Targeted Therapies for Hepatocellular Carcinoma

34

Susan Kralian and Brian I. Carr

Contents

S. Kralian
New York, NY, USA
e-mail: kralian@oncomedwriter.comkralsusa@yahoo.com

B.I. Carr
Izmir Biomedicine and Genome Center, Dokuz Eylül University, Izmir, Turkey

B.I. Carr (✉)
Lusaka Apex Medical University, Lusaka, Zambia
e-mail: brianicarr@hotmail.com

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Over the past 30 years, researchers have claimed victory in the war against cancer several times. Advances in molecular biology have led to an increased understanding of the discrete cellular pathways that promote or reduce cell division, cell survival, apoptosis, and angiogenesis. With the increased comprehension of the molecular etiology of cancer and these pathways, the era of rational therapy—the design of molecularly targeted agents that could modulate these cellular pathways (reactivate apoptosis and decrease cell growth, cell survival, and angiogenesis) to stabilize or halt the progress of cancer—began. Only in the past few years has this new knowledge and approach led to the production of pharmacologic agents that not only target a pathway but also produce clinical benefits.

Understanding molecular pathways can lead to the development of new drugs or improved drug regimens. Molecular pathways associated with hepatocarcinogenesis that modify apoptosis, cell division, cell survival, and angiogenesis include the rat sarcoma/rat sarcoma-activated factor/mitogenactivated protein kinase/extracellular regulated kinase (Ras/Raf/MAP/ERK) pathway, the phosphatidylinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway, Wnt/β-catenin, and the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway [1]. These pathways are the targets of rational drug design, with the objective of modulating them to prevent progression or worsening of hepatocellular carcinoma (HCC).

34.1 Molecular Pathways

34.1.1 Growth Factor Receptors

Growth factor receptors, such as epidermal growth factor receptor (EGFR), insulin-like growth factor receptor (IGFR), stem cell growth factor receptor (c-KIT), hepatocyte growth

factor and its respective receptor (HGF/c-MET), and the cytokine transforming growth factor-β (TGF-β1) receptor bind to their ligands and form receptor dimers. Dimerization initiates autophosphorylation of intracellular receptor domains, which then leads to the phosphorylation of intracellular second-messenger proteins [1, 2].

Mutations in growth factor receptor pathways have been found in tumors from patients with HCC. EGFR mRNA is upregulated in tissue samples from patients with HCC. Likewise, an increase in the amount of EGFR ligands that can activate these receptors, such as transforming growth factor alpha (TGF-α), has been found in HCC cell lines. Constitutively, activated growth factor receptors are another type of mutation associated with hepatocarcinogenesis; thus, even in the absence of ligand, the pathway can be activated [3].

34.1.2 Ras/Raf/MAP/ERK Pathway

When Ras, a GTPase, is covalently bound to a prenyl group, it is localized to and associates with the plasma membrane, where it couples with extracellular growth factor receptors [4, 5]. Binding of the extracellular receptor to the ligand induces receptor homodimerization or heterodimerization and autophosphorylation of intracellular receptor domains. Ras then undergoes a conformational change from an inactivated state, Ras- GDP, to an active state, Ras-GTP [4, 6]. The conformational change induces a series of intracellular phosphorylations: Ras phosphorylates Raf, which then phosphorylates MAP, and MAP phosphorylates numerous proteins, including ERK and several transcription factors, such as c-myc and c-jun [4, 6, 7]. Phosphorylated ERK translocates into the nucleus and activates several transcription factors [4, 7].

The Ras/Raf/MAP/ERK pathway has been implicated in numerous cancer types; 15–30 % of all cancers have Ras mutations [7–9]. Some cancer types, such as HCC, demonstrate an even greater vulnerability to mutations in this pathway. Tumor biopsies from patients with HCC were analyzed for *c-raf-1* gene and Raf-1 protein expression; the overexpression of the *c-raf-1* gene was observed in 50 % of samples and overactivity of Raf-1 was observed in 100 % of samples [10]. Furthermore, Raf mutations are frequently associated with hyperphosphorylated downstream effectors. Raf mutations associated with cancer were transfected into cell lines, and the majority of the various Raf mutations (82 %) had hyperphosphorylated ERK in the transfected cells [11].

The Ras pathway can also be controlled through inhibitors such as RASSF1A and NORE1A. The amount of these inhibitors is associated with the presence of HCC and disease status. RASSF1A was significantly decreased in the liver samples from patients with HCC (both good and poor prognosis) compared with liver samples from healthy patients. NORE1A, on the other hand, was decreased only in liver samples from patients with HCC and poor prognosis; there was no difference between the amount of NORE1A in the liver samples of healthy patients and patients with HCC and good prognosis, suggesting NORE1A may be a target to prevent worsening of HCC [12].

34.1.3 JAK/STAT Pathway

When growth factor receptors bind to their ligands, the receptors undergo dimerization and autophosphorylation of the intracellular cytoplasmic domains. JAK proteins are phosphorylated and JAK phosphorylates the cytoplasmic protein STAT. Phosphorylated STAT forms homodimers, and the STAT dimer translocates into the nucleus and acts as a transcription factor. STAT dimers are quickly inactivated by inhibitors of STAT, suppressors of cytokine signaling (SOCS) [13].

In tumors from patients with HCC, JAK and STAT were hyperphosphorylated; the phosphorylation levels of JAK1, JAK2, STAT3, and STAT5 were significantly higher in the liver samples from patients with HCC than in patients with normal livers. Mutations were found in many of the STAT inhibitors, such as SOCS1, SOCS2, and SOC3 [12].

34.1.4 The PI3K/Akt/mTOR Pathway

PI3K associates with the intracellular domain of many growth factor receptors. Upon binding of ligands to a growth factor receptor, the growth factor receptors form dimers, and intracellular domains of the growth factor receptors are phosphorylated. When the PI3K/Akt/mTOR pathway is activated, PI3K cleaves phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3) [6]. The accumulation of PIP3 induces a series of intracellular events, including the activation of Akt, and Akt in turn phosphorylates mTOR, a serine/threonine kinase [13–15]. Activated mTOR promotes the expression of *c-myc*, *cyclin D*, and other genes involved in cell proliferation and angiogenesis. Mutations that induce the constitutive activation of Akt, which then increase the activity of mTOR, have been found in several types of cancers [1]. Approximately half of the cases with HCC had overactivation of the PI3K/Akt/mTOR signaling pathway [16].

34.1.5 Wnt/β-Catenin

Wnts are secreted glycoproteins that bind to the extracellular receptors frizzled, LRP5, and LRP6. In the absence of the

ligand, some of the intracellular protein β-catenin forms a complex with E-cadherin, a complex responsible for cell–cell adhesion. β-Catenin also forms a complex with GSKβ, which is then degraded by a proteasome. Upon binding of Wnt to extracellular receptors, a downstream effector phosphorylates β-catenin. Phosphorylated β-catenin dissociates from many of the protein complexes, and this induces other cellular activities. When β-catenin dissociates from E-cadherin, cell motility is enhanced. When β-catenin is phosphorylated and free from the GSKβ complex, it translocates into the nucleus and acts as a coactivator to stimulate the transcription of genes, such as *c-myc*, *c-jun*, and *cyclin D2* [1, 3]. Approximately half of the cases with HCC had activation of the Wnt/β-catenin signaling pathway [17].

34.1.6 Transcription Factors

Transcription factors that induce the transcription of genes that promote cell division, cell survival, angiogenesis, or that inhibit apoptosis can lead to cancer. Nuclear factor-kappa B (NF-κB) is a transcription factor known to be associated with hepatocarcinogenesis that induces the transcription of anti-apoptotic genes [1].

In the inactive form, NF-κB remains in the cytoplasm and is bound to an inhibitory protein, inhibitory kappa B (IκB). There are several mechanisms that can remove IκB and, in turn, activate NF-κB. For example, inhibitor kappa kinase can phosphorylate IκB, and phosphorylated IκB dissociates from NF-κB. IκB can also be removed by a specialized proteasome degradation pathway. When no longer associated with IκB, NF-κB translocates into the nucleus and functions as a transcription factor [6, 18]. The PI3K/Akt pathway can also activate NF-κB; Akt phosphorylates numerous proteins and can also activate NF-κB [19]. Constitutively, active NF-κB has been found in some forms of cancer and has been associated with hepatocarcinogenesis [1, 20].

34.1.7 Proteasome

Cells remove intracellular proteins by a specialized proteasome degradation pathway. The protein to be degraded is covalently linked to ubiquitin molecules by ubiquitin ligases. The chain of ubiquitin molecules bound to the protein 'tags' the protein for a special degradation pathway, and the proteasome destroys the ubiquitinated protein. Proteasomes are essential for the regulation of cellular activities, such as cell division and gene expression. Cyclins, protein regulators of the cell cycle, are degraded at key steps by proteasomes; in this manner, the cell progresses to the next stage of the cell cycle. Gene expression is also controlled by proteasomes. For example, proteasomes degrade IκB, an inhibitor of NF-κB. In this manner, NF-κB is activated and can then function as a transcription factor [6, 21].

34.1.8 Angiogenic Targets: VEGFR, PDGFR, and FGFR

Activation of vascular endothelial growth factor receptors (VEGFRs), including VEGFR1 (FLT-1), VEGFR2 (FLK1-KDR), and VEGFR3 (FLT4), or platelet-derived growth factor receptors (PDGFR)-α or -β, promotes angiogenesis. Activation of VEGFR2 on endothelial cells in particular promotes a strong mitogenic, survival, and angiogenic signal. The intracellular molecular pathway is similar to that of growth factor receptors. Upon binding to the ligand, VEGFR forms dimers and activates the intracellular Ras/Raf/MAP/ERK and PI3/Akt/mTOR pathways (Fig. 34.1: Angiogenic Signaling Pathways) [3]. VEGF levels have been found to correlate with the amount of angiogenesis and poor prognosis. When tumor samples from patients with HCC were collected and analyzed, VEGF levels correlated with the amount of angiogenesis. Furthermore, higher preoperative VEGF serum levels correlated with shorter disease-free survival and overall survival [22].

