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Molecular approaches to treatment of HCC

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1. Introduction

Hepatocellular carcinoma (HCC) represents a major health problem worldwide. In Europe and the United States the incidence has increased dramatically over the past decades mainly due to the rise in hepatitis C virus (HCV) infection [1]. HCC has the third highest mortality rate among all cancers and is reported to be the sixth most common cancer worldwide [2]. At the time of presentation less than 40% of patients in the western world fulfill criteria for curative treatment (resection, transplantation, local ablation) and only 20% are eligible for chemoembolization [3,4]. Alternative or palliative treatment options are very limited due to resistance to conventional chemotherapy and radiotherapy. The multikinase inhibitor sorafenib represents a breakthrough advance in the treatment of HCC and is now considered standard of care for advanced HCC. Prior to sorafenib no effective systemic therapy was available to successfully treat this devastating disease [5].

Although major etiological factors of HCC have been identified (hepatitis B [HBV] and C infection, alcohol, and aflatoxin) the molecular pathogenesis of this neoplasm is still not well understood [1]. An urgent need for diagnostic/prognostic markers and novel treatment strategies in HCC has driven research on genetic and epigenetic alterations as well as specific signaling pathways. This article reviews the current knowledge of the molecular pathogenesis of HCC and gives a brief overview of preclinically and clinically tested molecular targeted therapies in the management of HCC.

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2. Molecular pathogenesis

Complex genetic and epigenetic alterations, chromosomal aberrations, mutations, and altered molecular pathways lead to the development of HCC. The exact sequence of hepatocarcinogenesis though, including the development of preneoplastic lesions and its progression to HCC, is not well understood [6,7].

Cirrhosis is present in 80% of those diagnosed with HCC and is therefore considered as a main risk factor. Infection with HBV and HCV account for the majority of cases of HCC; however whereas HCC in those infected with HCV is usually associated with cirrhosis, those infected with HBV can develop HCC in the absence of cirrhosis. Hepatitis C virus can act as a Wnt ligand, inactivates p53 and transactivates Ras [8]. In contrast, integration of hepatitis B virus DNA into the genome induces genomic instability and genetic alterations like activation of oncogenes or silencing of tumor suppressor genes (TSG) [9]. In general, any condition leading to chronic inflammation in the liver, such as chronic intake of alcohol or autoimmune hepatitis, causes genomic and mitochondrial damage to cells facilitating the development of neoplasms. In certain areas of Africa and Asia, aflatoxin B represents the most important risk factor for HCC through its strong association with p53 point mutations [1]. In addition, HCC is being increasingly recognized in individuals with chronic liver diseases associated with the Metabolic Syndrome (MS).

These abovementioned risk factors for HCC can cause initial damage to the liver, but several additional external or internal influences are needed to finally initiate the multistep process leading to the development of HCC. These events can start at the level of mature hepatocytes or liver stem cells, but in any of the circumstances, selected cell populations develop a growth advantage and proliferate into dysplastic nodules (DN) [6]. Additional genomic hits (genetic, epigenetic alterations, mutations) are then necessary to induce a malignant phenotype in these cells. It has been proposed by Vogelstein et al. that disruption of at least three different molecular pathways is required for the development of a solid tumor [10]. Any hit can therefore be the responsible mechanism altering crucial key genes of cancer pathways, which as a whole then lead to the development of a solid malignancy.

In order to fully understand the molecular pathogenesis of HCC, the following cancer pathways as well as genetic and epigenetic alterations have been studied extensively with the consequent development of molecular targeted anti-cancer molecules.

3. Signaling pathways

Wnt- β -catenin, hedgehog, c-Met, IGF, Ras-MAPK, PI3/Akt/mTOR, and apoptosis are the most important intracellular molecular signaling pathways in HCC (Fig. 1).

