



Personalized molecular targeted therapy for hepatocellular carcinoma in the era of genome sequencing

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Nowadays, hepatocellular carcinoma (HCC) represents a major health problem worldwide, being the second most frequent cause of cancer-related deaths (1). Among the different cancer types, HCC is the fifth most common in men and the seventh in women (1). In the liver, HCC is the first primary malignancy, accounting for about 90% of hepatic cancers. Its incidence is globally increasing, exceeding the 780,000 new cases in 2012 (2,3). Because of its high lethality, incidence and mortality of HCC are almost equal (1-3). Albeit with a global diffusion, HCC hits hard in some specific geographic areas, since over 80% of cases are diagnosed in the Sub-Saharan Africa and in East Asia, in contrast with North and South America, North Europe and Oceania, where HCC incidence is lower, whereas in the Mediterranean countries HCC risk is intermediate (4). Notably, although incidence of HCC progresses with age, reaching a peak between 60 and 75 years, its detection in young individuals is steadily increasing in the last decade, thus enhancing its socio-economic impact (4). In nearly 90% of cases, HCC develops on a background of chronic liver disease, generally cirrhosis (5,6), where the frequency of tumor development increases over the course of the disease (3.9% annual incidence), and HCC is actually the leading cause of death in patients with cirrhosis related to HCV infection (7). To date, a wide range of options can be offered for the treatment of these patients, including surgical resection, liver transplant, percutaneous ablation, transarterial chemoembolization, depending upon the residual hepatocellular function,

and the tumor development (size and stage). Despite this apparent richness of treatments, there is a startling lack of therapeutic effectiveness as the overall 5-year survival of HCC remains low (<20%), due to the severe dysfunction related to cirrhosis, the marked tumor aggressiveness, or even, the late diagnosis (8). In fact, there is still a high proportion of patients (up to 70–90% in Western countries and in Asia, respectively) (9), where regrettably, HCC is diagnosed at an already advanced stage, and thus, patients are eligible only for palliative therapies. At this stage, only one approved systemic therapy is available, sorafenib, which was introduced in the treatment algorithm of HCC nearly ten years ago (10,11). Sorafenib is a multi-tyrosine kinase inhibitor acting on both tumor cell proliferation and tumoral neoangiogenesis. The pleiotropic anti-tumoral activities exerted by sorafenib, depend upon its ability to target the Raf kinases (B-Raf, the oncogenic B-Raf V600E, and C-Raf), a key regulatory point of the mitogen-activated protein kinases (MAPK/ERK) pathway, and to inhibit the activation of several receptors, including the vascular endothelial growth factor receptors-1, -2, and -3 (VEGFR1–3), the platelet-derived growth factor receptor- β (PDGFR β), and the stem cell growth factor receptor c-Kit (CD117) (12). Unfortunately, after an initial enthusiasm, sorafenib failed to live up the expectations as shown in several following studies. In fact, the SHARP study showed in treated patients, a slight increase in the overall survival (3 months) concomitant with a reduced radiologic progression compared with placebo (11). Moreover, the

STORM trial, a phase III study, aimed at evaluating the efficacy of sorafenib as adjuvant treatment following liver resection or local ablation, demonstrated that it did not ameliorate the overall survival and the disease free survival from the first therapeutic intervention (13). Although with limited favorable results, sorafenib represents indeed a breakthrough in the HCC treatment, because it paves the way for a novel molecular-based approach targeting fundamental mechanisms of cancer invasiveness. Starting from the sorafenib experience, several clinical trials were then designed to test drugs and small molecules capable to specifically inhibit the signaling pathways known to be deregulated in HCC. In particular, a number of studies were first performed to test compounds able to interfere with the signal pathways activated by VEGF and PDGF, pivotal players of the tumoral neoangiogenesis dictating the early metastatic spread of HCC. Based on data derived from other epithelial tumors, such as ovary and breast cancer (14,15), the last years witnessed a general burst of translational studies testing a variety of molecular targets. Among them, some tyrosine kinases, including the receptors for the epidermal growth factor (EGFR), the fibroblast growth factor (FGFR), or the hepatocyte growth factor (cMet), the TGF β signaling, or some common downstream effectors, such as MAPK/ERK, or the mammalian target of rapamycin (mTOR), have been investigated. Unfortunately, results from these studies have been largely disappointing, showing no improving effects with respect to sorafenib or even, to the other more conventional chemotherapeutic regimens. However, the failure of these strategies should reflect the huge genetic heterogeneity featuring HCC where multiple mutations in several key oncogenic drivers are variably involved depending on the etiology of the underlying cirrhosis, thus making ineffective an approach based on the use of a molecule targeting a single receptor or signal pathway, among many differently deregulated.