Therapies that abrogate VEGFR signaling initially slow tumor growth and inhibit angiogenesis. Continuous treatment with anti-VEGFR agents, however, promotes the upregulation of activation of other proangiogenic signaling pathways, namely, PDGF/PDGFR and fibroblast growth factor ligands and receptors (FGF and FGFR) [23–26]. The FGF signaling pathway, which is comprised of 4 receptors (FGFR1-4) and over 20 ligands (FGF1-20), exerts activity via the intracellular Ras/Raf/MAP/ERK and PI3K/AKT/mTOR pathways. Dysregulation of the FGF/FGFR pathway has been implicated in promoting neoangiogenesis, therapy resistance, and disease recurrence [23–26].

34.1.9 Extracellular Matrix Changes

hanges in the extracellular matrix (ECM) can lead to tumor invasion, metastasis, and the worsening of HCC. HCC tissue has been found in association with overexpression of several types of matrix metalloproteinase (MMP) enzymes, such as MMP-2, MMP-7, and MMP-9, which digest ECM proteins.

In addition, changes in the expression of integrins, receptors that mediate cell–cell and cell–ECM adhesion, have been found in tissue from patients with many types of cancer, including HCC [1, 21, 27].

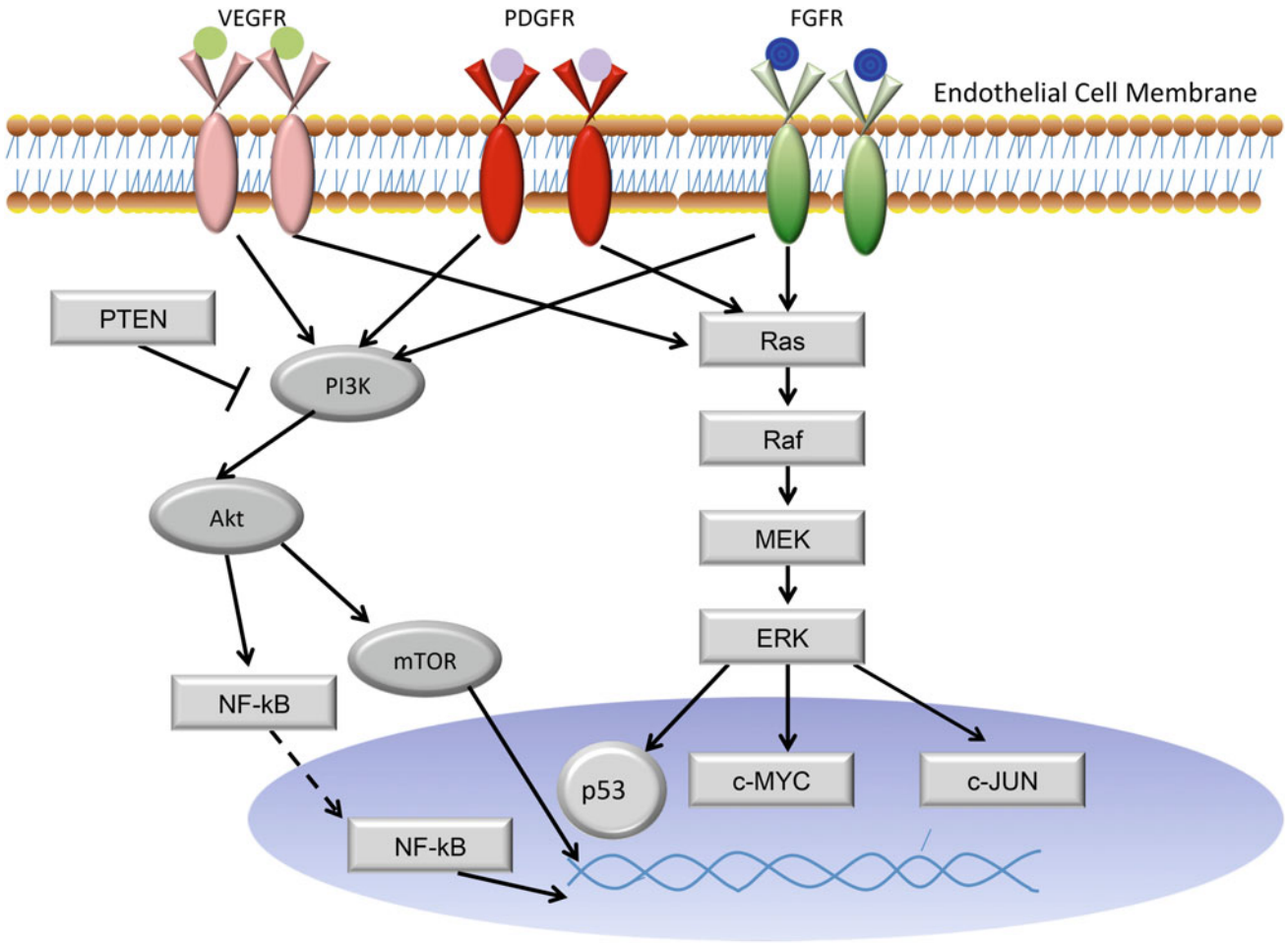

Fig. 34.1 Angiogenic signaling pathways for VEGFR, PDGFR, and FGFR

34.1.10 Apoptosis

Anti-apoptotic transcription factors activated by the second-messenger systems, such as the activation of growth factor receptors and the Ras/RAF/MEK/ERK pathway [13], can lead to inhibition of apoptosis.

Another protein that is essential to prevent cancer is the *p53* gene. This protein can induce apoptosis [22]. Similarly, *p53* plays an essential role in HCC; *p53* gene mutations are associated with 30–50 % of biopsies from patients with HCC. Furthermore, correlations between p53 mutations and shorter survival time have been observed [21, 28, 29].

34.1.11 Immune Checkpoints

An optimally functioning immune system maintains a balance between tolerating normal cells with self-antigens, and eliminating pathogens and damaged cells [30, 31]. Cancer tumors modulate the signaling cascades of helper T cells to evade detection by the immune system [30, 31]. Understanding the signaling cascade can provide potential targets to reactivate the immune system and eradicate tumors.

Immune checkpoints that inhibit the immune system upon activation and that have been identified as targets for HCC includes the T lymphocyte associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD1) receptor with its respective ligands, programmed cell death 1 ligand 1 (PD-L1) and PD-L2. CTLA-4 and PD1 are expressed on helper T cells, and tumor cells express PD-L1 and PD-L2 [31–33].

34.1.12 Inflammation

Epidemiological studies suggested that use of anti-inflammatory agents, such as aspirin, lowers the risk of developing HCC versus nonuse [34, 35]. Elucidating the inflammatory pathways might lead to the development of novel therapies. Cyclooxygenase (COX) enzymes have been implicated in inflammation and hepatocarcinogenesis; aspirin inhibits COX-1 and COX-2, COX-2 is expressed at

low levels in normal tissue, and COX-2 is upregulated and overexpressed in HCC [36].

34.1.13 Challenges to the Modification of These Pathways for the Treatment of HCC

Although researchers now understand many of these molecular pathways and have identified factors that could induce mutations that lead to intracellular changes, several challenges still exist. HCC is molecularly heterogeneous; in other words, the underlying pathology that leads to the development of HCC may be different from patient to patient, and a pharmacologic agent may only exhibit efficacy in a subgroup of patients. Another challenge is that some mutations with a constitutively active protein potentiate not one but several intracellular pathways. For example, dysregulation of the PI3K/Akt/mTOR signaling cascade correlated with overactivity of other signaling pathways, such as EGFR, in over half the HCC cases [16]. If a pharmacologic agent targets either the receptor or the point of signal transduction, then treatment necessitates a therapeutic agent that targets several pathways or the use of a combination of agents that target several pathways. Another challenge is that there is cross talk among many of these intracellular pathways. Therefore, successful modification of one pathway could lead to an increase or decrease in the activity of another pathway or even cause changes that lead to resistance of the pharmacologic agent [1]. For example, therapeutic inhibition of the VEGFR pathway in vitro leads to increased activation of the FGF/FGFR pathway, and ultimately, resistance to anti-VEGFR agents [23–26].

34.2 Rational Therapies

34.2.1 Targeting Growth Factor Receptors

Inhibiting or preventing the activation of growth factor receptors has been a strategy to prevent activation of intracellular molecular pathways, such as Ras/Raf/MEK/ERK and P13/Akt/mTOR.

34.2.1.1 Targeting EGFR

There are several pharmacologic agents in development that target one growth factor receptor in particular: EGFR. The two strategies that target the EGFR include antibodies that bind to an extracellular domain of the receptor and EGFR tyrosine kinase inhibitors.

Monoclonal anti-EGFR antibodies include cetuximab (Erbitux), a monoclonal IgG1 chimeric antibody, and panitumumab (Vectibix), a monoclonal IgG2 antibody. Both of these antibodies bind to a ligand-binding site on the extracellular domain of the EGFR and reduce activation of the EGFR [19, 37]. Although both cetuximab and panitumumab are antibodies, they have differing mechanisms of action. Cetuximab has been proposed to stimulate antibody-dependent cell-mediated cytotoxicity, whereas panitumumab is believed not to activate antibody-dependent cell-mediated cytotoxicity [19, 37, 38]. Another difference is the final destination of the receptors that bind to the antibodies. Cetuximab binds to receptors and stimulates endocytosis, but the antibodies are later returned to the cell surface, whereas receptors bound to panitumumab undergo endocytosis but are then degraded [19, 37]. Gefitinib (Iressa) and erlotinib (Tarceva) are EGFR tyrosine kinase inhibitors, which compete with the ATP intracellular domain of EGFR inhibitors and prevent activation of the intracellular cascade [37]. Other EGFR tyrosine kinase inhibitors in clinical development include lapatinib (Tykerb) and AC480.

Because some of the agents that target EGFR, such as gefitinib, erlotinib, and cetuximab, are approved for other cancer types, agents that similarly target EGFR are thought to have the potential to treat HCC. However, agents that target EGFR have mixed results in the treatment of other tumor types. Some patients do not respond to anti-EGFR therapy and other patients who initially respond develop resistance [39]. Thus, many current and recently completed clinical trials evaluate the efficacy and safety of anti-EGFR pharmacologic agents alone or in combination for patients with HCC [1]. Erlotinib was recently evaluated in a phase III study, and will be further discussed in the polypharmacy section of this chapter.