3.1 Pathways related with cell survival

Wnt signaling is a frequently activated pathway observed in HCC as well as in other cancers [6]. Wnt ligands stimulate Frizzled receptors (FZD) with or without coreceptors, so that either the canonical β -catenin pathway or the noncanonical c-Jun N-terminal kinase (JNK) and protein kinase C (PKC) pathways are activated. In contrast, secreted Frizzled-related proteins can bind to Wnt ligands and block the activation of FZD. If the pathway is activated, β -catenin can uncouple from E-cadherin and translocates into the nucleus where it regulates oncogenes like c-myc, survivin and cyclin D. β -catenin gene (CTNNB1) mutations represent the second highest frequency of known mutations in HCC and define a recently described molecular subclass of HCC associated to activation of Wnt signaling [11]. General activation of the pathway has been described in one third of HCCs, but mutations occur in

only 17% of cases [12]. Interestingly the Wnt signaling cascade is mainly activated in HCV positive HCCs.

The hedgehog (Hh) signaling pathway is another crucial pathway described in HCC among other cancers. It is important for embryonic liver development and regeneration, stem cell biology and cell differentiation. Sonic Hh (SHH) and smoothened (SMO) are frequently overexpressed in HCC, activate the pathway and contribute to hepatocarcinogenesis. Its negative regulator Hh-interacting protein (HHIP) is downregulated in HCC due to promoter hypermethylation and/or loss of heterozygosity (LOH).

3.2 Pathways related to cell proliferation

Hepatocyte growth factor (HGF) and its tyrosine protein kinase receptor c-Met (mesenchymal-epithelial transition factor) pathway play an important role in cellular responses, such as invasive growth in development and cancer, tissue regeneration, angiogenesis, proliferation, and migration. HGF overexpression and aberrant c-Met activation has been reported in HCC due to c-Met amplification, mutation, and transcriptional upregulation of HGF dependent autocrine loops [6]. C-Met expression showed an inverse correlation to patient survival and c-Met silencing led to decreased cell migration and proliferation in an in vitro HCC model. HGF expression was higher in cirrhotic livers and high serum levels were associated with worse outcome after tumor resection.

Activation of the insulin-like growth factor (IGF) signaling pathway is another common event in HCC. Overexpression of IGF-1 and IGF-2 receptor (IGF-1R/2R), and silencing of the IGF binding proteins (IGFBP-1-5) contribute to proliferation, anti-apoptosis, and invasive behavior. The binding of the ligands IGF-1 and IGF-2 to IGF-1R results in the activation of the intrinsic tyrosine kinase domain, initiating various signaling pathways, including the PI3K/Akt pathway and Ras/MAPK pathway.

The PI3K/PTEN/Akt/mTOR pathway itself is involved in many cellular processes including proliferation, differentiation, apoptosis, cell cycle progression, cell motility, tumorigenesis, tumor growth, and angiogenesis. Akt activation can happen through the EGF or IGF signaling cascade, prior PI3K activation or PTEN silencing. Aberrant mTOR signaling, another important mediator of the PI3K/PTEN/Akt pathway was present in 50% of HCCs and is associated with insulin-like growth factor pathway activation, epidermal growth factor up-regulation, and PTEN dysregulation [13].

The tumor suppressor gene PTEN can mediate cell growth, migration and invasion in HCC. The pathway seems to also be involved in invasion and metastasis through overexpression of MMP-9 in HCC.

The EGFR-Ras-mitogen-activated protein kinase (MAPK) pathway mediates cellular responses to growth signals and is frequently dysregulated in cancer. It is a complex pathway and regulator of cell growth and division. EGF, HGF, PDGF and VEGF are examples of ligands activating the cascade in general, whereas in HCC activation possibly results from aberrant EGFR and IGF signaling or from hypermethylation and subsequent silencing of Ras-binding TSGs (RASSF1A, NORE1A) [14]. Mutations of key regulators of this pathway are rather rare in HCC and seem to play an inferior role as activators.

