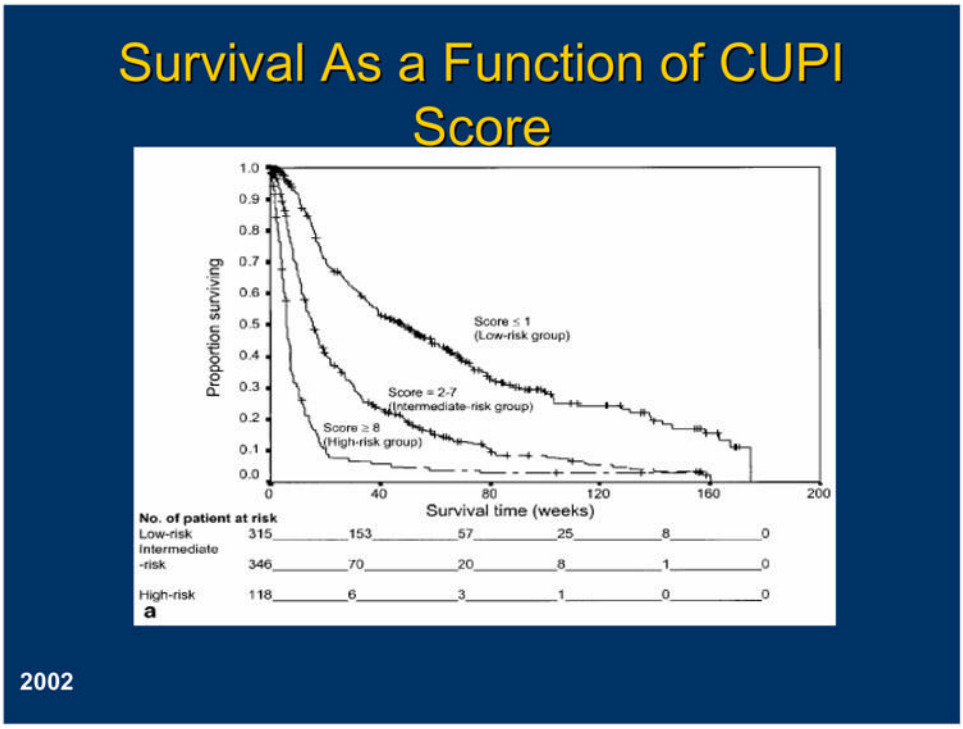

Figure 2.
Survival as a function of CUPI score. Abbreviations: CUPI, Chinese University Prognostic Index.

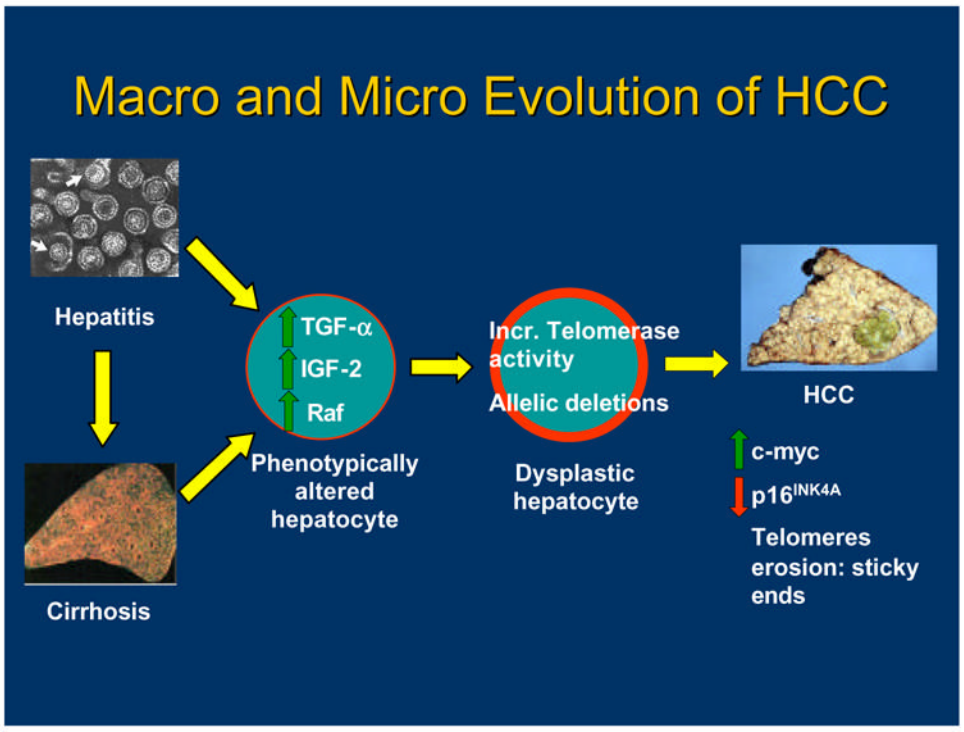

Figure 3.
Macro and micro evolution of HCC. Abbreviations: HCC, hepatocellular carcinoma; IGF, liver-derived insulin-like growth factor; TGF, transforming growth factor; Raf, rat sarcoma virus oncogene activated factor.