34.2.1.2 Targeting HGF/c-MET

Agents that target the c-MET signaling pathway are also in development. One of the more exciting potential therapies for HCC within the last few years is tivantinib, a c-MET tyrosine kinase inhibitor that abrogates downstream Ras/Raf/MEK/ERK and P13/Akt/mTOR signaling pathways [40, 41]. Preliminary findings from a phase II clinical study suggest that biomarkers can potentially identify patients who are most likely to be responsive to tivantinib [42–44]. Patients (n = 107) who had experienced disease progression and/or intolerance to sorafenib or sunitinib were randomized into a tivantinib (360 mg twice a day) or placebo arm at a 2:1 ratio [43]. Notably, patients with MET-high tumors exhibited improved median overall survival with tivantinib versus placebo (7.2 vs. 3.8 months, respectively; hazard ratio = 0.38; P = 0.01). There was no statistically significant difference in overall survival between the tivantinib and placebo arms for patients with MET-low tumors (5.0 vs. 9.0 months, respectively; hazard ratio = 1.33, P = 0.50) [42, 43]. Although these preliminary findings suggest the use of c-MET as a predictive marker of

responsiveness to tivantinib, patients with MET-positive tumors need to be prospectively enrolled in phase III studies. There are 2 phase III clinical studies that are recruiting patients with diagnostically c-MET-high tumors; these studies will evaluate the efficacy and safety of tivantinib in the second-line setting [45].

Other therapies that target the HGF/c-MET pathway are in development and being evaluated in clinical trials, such as emibetuzumab, a monoclonal anti-MET antibody that targets the extracellular receptor. [24, 45–47]. Unlike tivantinib, however, biomarkers are not being integrated into these studies [45]. Other agents that target c-MET in addition to other signaling pathways will be further discussed in the multitargeted kinase inhibitors section.

34.2.1.3 Targeting Other Growth Factors

Other agents in development target IGF-1R, such as anti-IGF-1R antibodies (i.e., cixutumumab, BIIB002); these agents are currently being evaluated in phase I studies in combination with other therapies [45]. A therapy that targets TGF-β1R (i.e., galunisertib) is also in development [48].

34.2.2 Targeting Ras/Raf/MAP/ERK

Numerous therapies that abrogate the intracellular Ras/Raf/MAP/ERK signaling cascade are in development. For example, donafenib, a ras inhibitor, is currently being evaluated in phase I/II studies [45]. The downstream MAP protein is an important target to evaluate. For example, even in the absence of a Ras or Raf mutation, constitutively activated MEK has been reported in HCC cases [47, 49]. MEK inhibitors in development include selumetinib (AZD6244), refametinib (BAY 86-9766), and trametinib, and are under evaluation in phase II clinical trials [28, 45, 50].

34.2.3 Targeting PI3K/Akt/mTOR

Several pharmacologic agents targeting the *PI3K/Akt/mTOR* pathway have been developed. Although some of the agents that inhibited the activity of PI3K (e.g., wortmannin and LY294002) were initially promising in tumor xenograft models, later studies demonstrated that they would not be appropriate as clinical agents because their pharmacokinetic properties were not favorable [51]. Other therapeutic agents in early clinical development, such as alkylphospholipid perifosine, target Akt [52].

There are many agents in development that block the downstream effector, mTOR. The mTOR inhibitors in development include everolimus, temsirolimus, and sirolimus [1, 19, 53]. Everolimus and temsirolimus are currently approved for other tumor types. There are several phase I/II trials evaluating temsirolimus, either administered alone or in combination with other therapies [45].

The mTOR inhibitor that has reached the most advanced stage of development is everolimus, which was recently evaluated in a phase III study in a second-line setting [54]. Although sorafenib has provided benefit by extending the median overall median survival of patients with advanced HCC by approximately 2–3 months, sorafenib has been unable to extend survival to 1 year [55, 56]. There is an unmet need for additional therapies for advanced HCC in the second-line setting; after patients experience disease progression with sorafenib, there are no currently approved targeted therapies to slow or halt disease progression. Moreover, approximately 30 % of patients discontinued therapy because of sorafenib-associated adverse events [57]. Therefore, safe and effective therapeutic options to be administered in the second-line setting are an unmet need in the management of advanced HCC. The efficacy and safety of everolimus was assessed in a phase III study (EVOLVE-1) (n = 546) [54]. After treatment failure with sorafenib, patients were randomized into an everolimus (everolimus at 7.5 mg/day plus best supportive care) or placebo (placebo plus best supportive care) arm at a 2:1 ratio. The primary end point, improved overall survival, was not achieved; there was no statistically significant difference in median survival between the everolimus and placebo arms (7.6 vs. 7.3 months, respectively, hazard ratio = 1.05; P = 0.68). The most common severe (grade 3) and life-threatening (grade 4) adverse events in the everolimus arm were anemia (7.8 %), asthenia (7.8 %), and decreased appetite (6.1 %) [54]. Everolimus is still being evaluated in a phase II clinical study, although it will be evaluated in combination with sorafenib [45].

34.2.4 Targeting Wnt/β-Catenin

Pharmacologic agents in development that target the Wnt/β-catenin signaling pathway are in preclinical development. These include anti-Wnt antibodies, which disrupt activity of the downstream Wnt effector, β-catenin, and promote apoptosis in cancer cell lines [1, 58–62]. Other therapies in development include ICG-001 and PMED-1; these agents disrupt the interaction between β-catenin and the transcription regulator CREB-binding protein, and ultimately inhibit downstream signaling [52, 59, 63].

34.2.5 Proteasome Inhibitors

In preclinical studies, proteasome inhibitors demonstrated efficacy when delivered with other agents; bortezomib was given as a pretreatment to cells followed by a tumor necrosis

factor-related apoptosis-inducing ligand (TRAIL) [64]. Apoptosis was induced only in HCC cells, whereas non-HCC hepatocytes did not exhibit apoptosis [64]. Proteasome inhibitors in development include bortezomib and oprozomib [65]. Proteasome inhibitors, in combination with other therapies, are under evaluation in phase II clinical studies [45].

34.2.6 Targeting Angiogenic Pathways: VEGFR, PDGFR, and FGFR

Because VEGFR and PDGFR stimulate proangiogenic pathways, pharmacologic agents that target these receptors can inhibit this process. A pharmaceutical agent in development is bevacizumab, an anti-VEGF antibody; by removing the VEGF ligand, the proangiogenic VEGFR signaling pathway should not be activated [21]. Although bevacizumab as a single agent exhibited activity in a phase II study evaluated patients with advanced HCC (i.e., a 13 % objective response rate was achieved), there are currently no plans for further development of bevacizumab as a single agent in phase III studies [47, 66]. The efficacy and safety of bevacizumab in combination with other therapies, however, is still under evaluation in ongoing phase II studies [45].

Ramucirumab (IMC-1121B), a fully human anti-VEGFR-2 monoclonal antibody, was recently evaluated in a phase III clinical study the second-line setting (REACH) [67, 68]. Patients (n = 565) were randomized at a 1:1 ratio into a ramucirumab (intravenous ramucirumab at 8 mg/kg plus best supportive care) or placebo (placebo plus best supportive care) arm in the second-line setting (i.e., experienced disease progression and/or intolerant to sorafenib) [68]. Patients in the ramucirumab arm failed to achieve the primary end point, improved overall survival; there was no statistically significant difference in survival between the ramucirumab and placebo arm (9.2 vs. 7.6 months, respectively; P = 0.14). The most common grade 3/4 adverse events in the ramucirumab arm included liver injury or failure (19 %), hypertension (12 %), and malignant neoplasm progression (6 %) [68]. Although ramucirumab alone failed to achieve improved survival as a single agent in a second-line setting, the efficacy and safety of ramucirumab in combination with other therapies is being investigated in ongoing clinical studies [45].

Other therapies that target proangiogenic signaling pathways include axitinib, a VEGFR-1,-2,-3 kinase inhibitor, and dovitinib, an FGFR3 kinase inhibitor [19, 45, 69, 70]. Therapies in development that target multiple proangiogenic signaling pathways will be discussed further in the multitargeted kinase inhibitors section.

Antiangiogenic therapies are shown in Fig. 34.2. Therapies that target other pathways are shown in Fig. 34.3.

34.2.7 Targeting Immune Checkpoints

Therapies that target the CTLA-4 and PD1/PD-L1 immune checkpoints are in development for HCC. Some of these agents have already exhibited efficacy against other malignancies; both ipilimumab (Yervoy), an anti-CTLA-4 antibody, and nivolumab (Opdivo), anti-PD-L1 antibody, have been approved by the FDA for melanoma [31, 71].

Antibodies that target CTLA-4 or PD1 abrogate activation of the inhibitory immune pathway. Anti-CTLA-4 antibodies (i.e., tremelimumab, ipilimumab) are currently under evaluation in clinical trials for HCC [31, 70]. The most advanced therapy for HCC that targets an immune checkpoint is nivolumab. In a recently reported phase I/II clinical study, nivolumab exhibited activity as assessed by reduction in tumor size in patients with HCC [72]. A phase III trial to evaluate the efficacy and safety of nivolumab in HCC has been registered [45].

34.2.8 Multitargeted Kinase Inhibitors

To date, the only multitargeted kinase agent to be FDA approved for the management of HCC is sorafenib. Over the last 5 years, other multitargeted kinase agents (i.e., sunitinib, linifanib, brivanib), have been evaluated; these agents failed to improve overall survival in phase III studies, and will be discussed in more detail below.