Most of the pathways described above are involved in either the regulation of cell proliferation or programmed cell death (apoptosis), a major phenomenon in cell biology. In cancer many apoptotic events are altered and uncontrolled cell proliferation leads to the formation of neoplasms [15]. Apoptosis can be activated either through an intrinsic (e.g.

DNA damage) or extrinsic pathway (binding of pro-apoptotic factors to cell surface) leading to the destruction of cells by proteins called caspases. In HCC some proapoptotic molecules are downregulated (Fas, p53, PTEN, Bax, Bid), but the imbalance between cell survival and death is mainly due to overexpression of antiapoptotic signals (Snail, β -catenin, NF- κ B, c-IAP1, Akt, Ras/ERKs, HGF, and EGFR ligands) [16].

4. Genetic alterations

Chromosomal amplifications and deletions, mutations and genomic instability are the most important structural changes in HCC (Table 1).

Mutations in p53 and β -catenin are probably best described and most prevalent in HCC. The tumor suppressor gene p53 is mutated in 27%, β -catenin in 0–44% of cases, leading to a disruption of cell cycle control, DNA repair, apoptosis and proliferation, and the activation of the Wnt pathway. Mutations in AXIN1, K-Ras, and PI3KCA have been described, but are less frequent [13]. Results of whole genome deep sequencing are expected to change the portrait of critical mutations in HCC.

Chromosomal amplifications (1q, 6p, 8q, 17q and 20q) and deletions (4q, 8p, 13q, 16q, and 17p) alter several candidate genes that have been identified to be crucial for hepatocarcinogenesis: c-myc, PTEN, E-cadherin, AXIN1, p53, Rb1, FGF3/4, Cyclin D1, and IGF-2R [17]. Among others the activation of telomerase in HCC is associated with increased genomic instability. Its activity is increased in more than 80% of HCCs resulting in telomere length alteration [18].

5. Epigenetic alterations

Epigenetic alterations are heritable changes in the pattern of gene expression mediated by mechanisms other than alterations in the DNA sequence. They consist of DNA methylation, histone modifications and nucleosome remodeling. Genome wide hypomethylation is a common event in cancer leading to genomic instability, which has also been described for HCC. Hypermethylation of CpG islands within the regulatory regions of genes usually leads to gene silencing. Hypermethylation and silencing of important tumor suppressor genes like p16, RASSF1, IGFBP3, E-cadherin and SOCS 1 have been reported among many others in HCC. Conversely, hypomethylation of promoter regions and reactivation of oncogenes (c-myc) was demonstrated in HCC [6]. Histone modifications are more complex alterations on the N-terminal tails (methylation, acetylation, ubiquitination, etc.) that can regulate gene expression either directly or through chromatin remodeling. Only very few genes regulated by histone modifications have been described in HCC (Table 2) [19,20]. In addition to this, HBx antigen has been shown to recruit the enzyme responsible for histone deacetylation (HDAC1) and to transcriptionally repress IGFBP-3. Table 2 summarizes all known epigenetic changes in HCC. In other malignancies whole genome changes in methylation have been correlated to clinical data and were used as prognostic factors, a phenomenon that still needs to be further explored for HCC.

6. Small RNAs

MicroRNAs (miRNAs) are small RNA molecules, approximately 22 nucleotides long, that negatively control their target gene expression posttranscriptionally. Their role in cancer is of increasing importance, since they can act as oncogenes or tumor suppressor genes. In HCC several miRNAs have been shown to be dysregulated. Some function as oncogenes and inhibit apoptosis (miR-221), promote cell invasion (miR-9) or silence c-Met and uPA-expression and by that inhibit migration and proliferation (miR23b). Others seem to have a tumor suppressor gene like function (miR-101, 195, 122, 338) and are silenced in HCC.

Downregulation of miR-122 was reported to correlate with suppression of the hepatic phenotype and gain of metastatic properties. Just recently, the upregulation of Lin28b in HCC and its consequent repression of miR-let7 has been described to be associated with advanced disease and poor clinical outcome [21].