The seminal paper of Schulze and colleagues (16), represents a key step for a better understanding of the complex scenario of genetic alterations and related pathways of HCC specifically associated with the etiological background, and consequently, for the development of molecularly-targeted therapies in a tailored approach to patients with HCC. Taking advantage of next generation sequencing (NGS) techniques, by whole exome sequencing (WES), the authors analyzed the whole coding sequence of 243 tumor samples at different stages recapitulating the entire multistep process from dysplastic macronodule to advanced tumor, obtained from several European centers. Such highly innovative and sophisticated technological

endeavor led to the identification of (I) specific mutations linked to known risk factors of HCC, such as alcohol and tobacco consumption, and hepatitis B virus (HBV) infection; (II) 11 deregulated signal pathways driven by 161 putative genes; and (III) specific mutational patterns through the different stages of the disease.

The main mutational signatures discovered by the authors, clustered in three major groups, namely *CTNNB1*, *AXIN1*, and *TP53*, each associated with specific environmental risk factors, in particular alcohol toxicity and hepatitis B virus infection. *CTNNB1* mutations were strongly associated to alcohol intake. *CTNNB1* is the gene encoding for β -catenin, a major component of the WNT/ β -catenin signaling, known to be frequently deranged in a variety of chronic inflammatory conditions and in different epithelial cancers. Perturbations of this signaling, derived from both canonical (WNT-related) and non-canonical stimulations, result in the activation of downstream effectors regulating many pro-invasive functions, such as epithelial to mesenchymal transition, cell proliferation and cell reshaping, and enhanced secretion of pro-inflammatory mediators (cytokines and chemokines). Moreover, inappropriate activation of β -catenin leads in the liver, to an expansion of the hepatic progenitor cell compartment along with its phenotype stabilization (17). *AXIN1* is another component of the WNT/ β -catenin pathway. It binds the cytoplasmic phosphoprotein Dishevelled (downstream of the WNT receptor Frizzled), and, together with other partners (adenomatous polyposis coli, casein kinase 1, and glycogen synthase kinase 3 β), retains β -catenin in the cytoplasm, whereby it can be ubiquitinated, thus preventing its nuclear import and subsequent transcriptional activity (18). Unlike *CTNNB1*, *TP53* gene mutations were significantly linked to HBV infection. *TP53* is the gene encoding p53, the tumor suppressor protein most frequently mutated in HCC, which regulates cell cycle and DNA repair mechanisms (for instance, by binding the growth arrest and DNA damage GADD45). The inactivation of p53 protein results in an unbalance of the proliferation/apoptosis ratio, thus leaving unchecked the cell proliferative mechanisms.

Starting from a large amount of genetic mutations [161], the study identifies 11 core pathways consequently aberrantly activated in HCC (Table 1). This finding is of particular importance in an attempt to select patients to the best pharmacological treatment. Notably, the 28% of the genetic alterations detected in HCC patients were potentially suitable of treatment with compounds already approved by the US Food and Drug Administration (FDA), while the percentage increased up to 86% when

Table 1 Genetic alterations responsible for the key oncogenic pathways in HCC, and FDA-approved drugs able to interfere with each deregulated signaling

Altered pathways	% of mutation	FDA-approved drugs
TERT promoter mutations	60	–
WNT/ β -catenin	54	–
PI3K-AKT-mTOR	51	Afatinib, Axitinib, Cabozantinib, Cetuximab, Erlotinib, Everolimus, Gefitinib, Lapatinib, Panitumumab, Pazopanib, Pertuzumab, Ponatinib, Regorafenib, Sunitinib, Sorafenib, Temsirolimus, Trastuzumab
TP53/cell cycle	49	–
MAPK	43	Aflibercept, Axitinib, Bevacizumab, Bosutinib, Cabozantinib, Dabrafenib, Dasatinib, Imatinib, Nilotinib, Nintedanib, Pazopanib, Ponatinib, Ramucicromab, Regorafenib, Sorafenib, Trametinib, Vemurafenib
Hepatic differentiation	34	–
Epigenetic regulation	32	–
Chromatin remodeling	28	–
Oxidative stress	12	–
IL-6/JAK-STAT	9	Ruxolitinib, Tocilizumab, Tofacitinib
TGF β	5	–

HCC, hepatocellular carcinoma; FDA, Food and Drug Administration; PI3K, phosphoinositide 3-kinase; MAPK, mitogen-activated protein kinase; IL, interleukin; TGF, transforming growth factor.

considering drugs actually under investigation in clinical trials of phase I–III. As many of these mutational signatures can be detected since the early clinical stages, this approach might identify potential therapeutic targets not limited to the advanced HCC, as it is now the case with sorafenib. However, it must be underlined that advanced HCCs display an increased number of mutations amenable of molecular targeting, and thus, the NGS-WES approach might open new opportunities in a clinical stage, which for the time being, is only considered for palliation. In addition to genomic alterations directly responsible for tumor development ('driver genes'), the NGS-WES analysis pinpoints the presence of genes modulating other critical features of tumor invasiveness, involving the sensitivity to chemotherapeutic drugs, as in *NQO1* activation induced by mutations in *KEAP1* or *NFE2L2*, or bursting further signal pathways already deregulated in HCC (RAS/MAPK), as observed with *RPS6KA3* mutations. *NQO1* encodes a reductase member of the NAD(P)H dehydrogenase family which increases sensitivity to HSP90 inhibitors, a class of potent chemotherapeutic drugs, and thus its modulation may represent a strategy to circumvent drug resistance, a major issue of HCC (19).