34.2.8.1 Sorafenib

Sorafenib (Nexavar) inhibits the Ras/Raf/MAP/ERK pathway, VEGFR-2 and -3, PDGFR-β, KIT, RET, and Flt-3 receptor tyrosine kinases [73–75]. In addition to blocking multiple pathways, sorafenib is the first systemic agent that has provided clinical benefit to patients with HCC. In a phase III trial (SHARP trial), 602 patients predominantly from Europe, Australia, and the United States and diagnosed with advanced HCC were randomized to receive either placebo or sorafenib at 400 mg twice a day. Patients in the placebo arm had an overall survival of 7.9 months, whereas patients in the sorafenib arm had an overall survival of 10.7 months (hazard ratio = 0.69; P < 0.001) [56]. Sorafenib was generally well tolerated. The most common (any grade) drug-related adverse events reported in 10 % or more of the sorafenib arm included diarrhea (39 %), fatigue (22 %), hand–foot skin reaction (21 %), rash/desquamation (16 %), alopecia (14 %), anorexia (14 %), and nausea (11 %) [56]. The most common grade 3/4 adverse events were hand–foot skin reaction (8 %) diarrhea (8 %), fatigue (3 %), hypertension (2 %), weight loss (2 %), and abdominal pain (2 %) [56]. Based on the improvements in health outcomes, such as overall survival, demonstrated in patients administered sorafenib in this phase III trial, sorafenib was granted FDA approval. Sorafenib is the first molecularly

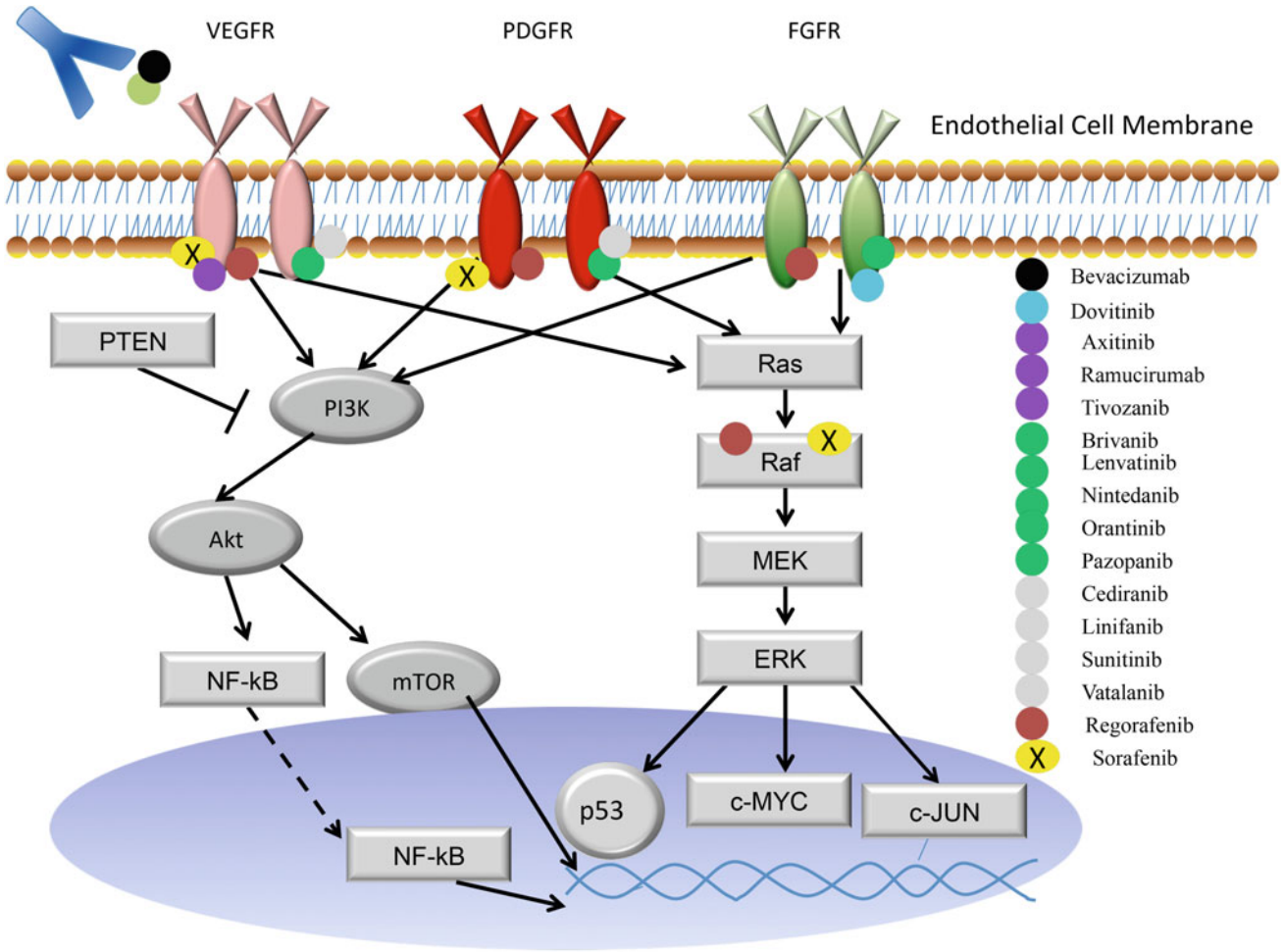

Fig. 34.2 Antiangiogenic therapies

targeted agent to reach the clinic for the treatment of HCC. Sorafenib has been integrated into the National Comprehensive Cancer Network (NCCN) guidelines; for patients who are not candidates for resection or a liver transplant, sorafenib is a treatment option [76]. Sorafenib is the only approved therapy for patients with metastatic HCC [76]. Moreover, within this treatment algorithm, sorafenib has a category 1 recommendation (i.e., high-level evidence and consensus among the NCCN panel members) for patients with Child-Pugh Class A [76].

Although this trial demonstrated that sorafenib significantly improved overall survival, it should be noted that 96 % of the patients in this trial were Child–Pugh class A. Thus, more studies are needed to evaluate the efficacy and safety of sorafenib in patients with Child–Pugh classes B and C [52]. Consensus guidelines by the NCCN reflect the need for future studies to assess the safety of sorafenib in patients with Child–Pugh class B and C status. The guidelines suggest that patients with inoperable HCC and either Child–Pugh class A or B status receive sorafenib, with the caveat that patients with Child–Pugh class B status be administered the drug with caution, because there are only limited safety data available with Child–Pugh class B status [76]. To further explore the role of sorafenib for patients with Child–Pugh class B, approximately 320 patients with Child–Pugh class B are being recruited to participate in a phase III study to evaluate the efficacy and safety of sorafenib [45].

The benefit of sorafenib has also been validated in another large (n = 226) randomized, placebo-controlled, phase III trial [55]. This trial was conducted in the Asia-Pacific region and many patients (73.0 %) had hepatitis B virus. Patients were randomized into a sorafenib or placebo arm at a 2:1 ratio [55]. Overall survival significantly improved among patients receiving sorafenib (P = 0.014); patients in the placebo arm had a median overall survival of 4.2 months, whereas patients in the sorafenib arm had a median overall survival of 6.5 months. Drug-related adverse events (any grade) reported by 10 % or more of the sorafenib arm included hand–foot skin reaction (45 %), diarrhea (26 %), alopecia (25 %), fatigue (20 %), rash (20 %),

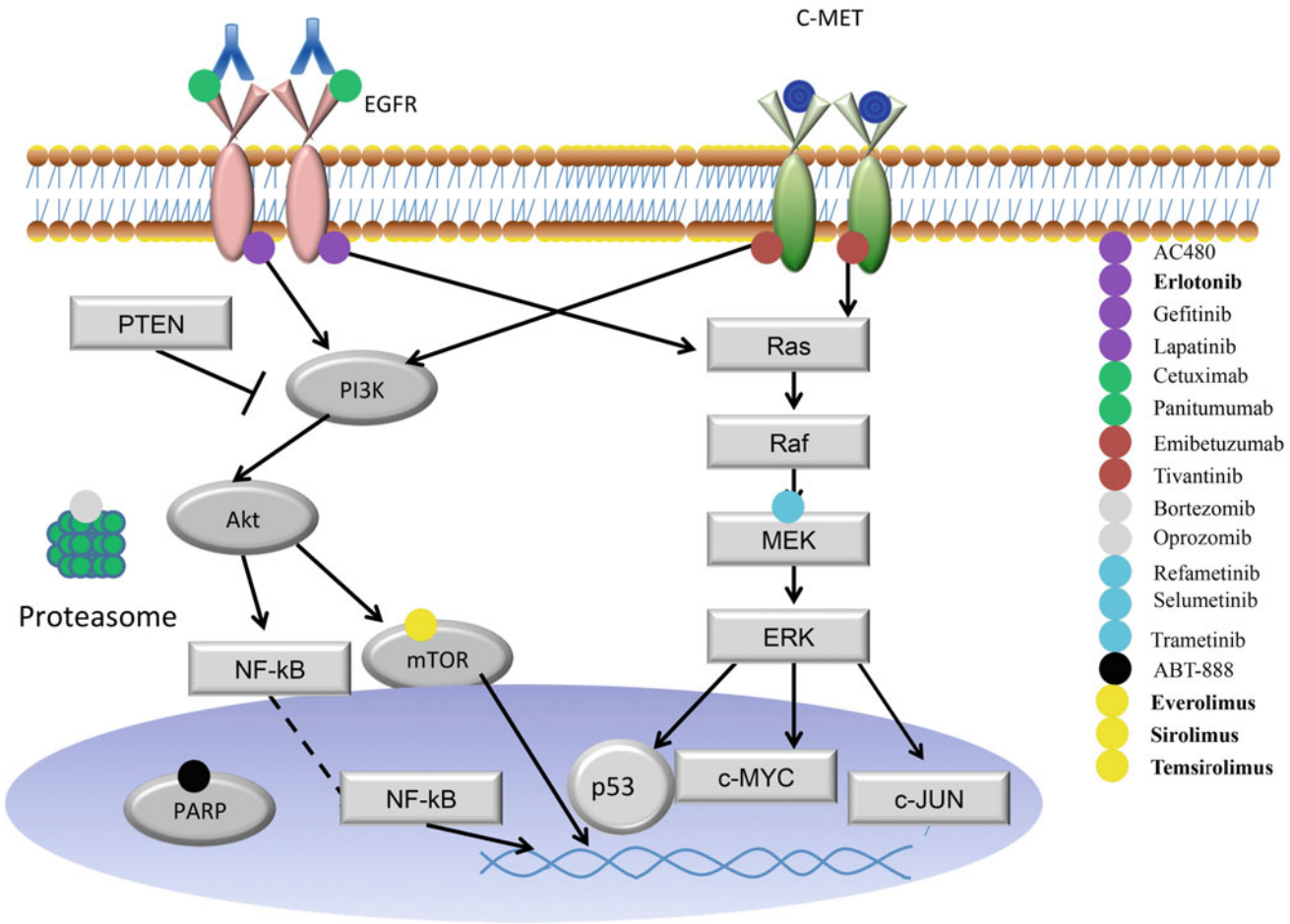

Fig. 34.3 Therapies with other molecular targets

hypertension (19 %), anorexia (13 %), and nausea (11 %) [55]. The most common grade 3/4 adverse events in the sorafenib arm included hand–foot skin reaction (10.7 %), diarrhea (6 %), fatigue (3.4 %), and hypertension (2 %) [55].