7. Molecular classification

There is no widely accepted molecular classification of HCC comparable to clinical staging and classification systems like the Barcelona Clinic Liver Cancer (BCLC) system [22]. Genome wide gene expression arrays have been used by several groups to identify different molecular subclasses of human HCC tissue samples [23]. The Wnt-signaling and proliferation subclass have been repeatedly described by different authors in different cohorts, whereas Chiang et al. just recently found a new molecular subclass of HCC related to broad gains in chromosome 7 [11].

8. Molecular therapies

Anti-cancer drugs targeting cancer specific molecules or gene alterations are designated molecular targeted therapies. Herein the protein kinase inhibitors represent the biggest group in oncology, followed by monoclonal antibodies.

Many molecular alterations in HCC display potential targets for molecular targeted drug therapy in HCC. Effective compounds are urgently needed to stop the progress or relapse of this fatal disease, particularly in advanced and incurable tumors, but also in the adjuvant setting after resection of HCC.

Sorafenib was the first compound that significantly improved the survival of patients with advanced HCC [5]. This multikinase inhibitor is active against Raf-1, VEGFR2, B-Raf, PDGFR and c-Kit as well as other receptor tyrosine and serine threonine kinases. Preclinical models showed significant anti-tumoral activity of sorafenib by reducing cell viability and inducing apoptosis in vitro and in vivo. Later, a phase II trial was conducted that demonstrated clinical benefit in treated patients. This led to the design of a large phase III clinical trial (SHARP, n = 602) that was terminated after the first interim analysis showed significant survival benefit for patients treated with sorafenib compared to placebo (10.7 versus 7.9 months, respectively) [5]. The FDA (US Food and Drug Administration) and EMEA (European Medicine Agency) approval of sorafenib followed shortly after, and a consensus of several HCC expert panels recommended that sorafenib should be the standard of care for the treatment of advanced HCC, as well as the control-arm that novel compounds for the treatment of HCC need to be tested against in future clinical trials [24]. These results truly are a milestone in the treatment of HCC and stimulated the search for similar compounds targeting other molecular alterations, in particular because combination therapies are postulated to be a promising approach through synergistic anti-tumoral effects [24].

Many novel compounds have now been tested in preclinical and in clinical studies for HCC as summarized in Tables 3 and 4, respectively. Most agents can be classified according to their main targets: EGFR, mTOR, angiogenesis and others including IGF signaling (Fig. 1).

Erlotinib, Gefitinib, Laptinib and Vandetanib are tyrosine kinase inhibitors targeting EGFR that have all been tested in clinical trials for HCC [13]. Erlotinib showed anti-tumoral activity in experimental models and clinical studies of HCC. Also treatment in combination with the VEGF-Inhibitor Bevacizumab showed promising results and is currently under investigation in a phase II trial compared to sorafenib alone. Although Gefitinib showed activity against HCC in preclinical models, the published data of the clinical trial reported

no activity in patients [17]. Lapatinib and Vandetanib are two dual blocking molecules against Her2/neu and VEGFR, respectively. Vandetanib inhibited HCC cell proliferation, migration and invasion in vitro, and is currently under investigation in a phase II trial for inoperable HCC. Lapatinib has been tested in clinical trials but showed only minimal antitumoral activity, possibly because Her2/neu overexpression is rather uncommon in HCC. Cetuximab, a monoclonal antibody inhibitor of EGFR, inhibited cell proliferation and induced cell cycle arrest and apoptosis in an HCC cell line.