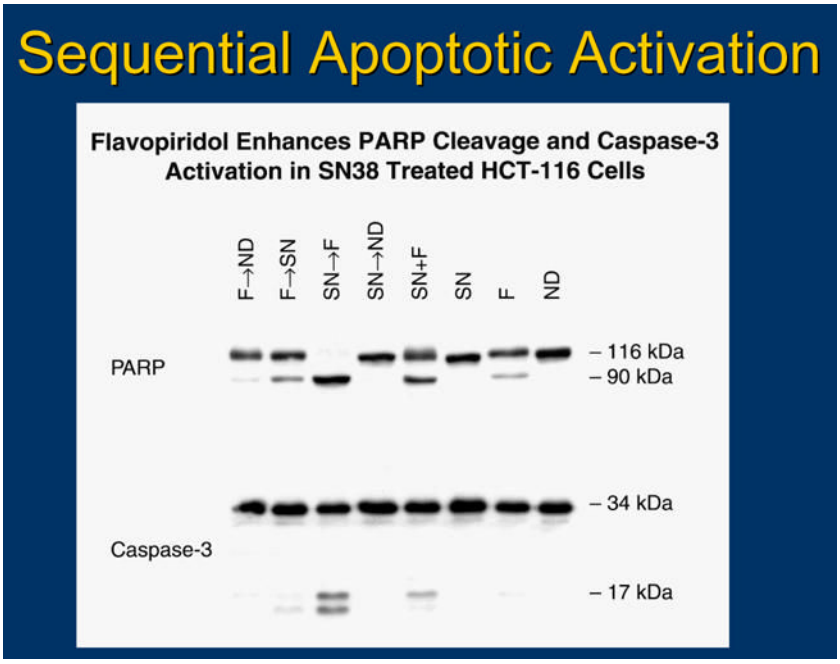

Figure 4.
Sequential apoptotic activation. Flavopiridol was given 7 hours after SN38, the active metabolite of irinotecan, resulting in cleavage of the Poly (ADP-ribose) polymerase (PARP) and caspase-3 activation.

Table 1

Child's Pugh Score of Liver Cirrhosis

Parameter	Points		
	1	2	3
Albumin (g/dL)	> 3.5	2.8–3.5	< 2.8
Bilirubin (mg/dL)	< 2	2 – 3	> 3
Ascites	Absent	Slight	Moderate
Encephalopathy	None	I – II	III – IV
PT (INR)	< 1.7	1.8 – 2.3	> 2.3
Score	A	B	C
Points	5 – 6	7 – 9	10 – 15

Modified from Child CG, Turcotte JG.[4]

Abbreviations: INR, international normalized ratio; PT, prothrombin time.

Table 2

CLIP Scoring System for HCC

Parameter			Score		
			0	1	2
Child's Pugh score			A	B	C
Tumor Morphology			Uni-nodular & Extension=50%	Multi-nodular & extension=50%	Massive or extension>50%
Portal Vein Thrombosis			no	yes	
AFP (ng/dL)			< 400	= 400	
Score	0	1	2	3	4–6

Modified from Reference 5. Abbreviations: AFP, alpha-fetoprotein; CLIP, Cancer of the Liver Italian Program; HCC, hepatocellular carcinoma.

Table 3

CUPI Risk Groups in HCC

Parameter	Weight (CUPI Score)					
Bilirubin (mg/dL)	**< 1.9**	**0**	**1.9 – 2.8**	**3**	**= 2.9 .**	**4**
Ascites	**Present**	**3**				
Alk. phosphatase	**= 200 IU/L**	**3**				
TNM Stage	**I & II**	**− 3**	**IIIa & IIIb**	**− 1**	**IVa & IVb**	**0**
AFP (ng/ml)	**= 500**	**2**				
Disease symptoms on presentation	**None**	**− 4**				
Risk Group	Low		Intermediate		High	
Score	− 7 to 1		2 to 7		8 to 12	

Modified from Leung et al.[7] Abbreviations: AFP, alpha-fetoprotein; alk. phosphatase, alkaline phosphatase; CUPI, Chinese University Prognostic Index; HCC, hepatocellular carcinoma.