Managing sorafenib-associated adverse events remains challenging, and minimizing the toxicity of sorafenib might further improve the therapeutic index. Hypertension (any grade) was reported at an incidence of 19 % of the sorafenib arm in the pivotal phase III study [55], and grade 3/4 hypertension was reported at an incidence of 2 % of the sorafenib arms of both phase III pivotal sorafenib studies [55, 56]. Within the first few weeks of treatment with sorafenib, sorafenib-associated hypertension can occur [77]. Patients receiving sorafenib should be monitored weekly for hypertension [78]. Moreover, patients who develop hypertension should be managed with typical antihypertensive agents and if hypertension persists, sorafenib should be discontinued, either temporarily or permanently [78]. Hand–foot skin reaction is one of the most common (any grade) adverse events associated with sorafenib and is a dose-limiting toxicity [55, 56, 78, 79]. Hand–foot skin reaction typically occurs within the first few weeks of sorafenib therapy [78, 80]. Although suggestions have been made to reduce the likelihood of developing hand–foot skin reaction by minimizing exposure of a patient's hands and feet to hot water or excessive friction, and using topical agents if hand–foot skin reaction develops, there are no consensus guidelines or clinical trials to evaluate the management of hand–foot skin reaction [81, 82]. Severe hand–foot skin reaction may necessitate dose modification and/or discontinuation of therapy [78]. Approximately 30 % of patients have needed to discontinue therapy due to sorafenib-associated adverse events [57].

34.2.8.2 Sunitinib

Sunitinib (Sutent) inhibits VEGFR-1 and -2, PDGFR-α and -β, stem cell factor receptor c-KIT, and the FLT3 and RET kinases [2]. The efficacy and safety of sunitinib versus sorafenib was evaluated in an open-label phase III trial (n = 1074); overall survival was the primary end point [83]. Patients were randomized at a 1:1 ratio to receive sunitinib at

37.5 mg once a day or sorafenib at 400 mg twice a day. Patients in the sorafenib arm achieved superior overall survival; median overall survival in the sunitinib arm was 7.9 months overall, whereas the median overall survival for patients in the sorafenib arm was 10.2 months (hazard ratio = 1.30; one-sided $P = 0.9990$, two-sided $P = 0.0014$) [83]. The majority of adverse events reported in both study arms were mild (grade 1) to moderate (grade 2) in severity. A higher proportion of patients in the sunitinib arm (82.1 %) versus the sorafenib arm (74.2 %) had grade 3/ 4 adverse events. The most common grade 3/4 adverse events in the sunitinib arm included thrombocytopenia (29.7 %), neutropenia (25.7 %), and hand-food syndrome (13.3 %), whereas in the sorafenib arm this included hand-food syndrome (21.3 %) [83]. Due to lack of efficacy and safety concerns, the study was terminated early [83].

34.2.8.3 Linifanib

Another multitargeted kinase inhibitor in development is linifanib (ABT-869), a VEGFR and PDGFR tyrosine kinase inhibitor [13, 47].

In an open-label phase III study (LIGHT) ($n = 1035$), at a 1:1 ratio, patients were administered linifanib at 17.5 mg per day or sorafenib at 400 mg twice a day [84]. Linifanib failed to achieve the primary end point, overall survival; patients in the linifanib had a median overall survival of 9.1 months and patients in the sorafenib arm had a median overall survival of 9.8 months (hazard ratio = 1.046) [84]. Patients in the linifanib versus sorafenib arm had a higher frequency of grade ≥ 3 adverse events (85.3 % vs. 75.0 %, respectively; $P < 0.001$) and adverse events leading to drug discontinuation (36.3 % vs. 25.4 %, respectively; $P < 0.001$). The most common grade 3/4 adverse events experienced by patients in the linifanib arm included hypertension (20.8 %), palmar-plantar erythrodysesthesia syndrome (13.7 %), AST increased (12.2 %), and diarrhea (12.0 %), whereas the most common grade 3/4 adverse events experienced by patients in the sorafenib arm included palmar-plantar erythrodysesthesia syndrome (14.8 %), AST increased (12.5 %), and hypertension (10.6 %) [84].

34.2.8.4 Brivanib

Another multitargeted kinase inhibitor in development for HCC includes brivanib (AEE788)—an inhibitor of the FGFR-1, PDGFRβ, and VEGFR-2 pathways [21, 85, 86].

In a phase III noninferiority study (BRISK-FL study), at a 1:1 ratio, brivanib versus sorafenib was evaluated in the first-line setting [87]. Patients ($n = 1150$) were administered brivanib at 800 mg once a day or sorafenib at 400 mg twice a day [87]. Brivanib failed to achieve the primary endpoint, noninferior overall survival; the median overall survival of patients in the brivanib arm was 9.5 months versus 9.9 months in the sorafenib arm (hazard ratio = 1.06, with the prespecified margin upper limit for HR $\leq$ 1.08) [87]. The most common grade 3/4 adverse events experienced by patients in the brivanib arm included hyponatremia (23 %), AST increased (15 %), fatigue (14.5 %), hypertension (13.3 %), and hyperbilirubinemia (12 %), whereas the most common grade 3/4 adverse events in the sorafenib arm included AST increased (17 %) and hand–foot skin reaction (15 %) [87].

In a phase III study (BRISK-PS trial), the efficacy and safety of brivanib in a second-line setting was evaluated [88]. Patients who experienced disease progression with sorafenib or were intolerant to sorafenib ($n = 395$) were enrolled and randomized at a 2:1 ratio to a brivanib (brivanib at 800 mg per day plus best supportive care) or a placebo (placebo plus best supportive care) arm [88]. Patients receiving brivanib failed to achieve the primary end point, improved median overall survival; the median overall survival for patients in the brivanib arm was 9.4 and 8.2 months in the placebo arm (hazard ratio, 0.89; $P = 0.3307$) [88]. The most common grade 3/4 adverse events experienced by patients in the brivanib arm included hypertension (17 %), fatigue (13 %), and hyponatremia (11 %) [88].

34.2.8.5 Other Multitargeted Kinase Inhibitors

Other multitargeted kinase inhibitors in earlier stages of clinical development for HCC include the following: vatalanib, a VEGFR, PDGFR, and c-KIT tyrosine kinase inhibitor; cediranib, a VEGFR-1,-2, and -3 and PDGFR-α and -β kinase inhibitor; pazopanib, a VEGFR-1,-2,-3, PDGFR-α,-β, FGFR-1,-3, and c-kit inhibitor; and orantinib, a PDGFR, FGFR, and VEGFR inhibitor [13, 45, 47, 69, 89–91].

Other multitargeted kinase inhibitors, which are currently being evaluated in phase III studies, include the following: Regorafenib, a VEGFR-1, -2,-3, FGFR-1,-2, PDGFR, RET, kit, RAF-1, BRAF, and BRAFv600 inhibitor; cabozantinib, a VEGR2 and c-MET inhibitor; and lenvatinib, a VEGFR-1,-2,-3, FGFR-1,-2,-3,-4, PDGFR, RET, and kit inhibitor [45, 47, 92].

The mechanisms of action of the various molecularly targeted agents in development are summarized in Table 34.1.

34.2.9 Polypharmacy

Another strategy under evaluation to improve survival in advanced HCC is polypharmacy. Even if a molecular signaling pathway is successfully abrogated, because of cross-talk, other signaling pathways can be dysregulated; for example, inhibiting the VEGF/VEGFR signaling pathway activates the PDGF/PDGFR and FGF/FGFR proangiogenic pathways [23–26]. Therefore, to improve outcomes in HCC, combination therapies that can abrogate more than one

Table 34.1 Overview of mechanisms of action of pharmacologic agents

Agent	Mechanism of action									
	VEGF	VEGFR	PDGFR	FGFR	EGFR	mTOR	MEK	c-MET	Ras	c-kit
AC480 (BMS-599626)					•					
Axitinib[a,s] (AG-013736, Inlyta)		•								
Bevacizumab[b] (Avastin)	•									
Brivanib (BMS-582664)[s]		•	•	•						
Cabozantinib[c] (XL184, Cometriq)		•						•		
Cediranib (Recentin)[s]		•	•							
Cetuximab[d] (Erbitux)					•					
Donafenib									•	
Dovitinib				•						
Emibetuzumab (LY2875358)								•		
Erlotinib[e] (Tarceva)					•					
Everolimus[f] (Certican, Zortress, Afinitor, RAD001)						•				
Gefitinib[g] (Iressa)					•					
Lapatinib[h] (Tykerb)					•					
Lenvatinib[i] (E7080, Lenvima)[s]		•	•	•						•
Linifanib (ABT-869)[s]		•	•							
Nintedanib (BIBF 1120, OFEV)		•	•	•						
Orantanib (TSU-68)[s]		•	•	•						
Panitumumab[j] (Vectibex)					•					
Pazopanib[k] (Votrient)[s]		•	•	•						•
Ramucirumab[l] (IMC-112B, Cyramza)		•								
Refametinib (BAY 869766, BAY86-9766)							•			
Regorafenib[m] (Stivarga)[s]		•	•	•						•
Selumetinib (AZD6244)							•			
Sirolimus (Rapamune)						•				
Sorafenib[s,n](Nexavar)		•	•						•	•
Sunitinib[s,o] (Sutent)		•	•							
Temsirolimus[p] (Torisel)						•				
Tivantinib (ARQ197)								•		
Tivozanib		•								
Trametinib[q] (Mekinist)							•			
Vatalanib (PTK787)		•	•							•
Vandetanib[r,s] (Zactima, Caprelsa)		•			•					