MTOR-inhibitors are of increasing interest for the treatment of HCC, particularly since activation of the PI3K/Akt/mTOR pathway was reported for as many as 50% of HCCs [25]. The same study successfully inhibited tumor growth and improved survival with the mTOR-inhibitor everolimus in vitro and in a xenograft model. Ongoing clinical trials are studying everolimus alone and in combination to sorafenib for HCC. The VEGF inhibitor bevacizumab is currently under investigation in a combination with the mTOR-inhibitor rapamycin (see <http://www.clinicaltrials.gov>), an analog that has activity against HCC in xenograft models [26]. Temsirolimus is a novel mTOR-inhibitor that was only tested in a preclinical model for HCC

Angiogenesis and the overexpression of angiogenic factors such as VEGF, PDGF, FGF-2, or angiopoietin are early and frequent events in hepatocarcinogenesis [27]. Therefore, blocking angiogenesis directly or indirectly by targeting microvascular endothelial cells or protein activators of angiogenesis, respectively, is an important strategy in preventing tumor progression. The humanized monoclonal antibody bevacizumab (anti-VEGF) is approved for the treatment of breast cancer and colorectal liver metastases, and showed modest anti-tumoral activity in a phase I/II trial of 33 selected patients and in a study that combined treatment with the chemotherapies gemcitabine and oxaliplatin [28]. Vatalanib, a small molecule inhibitor of VEGFR1 and -2 has only been tested in vitro up to now, where it inhibited cell proliferation and induced apoptosis. The multikinase inhibitor sunitinib targets the angiogenic factors VEGFR1, -2 and PDGFR and is approved for renal cell carcinoma and gastrointestinal stromal tumors. Some anti-tumoral activity against HCC has been reported in two phase II studies, but an important reported side-effect is gastrointestinal bleeding, and potentially life-threatening complications [29]. Brivanib is a small molecules inhibiting FGF and VEGFR that is currently under investigation in phase III trials after showing promising results as a second line agent after sorafenib failure. TSU-68 is a small molecule inhibitor of the angiogenic receptor tyrosine kinases Flk-1/KDR, PDGFR β , and FGFR1, currently in phase I/II clinical trials for HCC, although there has been no in vitro data published so far.

The IGF signaling axis is frequently activated in HCC and several IGF-1R tyrosine kinase inhibitors and molecular antibodies have been tested preclinically for HCC and other cancers (Table 3). BIIB022 and AVE 1642 are two novel drugs in phase I/II studies as monotherapies or in combination with sorafenib and erlotinib, respectively, whereas the monoclonal antibody IMC-A12 has been investigated only in combination with sorafenib in a phase II clinical trial.

The hedgehog and Wnt- β -catenin pathway are commonly deregulated in hepatocarcinogenesis, but effective drugs are still under development. The hedgehog antagonist cyclopamine effectively inhibited hepatoblastoma cell growth and induced apoptosis. RNA interference-mediated knockdown of CDH17 inactivated Wnt signaling by inhibiting proliferation of primary and highly metastatic HCC cell lines in vitro and in vivo. Both preclinical studies demonstrate very nicely how important it is to find clinically usable drugs for the inhibition of the Wnt-signaling and hedgehog pathway. So far there are no ongoing clinical trials with these compounds in HCC.

One further approach in the treatment of HCC is targeting the epigenetic changes that inactivate important tumor suppressor genes or pathway antagonists. These epigenetic drugs consist of two major groups: demethylating agents and histone deacetylase inhibitors (HDACi). Both are able to reexpress epigenetically silenced genes, either by demethylation of methylated promoter regions or by histone acetylation [30].

Several novel compounds (MS-275, SAHA, Tributyrin, Valproic Acid) have been tested preclinically in HCC and other cancers showing promising results mainly when used as combination therapy. The novel HDACi LBH589 is now tested in combination with sorafenib in a phase I trial for advanced HCC, although preclinical results have only been reported for other cancers (Table 4). Demethylating agents like Dacogen and Vidaza have first been FDA approved for the treatment of myelodysplastic syndrome and are currently under preclinical and clinical investigation for several other hematological and solid malignancies. Although SAHA and Dacogen have been tested successfully in a preclinical setting for HCC and other solid tumors, none of these compounds have moved to clinical trials yet.

The potential of antagomirs or reconstitutive miRNA compounds in the treatment of cancer is also a very appealing new approach, with promising preclinical results. Nevertheless, this area needs further exploration before it can be studied in clinical trials.