Furthermore, the study unveils for the first time, gene mutations critically involved in the pathogenetic sequence leading from pre-neoplastic lesions (dysplastic macronodule) to advanced tumor (poor-prognosis HCC) and featuring distinct steps of this process (*Figure 1*). Whereas mutations of the telomerase reverse transcriptase (TERT) promoter were present in pre-tumoral lesions and in the early stages of HCC, other mutations increased through the progression of the disease (CTNNB1 and TP53); typically, focal mutations of FGF-CCND1 characterized instead the later stages of advanced HCC. Mutations in the TERT promoter represent in particular, a finding of great interest. In fact, TERT promoter mutations were significantly associated with both dysplastic macronodules and early HCC, thus suggesting a pivotal role in the initial phases of malignant transformation. TERT is the catalytic subunit of telomerase, whose function is to restore shortening telomeres, thus preventing replicative senescence, a tumor protective mechanism, and inducing instead a hectic proliferative behavior (20). Reactivation of TERT has been frequently found in various tumors, including HCC, where it was reported in more than 60% of cases (21,22). Interestingly, TERT reactivation has been described also

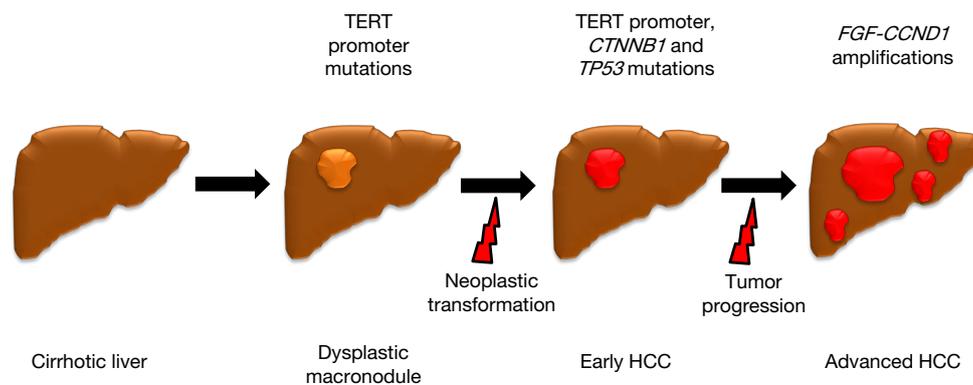


Figure 1 Molecular signatures underpinning the multistep process from dysplastic macronodule to advanced HCC. See text for further details. HCC, hepatocellular carcinoma.

in premalignant lesions of epithelial tumors, such as breast and colon cancer (23), but this is the first time that it is reported in dysplastic foci of liver cirrhosis. Since about 4% of cirrhotic patients will be developing HCC in a year, it is tempting to advance the possibility to identify the dysplastic nodules at major risk of progression to HCC by assessing the TERT promoter mutational signature. This might provide a crucial tool in the generation of new algorithms for the follow-up and treatment of cirrhotic patients. In fact, the finding of TERT promoter reactivation in cirrhotic nodules, especially in diseases related to HBV infection, could orient clinicians to adopt closer surveillance strategies or even lead to very early tailored therapies for these patients.

Altogether, the future perspectives of this work are many and of great translational relevance. Within the huge diversity of the HCC world, mutational signatures related to a specific liver background revealed by the NGS-WES analysis, could be used as ‘fingerprints’ to design more appropriately clinical trials, where patients can be better allocated, and to assemble more homogeneous sample cohorts, characterized by single mutational signatures. More in particular, this molecular-based stratification could be a valuable approach to accurately test the efficacy of one or more drugs able to target selectively the deregulated pathway, without confounding factors related to the presence of HCC of different etiologies or harboring different mutational signatures. Moreover, in clinical practice, it would also be possible for the clinician to screen the heterogeneous HCC patient population in distinct subsets to provide them with the most effective personalized treatment and hopefully, to limit the severe side effects caused by the conventional chemotherapy treatments.

This approach is predicted to have likely a strong impact on overall and disease free survival of HCC patients in the next years. Unfortunately, the clinical applicability of this approach is actually limited, at least in several European centers. Whilst the costs for the NGS-WES reagents and equipment are continuously decreasing, lowering to about 1,000€ for each sample analysis, there are some major issues that must be contemplated. They include the relative limited availability of instruments even in tertiary care hospitals, and the need of close interactions among different very specialized professional figures, including clinicians, biotechnologists and biostatisticians, which is still difficult to meet. In particular, there is a strong need of skilled biostatisticians, a professional figure actually hard to find, but definitely necessary to allow a fast and thorough analysis of data generated by NGS-WES. Building such a highly specialized team is mandatory for taking full advantage of the wealth of information that this cutting-edge technology may generate for patient care, pursuing the dream of the personalized cure.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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