[a]Approved for advanced renal cell carcinoma after failure of one prior systemic therapy
[b]Approved for the following: metastatic colorectal cancer; non-squamous non-small cell lung cancer; metastatic renal cell carcinoma; glioblastoma; and persistant, recurrent, or metastatic carcinoma of the cervix; platinum-resistant recurrent epithelial ovarian, fallopian, or primary peritoneal cancer
[c]Approved for progressive, metastatic medullary thyroid cancer
[d]Approved for squamous cell carcinoma of the head and neck and EGFR-expressing, K-Ras mutation-negative metastatic colorectal carcinoma
[e]Approved for non-small cell lung cancer and pancreatic cancer
[f]Approved for the following: advanced hormone receptor-positive, HER2-negative breast cancer; neuroendocrine tumors of pancreatic origin (PNET); advanced renal cell carcinoma after failure of treatment with sunitinib or sorafenib; renal angiomyolipoma and tuberous sclerosis complex
[g]Approved for first-line treatment of patients with metastatic non-small cell lung cancer whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test
[h]Approved for HER2-positive breast cancer

[i]Approved for locally recurrent or metastatic radioactive iodine-refractory differentiated thyroid cancer
[j]Approved for EGFR-expressing metastatic colorectal carcinoma
[k]Approved for renal cell carcinoma and soft tissue sarcoma
[l]Approved for advanced gastric or gastro-esophageal junction adenocarcinoma, metastatic non-small cell lung cancer, and metastatic colorectal cancer
[m]Approved for metastatic colorectal cancer and gastrointestinal stromal tumor
[n]Approved for HCC and renal cell carcinoma
[o]Approved for renal cell carcinoma, pancreatic neuroendocrine tumors, and gastrointestinal stromal tumor after disease progression on or intolerance to imatinib
[p]Approved for advanced renal cell carcinoma
[q]Approved for melanoma with BRAF V600E or V600K mutations as detected by an FDA-approved test
[r]Approved for medullary thyroid cancer
[s]Multitargeted tyrosine kinase inhibitor

Table 34.2 Ongoing phase II and III clinical evaluating single therapies

Pharmacologic agent	Mechanism of action	Phase	*N*	End points
Axitinib	VEGFR-1,-2,-3 inhibitor	II	29	OS, PFS, QoL, safety, response
Brivanib	Multitargeted TKI; VEGFR-2; FGFR-1; PDGFRβ	III	414	OS, TTP, response, DCR, DOR, DCR, safety
Cabozantinib[a]	VEGFR-2, c-MET inhibitors	III	760	OS, PFS, response
Donafenib	Ras inhibitor	I/II	106	TTP, safety
Gefitinib	TKI; EGFR inhibitor	II	40	Recurrence-free survival, biomarkers, safety
Lenvatinib	Multitargeted TKI: VEGFR-1,-2,-3; FGFR-1,-2,-3,-4; PDGFR; RET, kit	III	954	OS, PFS, TTP, response, QoL
Nintedanib	FGFR, VEGFR, and PDGFR inhibitor	I/II	134	TTP, MTD, OS, PFS, response, safety
Nintedanib	FGFR, VEGFR, and PDGFR inhibitor	II	124	TTP, MTD, OS, PFS, response, safety
Nivolumab	Anti-PD-L1 antibody	III	726	TTP, OS, PFS, response
Regorafenib[a]	VEGFR-1,-2,-3; FGFR-1,-2; PDGFR, RET, kit, RAF	III	560	OS, TTP, PFS, DCR, response
Tivantinib	c-MET inhibitor	III	160	PFS, OS
Tivantinib	c-MET inhibitor	III	346	PFS, OS, safety
Temsirolimus	mTOR inhibitor	I/II	50	MTD, PFS, response, safety, DOR
Temsirolimus	mTOR inhibitor	II	25	Safety, response, circulating tumor cells
Tivozanib	VEGFR inhibitor	I/II	49	PFS, OS, response, safety

DCR disease control rate; *DOR* duration of response; *OS* overall survival; *MTD* maximum tolerated dose; *PFS* progression-free survival; *QoL* quality of life; *TTP* time to progression
[a]Agents will be evaluated in the second-line setting

signaling pathway is being explored in clinical studies. Another rational for combination therapy is that together, two or more therapies might work synergistically to modulate signaling pathways [83, 84]. For example, interferon-α (IFN-α) activates the JAK1/STAT1 pathway and induces apoptosis in HCC models. But when IFN-α is used in combination with aspirin, significantly more STAT1 is activated and more apoptosis is induced [93].

Because sorafenib is the first agent to reach the clinic and improve overall survival in patients with HCC, clinical trials are currently in progress to evaluate whether the benefits of sorafenib can be improved. Post-transarterial chemoembolization (TACE) has been associated with an activation of proangiogenic signaling pathways, such as an upregulation and increase in VEGF and FGF levels [94, 95]. Strategies under evaluation to improve outcomes include administering sorafenib after TACE. In a phase III study, patients were randomized into a sorafenib or placebo arm at a 1:1 ratio after TACE. Sorafenib failed to improve survival after TACE; the investigators attributed this failure to an inadequate dose of sorafenib and/or a delay in the initiation of sorafenib therapy [96]. A high proportion of patients in the

sorafenib arm (73 %) required sorafenib dose reduction. Moreover, the median daily dosage of sorafenib in the TACE study was lower than the median dosage of sorafenib in the pivotal SHARP and the Asia-Pacific studies [55, 56, 96]. Patients in the TACE study received a median daily dosage of 386 mg sorafenib, whereas patients in the sorafenib arms of SHARP and the Asia-Pacific study received a median daily dosage of 797 and 795 mg sorafenib, respectively [55, 56, 96]. Further confounding the findings of this study, approximately 60 % of patients in the sorafenib arm of the TACE study did not initiate sorafenib until 9 weeks or more post TACE [96]. Administering sorafenib with TACE will continue to be evaluated, although different scheduling strategies will be used. For example, patients are being recruited for a phase III to evaluate the use of sorafenib after TACE, and sorafenib will be administered within 72 h of randomization. In another ongoing phase III study, TACE will be initiated within 2 weeks of receiving a stable dose of sorafenib [45].

Another strategy to improve outcomes was the administration of sorafenib post resection or ablation in patients with an intermediate-to-high recurrence risk, which was evaluated in a phase III study (STORM) [82]. Patients (n = 1114) were randomized into a sorafenib (400 mg twice/daily) or placebo arm at a 1:1 ratio [82]. The primary endpoint, recurrence-free survival, was not achieved; recurrence-free survival was similar between the sorafenib and placebo arm (33.4 months vs. 33.8 months, respectively; P = 0.26) [82]. Similarly, there was no statistically significant difference between treatment arms for time to recurrence and OS [82]. Discontinuation rates due to AEs were much higher in the sorafenib versus placebo arm (i.e., 24 % vs. 7 %, respectively) [82].

In a phase III trial (SEARCH study) (n = 720), patients were randomized into a sorafenib plus placebo arm or sorafenib plus erlotinib arm at a 1:1 ratio [97]. Median overall survival was similar across the sorafenib and sorafenib plus erlotinib arms (9.5 months vs. 8.5 months, hazard ratio = 0.929; P = 0.18). The most common grade 3/4 adverse events in the sorafenib versus sorafenib plus erlotinib arms, respectively, included fatigue (17.5 % vs. 17.7 %), hand–foot skin reaction (17.5 % vs. 10.2 %), diarrhea (11.8 % vs. 19.3 %), AST (11.8 % vs. 13.8 %), and hyperbiliruminemia (11.5 % vs. 11.9 %) [97].

A strategy under evaluation to reduce HCC involves the use of vaccines against hepatitis B virus in populations at high risk for acquiring this virus; preventing infection with hepatitis B virus (HBV) would reduce the likelihood of developing HCC [98]. Among patients who develop both HBV and HCC, a strategy to reduce the risk of recurrence has included the use of antiviral agents. Among patients seropositive for HBV, postoperative treatment with an antiviral regimen (adefovir dipivoxil plus lamivudine or entacavir) reduced HCC recurrence [99].

There are numerous ongoing studies evaluating the efficacy and safety of sorafenib in combination with other chemotherapeutic agents or other targeted therapies [33].

34.3 The Future

Although the last 5 years have been disappointing, with novel, targeted agents evaluated in phase III studies for HCC failing to meet their primary endpoint, OS, in both the first-line (i.e., sunitinib, linifanib, erlotinib plus sorafenib, brivanib) or second-line setting (brivanib, everolimus, ramucirumab) [54, 68, 83, 84, 87, 88, 97], there is hope to further improve outcomes in HCC. Notably, there are numerous ongoing clinical trials evaluating single (Table 34.2) and combination therapies (Table 34.3).

Table 34.3 Ongoing phase II and III combination trials with targeted therapies

Treatment	Phase	*N*	End points
Aspirin + lamivudine after surgery	III	112	Recurrence-free survival, OS, safety
Bevacizumab + erlotonib	II	44	PFS at 16 weeks
Bevacizumab + erlotonib (vs. sorafenib)	II	120	Response, safety, OS
Bevacizumab + floxuridine + dexamethasone	II	55	Response, safety
Brivanib + TACE	III	870	OS, TTDP, safety
Galunisertib (LY2157299) + nivolumab	I/II	100	MTD, PFS, DOR, OS, response
Ipilimumab + SBRT	I/II	100	MTD, response
Ramucirumab + Emibetuzumab (LY2875358)	I/II	70	Response, safety
Sorafenib + capecitabine + oxaliplatin	II	52	PFS, OS, tumor response, safety
Sorafenib + doxorubicin	II	170	TTP, OS, response, QoL, biomarkers
Sorafenib + doxorubicin	III	480	OS, TTP, PFS, response

(continued)

Table 34.3 (continued)