Conclusion

Molecular targeted therapies for the treatment of cancer including HCC are a very promising approach, clearly demonstrated by the breakthrough results of the multikinase inhibitor sorafenib [5]. The success of sorafenib is proof of the principle that molecular therapy plays an important role in the treatment of advanced HCC. Research focused on understanding the molecular pathogenesis and pathways in HCC over the past years has helped to identify many novel potential targets that need to be further evaluated in preclinical and clinical trials. Nonetheless, the ultimate biomarker to identify treatment responses has not been identified. Increasing evidence now justifies the testing of combination therapies targeting different molecules also in adjuvant settings. Even the combination of novel epigenetic drugs or anti-miRNA compounds with small molecules like sorafenib could be a promising new approach in treating this complex disease. Last but not least, the increasing knowledge of molecular subclasses of HCC will help to identify which patients will benefit best from certain compounds, an important step towards personalized medicine in the treatment for HCC. The identification of robust molecular subclasses and specific oncogenic loops is of special relevance in HCC since high molecular heterogeneity is observed. Certain molecular targeted therapies could be evaluated in patients selected according to their molecular profile in order to optimize clinical benefits for specific molecular subgroups.

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Figure 1. Relevant signaling pathways target of therapies, and molecular targeted therapies currently tested in preclinical and clinical trials in HCC. Only sorafenib has been approved by regulatory agencies.

Table 1Genetic Alterations in Hepatocellular Carcinoma (Modified from Villanueva et al⁶, and Llovet et al¹³)

	Gene	Gene Expression	Mutation/Copy Alteration	Frequency
Cell cycle / Apoptosis	p53	Decreased	Mutation / LOH	27% (0-67%)
	p16	Decreased	Mutation	13%
	p27kip	Decreased		
	Survivin	Increased		
Proliferation/differentiation	β-Catenin	Increased	Mutation	17% (0-44%)
	E-Cadherin	Decreased	LOH	
	c-myc	Increased	Amplification	
	APC	Decreased		
	SHH	Increased		60%
	Frizzled-7	Increased		90%
	AXIN 1	Decreased	Mutation / LOH	4-14%
Growth Factors / Receptors	IGF-2	Increased		12-44%
	IGF-1R	Increased		20-27%
	IGF-2R	Decreased	Mutation / LOH	25% / 60%
	EGF	Increased		
	EGFR	Increased		4-52%
	TGF-α	Increased		
	KRAS	Increased	Mutation	11% (3-42%)
	RASSF1	Decreased		
	PTEN	Decreased	Mutation / LOH	0-11%
	PIK3CA	Increased	Mutation	12% (0-35%)
Angiogenesis	HGF	Increased		
	VEGFR	Increased	Amplification	5%
	PDGF	Increased		
	Angiopoietin-2	Increased		11% (3-42%)
Metastasis	MMP-9	Increased		
	Osteopontin	Increased		
	MMP-14	Increased		
	Cyclin D1	Decreased	Amplification	7%
	Rb1	Increased	Mutation / LOH	15%
Telomerase reactivation	TERT/TERC	Increased		80%

Abbreviations: APC: adenomatous polyposis coli; AXIN: axis inhibition protein; EGF: epidermal growth factor; FGF: fibroblast growth factor; HGF: hepatocyte growth factor; LOH: loss of heterozygosity; IGF: insulin-like growth factor; KRAS: v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MMP: matrix metalloproteinase; PDGF: platelet-derived growth factor; PIK3CA: phosphoinositide-3-kinase, catalytic, alpha polypeptide; PTEN: phosphatase and tension homolog; -R: receptor; RASSF: Ras association domain family member; Rb: Retinoblastoma; SSH: sonic hedgehog homolog; TERT: telomerase reverse transcriptase; TERC: telomerase RNA component; TGF: transforming growth factor; VEGF: vascular endothelial growth factor receptor. Adapted with permission [13,17].

Table 2**Epigenetic Alterations in Hepatocellular Carcinoma**

	Gene	Gene Expression	Epigenetic Alteration	Frequency
Cell cycle / Apoptosis	p16	Decreased	Hypermethylation	56-83%
	p27	Decreased	Hypermethylation	48.3%
	p15	Decreased	Hypermethylation	42.5-49%
	p53	Decreased	Hypermethylation	14.2%
	p14	Decreased	Hypermethylation	8-20%, 25-30%
	CASP8	Decreased	Hypermethylation	72%, 43-50%
Proliferation/differentiation	E-Cadherin	Decreased	Hypermethylation	33-67%
	c-myc	Increased	Hypomethylation	30%
	APC	Decreased	Hypermethylation	45.2-77%, 78-91%
	T-Cadherin	Decreased	Hypermethylation / Histone Deacetylation	
	SFRP1	Decreased	Hypermethylation	52.6-75%
	SFRP2	Decreased	Hypermethylation	11.1-30%
Growth Factors / Receptors	SFRP5	Decreased	Hypermethylation	28.6%
	IGFBP-3	Decreased	Hypermethylation	70%
	RASSF1	Decreased	Hypermethylation	85%, 54-95%
	RASSF2	Decreased	Hypermethylation	5.6-48%
Other	SOCS1	Decreased	Hypermethylation	53-65%, 66-67%
	SOCS3	Decreased	Hypermethylation	33%
	CDH1	Decreased	Hypermethylation	39-46%
	PRDM2	Decreased	Hypermethylation	4.5-33%, 64.3%
	SPEN2	Decreased	Hypermethylation	0-39%
	SPEN1	Decreased	Hypermethylation	5.6-46%
	HIC-1	Decreased	Hypermethylation	78-86%
	DCC	Decreased	Hypermethylation	6-9%
	RPRM	Decreased	Hypermethylation	6-30%
	CACNA1G	Decreased	Hypermethylation	3-89%
	RUNX3	Decreased	Hypermethylation	41.1-47%, 39-82%
	PTGS2	Decreased	Hypermethylation	17-46%
	DLC1	Decreased	Hypermethylation	24%
	PGR	Decreased	H3-K27 methylation	
	ER α	Decreased	H3-K27 methylation	
DNA repair	PYCARD	Decreased	H3-K9 hypoacetylation and trimethylation	
	MGMT	Decreased	Hypermethylation	22-39%
	GSTP1	Decreased	Hypermethylation	41-85%

Abbreviations: APC: adenomatosis polyposis coli; CACNA: calcium channel, voltage-dependent, T type, alpha; CDH: cadherin; CASP: caspase; HIC: hypermethylated in cancer; DCC: deleted in colorectal carcinoma; DLC: deleted in liver cancer; ER: estrogen receptor; GSTP: glutathione S-transferase pi; IGFBP: insulin-like growth factor binding protein; MGMT: O-6-methylguanine-DNA methyltransferase; PRDM: PR domain containing; PGR: progesterone receptor; PTGS: prostaglandin-endoperoxide synthase 2; PYCARD: PYD and CARD domain containing; RASSF: Ras association domain family member; RPRM: reprimin, RUNX: runt-related transcription factor; TP53 dependent G2 arrest mediator candidate; SFRP: secreted frizzled-related protein 1; SOCS: suppressor of cytokine signaling; SPEN: spen homolog [17,20].

Table 3Molecular Therapies in preclinical studies for HCC (Modified from Villanueva et al⁶, and Llovet et al¹³)