Treatment	Phase	*N*	End points
Sorafenib + everolimus	II	106	PFS, response, TTP, OS, safety
Sorafenib + gemcitabine + oxaliplatin	II	78	PFS, response
Sorafenib + mapatumumab	II	101	TTP, OS, PFS, DOR, safety
Sorafenib + melphalan	II	31	response, PFS, safety
Sorafenib + oprozomib	Ib/II	140	MTD, TTP, OS, PFS, response, safety
Sorafenib + oxilapltin + S-1	II	100	OS, time to recurrence
Sorafenib + pravastatin	III	323	OS, PFS, TTP, QoL
Sorafenib + refametinib	II	14	OS, DOR, Response, PFS, safety
Sorafenib + temsirolimus	II	106	PFS, OS, TTP, response, safety
Sorafenib + temsirolimus	II	28	OS, PFS, TTP, response, safety
Sorafenib + SBRT	III	368	OS, TTP, PFS, QoL, safety
Sorafenib + TACE	II/III	246	OS, TTP, tumor response, safety
Sorafenib + TACE	II	63	TTP, safety
Sorafenib + TACE	II	228	TTP, OS, response, safety
Sorafenib + TACE	III	240	OS, recurrence
Sorafenib + TACE	III	412	PFS, OS, TTP, safety, QoL
Sorafenib + TACE + radiation	I/II	30	TTP, PFS, PS, safety
Sorafenib + TRC105	I/II	39	MTD, PFS, response, safety
Sunitinib + TACE	II/III	190	OS, relapse-free survival, QoL, safety
Trametinib + capecitabine + fluorouracil + leucovorin	II	89	Response, safety, OS
Tremelimumab + MEDI4736	I/II	129	OS, DOR, response, safety
Veliparib (ABT-888) + temozolomide	II	49	PFS, OS, safety, biomarker, benefit rate

DCR disease control rate; *DOR* duration of response; *MTD* maximum tolerated dose, *OS* overall survival; *PFS* progression-free survival; *QoL* quality of life; *SBRT* stereotactic body radiation therapy; *TACE* transcatheter arterial chemoembolization; *TTDP* time to disease progression; *TTP* time to progression

To improve the outcomes of patients with advanced HCC, the underlying genetic and molecular signaling pathways needs to be further defined and elucidated. Although some aberrant signaling pathways promote the initiation of tumors, other signaling pathways associated with oncogene addiction sustain the tumor; identifying and abrogating a signaling pathway that sustains the tumor would be more likely to achieve optimal tumor reduction [48, 100].

It is essential to conduct a biomarker analysis in clinical trials to assess whether biomarkers might identify subsets of patients more likely to respond to therapy, and prospectively enroll these patients into a clinical study. Unfortunately, recently reported phase III studies evaluating new treatments for HCC did not incorporate or report findings from a biomarker analysis [43, 100]. Despite this shortcoming, the promising preliminary findings from the responsiveness of patients with MET-high tumors to tivantinib is promising [43]. Although this suggests that personalized medicine may finally enter HCC treatment algorithms, the initial findings need to be verified by prospectively enrolling patients with MET-high tumors into phase III studies.

With the integration of biomarkers and the continued evaluation of targeted therapies, over the next few years, it is expected that the knowledge gained from advances in molecular biology will finally translate to real victories in the war against cancer and provide pharmacologic agents that can provide benefit to the patient, such as improved survival, better management of symptoms, and preservation of quality of life.

References

1. Avila MA, Berasain C, Sangro B, Prieto J. New therapies for hepatocellular carcinoma. Oncogene. 2006;25(27):3866–84.
2. Chow LQ, Eckhardt SG. Sunitinib: from rational design to clinical efficacy. J Clin Oncol. 2007;25(7):884–96.
3. Croce CM. Oncogenes and cancer. N Engl J Med. 2008;358(5):502–11.
4. Lyons JF, Wilhelm S, Hibner B, Bollag G. Discovery of a novel Raf kinase inhibitor. Endocr Relat Cancer. 2001;8(3):219–25.
5. Sridhar SS, Hedley D, Siu LL. Raf kinase as a target for anticancer therapeutics. Mol Cancer Ther. 2005;4(4):677–85.

6. Adjei AA, Hidalgo M. Intracellular signal transduction pathway proteins as targets for cancer therapy. J Clin Oncol. 2005;23 (23):5386–403.
7. Friday BB, Adjei AA. Advances in targeting the Ras/Raf/MEK/Erk mitogen-activated protein kinase cascade with MEK inhibitors for cancer therapy. Clin Cancer Res. 2008;14 (2):342–6.
8. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nat Rev Cancer. 2003;3(6):459–65.
9. Bos JL. Ras oncogenes in human cancer: a review. Cancer Res. 1989;49(17):4682–9.
10. Hwang YH, Choi JY, Kim S, et al. Over-expression of c-raf-1 proto-oncogene in liver cirrhosis and hepatocellular carcinoma. Hepatol Res. 2004;29(2):113–21.
11. Wan PT, Garnett MJ, Roe SM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004;116(6):855–67.
12. Calvisi DF, Ladu S, Gorden A, et al. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. Gastroenterology. 2006;130(4):1117–28.
13. Hopfner M, Schuppan D, Scherubl H. Growth factor receptors and related signalling pathways as targets for novel treatment strategies of hepatocellular cancer. World J Gastroenterol. 2008;14(1):1–14.
14. Clauss M. Molecular biology of the VEGF and the VEGF receptor family. Semin Thromb Hemost. 2000;26(5):561–9.
15. Seeliger H, Guba M, Kleespies A, Jauch KW, Bruns CJ. Role of mTOR in solid tumor systems: a therapeutical target against primary tumor growth, metastases, and angiogenesis. Cancer Metastasis Rev. 2007;26(3–4):611–21.
16. Villanueva A, Chiang DY, Newell P, et al. Pivotal role of mTOR signaling in hepatocellular carcinoma. Gastroenterology. 2008;135 (6):1972–83, 1983.e1-11.
17. Kim YD, Park CH, Kim HS, et al. Genetic alterations of Wnt signaling pathway-associated genes in hepatocellular carcinoma. J Gastroenterol Hepatol. 2008;23(1):110–8.
18. Jianhonb W, Qingke H, Minxin C. The role of NF-êB in hepatocellular carcinoma cell. Chinese Med J. 2003;116(5):747–52.
19. Rocha-Lima CM, Soares HP, Raez LE, Singal R. EGFR targeting of solid tumors. Cancer Control. 2007;14(3):295–304.
20. Okamoto T, Sanda T, Asamitsu K. NF-kappa B signaling and carcinogenesis. Curr Pharm Des. 2007;13(5):447–62.
21. Thomas MB, Abbruzzese JL. Opportunities for targeted therapies in hepatocellular carcinoma. J Clin Oncol. 2005;23(31):8093–108.
22. Chao Y, Li CP, Chau GY, et al. Prognostic significance of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin in patients with resectable hepatocellular carcinoma after surgery. Ann Surg Oncol. 2003;10(4):355–62.
23. Eikesdal HP, Kalluri R. Drug resistance associated with antiangiogenesis therapy. Semin Cancer Biol. 2009;19:310–7.
24. Torrecilla S, Llovet JM. New molecular therapies for hepatocellular carcinoma. Clin Res Hepatol Gastroenterol. 2015;39(Suppl 1):S80–5.
25. Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell. 2005;8(4):299–309.
26. Finn RS. Emerging targeted strategies in advanced hepatocellular carcinoma. Semin Liver Dis. 2013;33(Suppl 1):S11–9.
27. Mizejewski GJ. Role of integrins in cancer: survey of expression patterns. Proc Soc Exp Biol Med. 1999;222(2):124–38.
28. Vautier G, Bomford AB, Portmann BC, Metivier E, Williams R, Ryder SD. p53 mutations in british patients with hepatocellular carcinoma: clustering in genetic hemochromatosis. Gastroenterology. 1999;117(1):154–60.
29. Kazachkov Y, Khaoustov V, Yoffe B, Solomon H, Klintmalm GB, Tabor E. p53 abnormalities in hepatocellular carcinoma from United States patients: analysis of all 11 exons. Carcinogenesis. 1996;17(10):2207–12.
30. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer. 2006;6(1):24–37.
31. Hato T, Goyal L, Greten TF, Duda DG, Zhu AX. Immune checkpoint blockade in hepatocellular carcinoma: current progress and future directions. Hepatology. 2014;60(5):1776–82.
32. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A. 2002;99(19):12293–7.
33. Blank C, Gajewski TF, Mackensen A. Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy. Cancer Immunol Immunother. 2005;54(4):307–14.
34. Sahasrabuddhe VV, Gunja MZ, Graubard BI, et al. Nonsteroidal anti-inflammatory drug use, chronic liver disease, and hepatocellular carcinoma. J Natl Cancer Inst. 2012;104(23):1808–14.
35. Petrick JL, Sahasrabuddhe VV, Chan AT, et al. NSAID use and risk of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: the liver cancer pooling project. Cancer Prev Res (Phila). 2015. pii: canprevres.0126.2015 [Epub ahead of print].
36. Cervello M, Montalto G. Cyclooxygenases in hepatocellular carcinoma. World J Gastroenterol. 2006;12(32):5113–21.
37. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med. 2008;358(11):1160–74.
38. Kawaguchi Y, Kono K, Mimura K, Sugai H, Akaike H, Fujii H. Cetuximab induce antibody-dependent cellular cytotoxicity against EGFR-expressing esophageal squamous cell carcinoma. Int J Cancer. 2007;120(4):781–7.
39. Camp ER, Summy J, Bauer TW, Liu W, Gallick GE, Ellis LM. Molecular mechanisms of resistance to therapies targeting the epidermal growth factor receptor. Clin Cancer Res. 2005;11 (1):397–405.
40. Munshi N, Jeay S, Li Y, Chen CR, et al. ARQ 197, a novel and selective inhibitor of the human c-MET receptor tyrosine kinase with antitumor activity. Mol Cancer Ther. 2010;9(6):1544–53.
41. You H, Ding W, Dang H, Jiang Y, Rountree CB. c-Met represents a potential therapeutic target for personalized treatment in hepatocellular carcinoma. Hepatology. 2011;54(3):879–89.
42. Llovet JM, Hernandez-Gea V. Hepatocellular carcinoma: reasons for phase III failure and novel perspectives on trial design. Clin Cancer Res. 2014;20(8):2072–9.
43. Santoro A, Rimassa L, Borbath I, et al. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. Lancet Oncol. 2013;14(1):55–63.
44. Abou-Alfa GK. Approaching the era of personalised therapy for liver cancer? Lancet Oncol. 2013;14(1):7–8.
45. Website for registered clinical trials, clinicaltrials. gov.
46. Goyal L, Muzumdar MD, Zhu AX. Targeting the HGF/c-MET pathway in hepatocellular carcinoma. Clin Cancer Res. 2013;19 (9):2310–8.
47. Zhu AX. New agents on the horizon in hepatocellular carcinoma. Ther Adv Med Oncol. 2013;5(1):41–50.
48. Llovet JM, Villanueva A, Lachenmayer A, Finn RS. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. Nat Rev Clin Oncol. 2015;12(7):408–24.
49. Huynh H, Nguyen TT, Chow KH, Tan PH, Soo KC, Tran E. Over-expression of the mitogen-activated protein kinase (MAPK)