Function	Target	Compound	Other targets
Growth factors	Her2/neu	Trastuzumab	
	EGFR	XL-647	Her2/neu, VEGFR
	IGF-IR	XL-228	Aurora A/B
		PPP	
		NVP-AEW541	
		AG1024	
	c-MET	SU5416	VEGFR, PDGFR, c-KIT
		SU11274	
	mTOR	Temsirolimus	
		XL-765	PI3KCA
Angiogenesis	β-Catenin	ICG-001	
	VEGFR	Vatalanib	Fit-1, PDGFR-β, Flt-4, c-KIT, c-fms
	PDGFR	Imatinib	
	STAT3	NSC 74859	
	TGFβ	LY2109761	
	Akt	OSU-A9	NF-kappaB
	Cdk	Xylocyline	
	FAK	PF-562,271	PTK2B
	JNK	SP600125	
Methylation	DNMT	Dacogen	
		Zebularine	
Histone Acetylation	HDAC	Tributylin	
		Valproic Acid	
		MS-275	
		Belinostat	
		SAHA	

Abbreviations::CDK: cyclin-dependent kinases; DNMT: DNA methyltransferase; EGF: epidermal growth factor; FAK: focal adhesion kinase; HDAC: histone deacetylase; HER: human epidermal growth factor receptor; IGF: insulin-like growth factor; JNK: c-Jun N-terminal kinase; mTOR: mammalian target of rapamycin; NF: nuclear factor; PDGF: platelet-derived growth factor; PIK3CA: phosphoinositide-3-kinase; PTK: protein tyrosine kinase; -R: receptor; STAT: Signal transducer and activator of transcription; TGF: transforming growth factor; VEGF: vascular endothelial growth factor receptor. Adapted with permission [14].

Table 4

Molecular Therapies in clinical trials for HCC (Modified from Llovet et al¹³)

Function	Target	Compound	Other targets	Class	Phase
Growth Factors	EGFR	Gefitinib		small molecule	II
		Erlotinib		small molecule	II
		Cetuximab		monoclonal AB	II
		Vandetanib	Her2/neu	small molecule	II
	RAF	Sorafenib	VEGFR, PDGFR, c-KIT	small molecule	FDA approved
	MAP3K1	AZD 6244		small molecule	II
	Proteosome	Bortezomib		small molecule	II
	Her2/neu	Lapatinib	EGFR	small molecule	II
	IGFR	IMC-A12		monoclonal AB	II
		BIIB022		monoclonal AB	I/II
Angiogenesis	mTOR	AVE 1642		monoclonal AB	I/II
		Everolimus		small molecule	I/II
		Sirolimus		small molecule	II/III
		Bevacizumab		monoclonal AB	III
	VEGFR	Sunitinib	PDGF, FLT3, c-KIT	small molecule	III
		Brivanib	FGFR	small molecule	III
		Cediranib	PDGFR, c-KIT, FLT	small molecule	II
		PI-88	FGF1/2	small molecule	II
		IMC 1121B		monoclonal AB	II
		ABT 869	PDGF, FLT3	small molecule	II
Apoptosis	PDGFR	TSU-68	Flk-1/KDR, FGFR1	small molecule	I/II
		Imatinib	c-KIT	small molecule	II
		Dasatinib	Src	small molecule	II
		Mapatumumab		monoclonal AB	I/II
	TRAIL-R1				
	BCL-2		Oblimersen	ASO	II
	CDK		Flavopiridol	small molecule	II
	HDAC		LBH 589	small molecule	I

Abbreviations: ASO: antisense oligodeoxynucleotide; AB: antibody, BCL: B-cell CLL/lymphoma; FDA: US Food and Drug Administration; CDK: cyclin dependent kinases; EGF: epidermal growth factor; FDA: US Food and Drug Administration; FGF: fibroblast growth factor; FKL: Protein-tyrosine kinase receptor Flk-; FLT: fms-related tyrosine kinase; HDAC: histone deacetylase; HER: human

epidermal growth factor receptor; IGF: insulin-like growth factor; MAP3K1: mitogen-activated protein kinase kinase kinase 1; mTOR: mammalian target of rapamycin; PDGF: platelet-derived growth factor; RAF: v-raf-1 murine leukemia viral oncogene homolog; src: sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog; TRAIL: TNF-related apoptosis-inducing ligand; VEGF: vascular endothelial growth factor receptor. [<http://clinicaltrials.gov>]. Adapted with permission [13,17]