kinase (MEK)-MAPK in hepatocellular carcinoma: its role in tumor progression and apoptosis. BMC Gastroenterol. 2003;3:19.
50. Klein PJ, Schmidt CM, Wiesenauer CA, et al. The effects of a novel MEK inhibitor PD184161 on MEK-ERK signaling and growth in human liver cancer. Neoplasia. 2006;8(1):1–8.
51. Amaravadi R, Thompson CB. The survival kinases Akt and Pim as potential pharmacological targets. J Clin Invest. 2005;115 (10):2618–24.
52. Pang RW, Poon RT. From molecular biology to targeted therapies for hepatocellular carcinoma: the future is now. Oncology. 2007;72(Suppl 1):30–44.
53. Peralba JM, DeGraffenried L, Friedrichs W, Fulcher L, Grünwald V, Weiss G, Hidalgo M. Pharmacodynamic evaluation of CCI-779, an inhibitor of mTOR in cancer patients. Clin Cancer Res. 2003;9(8):2887–92.
54. Zhu AX, Kudo M, Assenat E, et al. Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial. JAMA. 2014;312(1):57–67.
55. Cheng A, Kang Y, Chen Z, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009;10(1):25–34.
56. Llovet J, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378–90.
57. Lencioni R, Kudo M, Ye SL, et al. GIDEON (Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and Of its treatment with sorafeNib): second interim analysis. Int J Clin Pract. 2014;68(5):609–17.
58. You L, He B, Xu Z, et al. Inhibition of Wnt-2-mediated signaling induces programmed cell death in non-small-cell lung cancer cells. Oncogene. 2004;23(36):6170–4.
59. Emami KH, Nguyen C, Ma H, et al. A small molecule inhibitor of betacatenin/CREB-binding protein transcription [corrected]. Proc Natl Acad Sci USA. 2004;101(34):12682–7.
60. You L, He B, Uematsu K, et al. Inhibition of Wnt-1 signaling induces apoptosis in betacatenin-deficient mesothelioma cells. Cancer Res. 2004;64(10):3474–8.
61. Yount S, Cella D, Webster K, et al. Assessment of patient-reported clinical outcome in pancreatic and other hepatobiliary cancers: the FACT hepatobiliary symptom index. J Pain Symptom Manag. 2002;24(1):32–44.
62. Wei W, Chua MS, Grepper S, So SK. Blockade of Wnt-1 signaling leads to anti-tumor effects in hepatocellular carcinoma cells. Mol Cancer. 2009;8:76.
63. Delgado ER, Yang J, So J, et al. Identification and characterization of a novel small-molecule inhibitor of β-catenin signaling. Am J Pathol. 2014;184(7):2111–22.
64. Ganten TM, Koschny R, Haas TL, et al. Proteasome inhibition sensitizes hepatocellular carcinoma cells, but not human hepatocytes, to TRAIL. Hepatology. 2005;42(3):588–97.
65. Zang Y, Thomas SM, Chan ET, et al. The next generation proteasome inhibitors carfilzomib and oprozomib activate prosurvival autophagy via induction of the unfolded protein response and ATF4. Autophagy. 2012;8(12):1873–4.
66. Siegel AB, Cohen EI, Ocean A, et al. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. J Clin Oncol. 2008;26(18):2992–8.
67. Spratlin JL, Cohen RB, Eadens M, et al. Phase I pharmacologic and biologic study of ramucirumab (IMC-1121B), a fully human immunoglobulin G1 monoclonal antibody targeting the vascular endothelial growth factor receptor-2. J Clin Oncol. 2010;28 (5):780–7.
68. Zhu AX, Park JO, Ryoo BY, et al. Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): a randomised, double-blind, multicentre, phase 3 trial. Lancet Oncol. 2015;16(7):859–70.
69. van Geel RM, Beijnen JH, Schellens JH. Concise drug review: pazopanib and axitinib. The Oncologist. 2012;17(8):1081–9.
70. Llovet JM, Villanueva A, Lachenmayer A, Finn RS. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. Nat Rev Clin Oncol. 2015;12(8):436.
71. John L, Cowey CL. The rapid emergence of novel therapeutics in advanced malignant melanoma. Dermatol Ther (Heidelb). 2015;5 (3):151–69.
72. El-Khoueiry AB, Melero I, Crocenzi TS, et al. Phase I/II safety and antitumor activity of nivolumab in patients with advanced hepatocellular carcinoma (HCC): CA209-040. Prersented a the American Society of Clinical Oncology (ASCO) Annual Meeting in Chicago, Illinois. J Clin Oncol 33, 2015 (suppl; abstr LBA101).
73. Wilhelm S, Chien DS. BAY 43-9006: preclinical data. Curr Pharm Des. 2002;8(25):2255–7.
74. Wilhelm SM, Carter C, Tang L, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004;64 (19):7099–109.
75. Carlomagno F, Anaganti S, Guida T, et al. BAY 43-9006 inhibition of oncogenic RET mutants. J Natl Cancer Inst. 2006;98 (5):326–34.
76. National Comprehensive Cancer Network (NCCN) Clinical practice guidelines in oncology: hepatobiliary cancers version 2. 2015. Accessed online at: http://www.nccn.org/professionals/physician_gls/pdf/hepatobiliary.pdf.
77. Akutsu N, Sasaki S, Takagi H, et al. Development of hypertension within 2 weeks of initiation of sorafenib for advanced hepatocellular carcinoma is a predictor of efficacy. Int J Clin Oncol. 2015;20(1):105–10.
78. Li Y, Gao ZH, Qu XJ. The adverse effects of sorafenib in patients with advanced cancers. Basic Clin Pharmacol Toxicol. 2015;116 (3):216–21.
79. Azad NS, Aragon-Ching JB, Dahut WL, et al. Hand-foot skin reaction increases with cumulative sorafenib dose and with combination anti-vascular endothelial growth factor therapy. Clin Cancer Res. 2009;15(4):1411–6.
80. Lacouture ME, Wu S, Robert C, et al. Evolving strategies for the management of hand-foot skin reaction associated with the multitargeted kinase inhibitors sorafenib and sunitinib. Oncologist. 2008;13(9):1001–11.
81. Chu D, Lacouture ME, Fillos T, Wu S. Risk of hand-foot skin reaction with sorafenib: a systematic review and meta-analysis. Acta Oncol. 2008;47(2):176–86.
82. Bruix J, Takayama T, Mazzaferro V, et al. STORM: A phase III randomized, double-blind, placebo-controlled trial of adjuvant sorafenib after resection or ablation to prevent recurrence of hepatocellular carcinoma (HCC) J Clin Oncol. 2014 ASCO annual meeting abstracts. Vol 32, No 15_suppl (May 20 Supplement), 2014: Abstract 4006.
83. Cheng AL, Kang YK, Lin DY, et al. Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. J Clin Oncol. 2013;31(32):4067–75.
84. Cainap C, Qin S, Huang WT, et al. Linifanib versus sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. J Clin Oncol. 2015;33(2):172–9.
85. Nakamura I, Zakharia K, Banini BA, et al. Brivanib attenuates hepatic fibrosis in vivo and stellate cell activation in vitro by inhibition of FGF, VEGF and PDGF signaling. PLoS ONE. 2014;9(4):e92273.

86. Huynh H, Ngo VC, Fargnoli J, et al. Brivanib alaninate, a dual inhibitor of vascular endothelial growth factor receptor and fibroblast growth factor receptor tyrosine kinases, induces growth inhibition in mouse models of human hepatocellular carcinoma. Clin Cancer Res. 2008;14(19):6146–53.
87. Johnson PJ, Qin S, Park JW, et al. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. J Clin Oncol. 2013;31(28):3517–24.
88. Llovet JM, Decaens T, Raoul JL, et al. Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase III BRISK-PS study. J Clin Oncol. 2013;31(28):3509–16.
89. Brave SR, Ratcliffe K, Wilson Z, et al. Assessing the activity of cediranib, a VEGFR-2/3 tyrosine kinase inhibitor, against VEGFR-1 and members of the structurally related PDGFR family. Mol Cancer Ther. 2011;10(5):861–73.
90. Hurwitz HI, Dowlati A, Saini S, et al. Phase I trial of pazopanib in patients with advanced cancer. Clin Cancer Res. 2009;15(12):4220–7.
91. Chuma M, Terashita K, Sakamoto N. New molecularly targeted therapies against advanced hepatocellular carcinoma: From molecular pathogenesis to clinical trials and future directions. Hepatol Res. 2014. doi:10.1111/hepr.12459. [Epub ahead of print].
92. Bruix J, Tak WY, Gasbarrini A, et al. Regorafenib as second-line therapy for intermediate or advanced hepatocellular carcinoma: multicentre, open-label, phase II safety study. Eur J Cancer. 2013;49(16):3412–9.
93. Li T, Dong ZR, Guo ZY, et al. Aspirin enhances IFN-α-induced growth inhibition and apoptosis of hepatocellular carcinoma via JAK1/STAT1 pathway. Cancer Gene Ther. 2013;20(6):366–74.
94. Xiong ZP, Yang SR, Liang ZY, et al. Association between vascular endothelial growth factor and metastasis after transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int. 2004;3(3):386–90.
95. Sergio A, Cristofori C, Cardin R, et al. Transcatheter arterial chemoembolization (TACE) in hepatocellular carcinoma (HCC): the role of angiogenesis and invasiveness. Am J Gastroenterol. 2008;103(4):914–21.
96. Kudo M, Imanaka K, Chida N, et al. Phase III study of sorafenib after transarterial chemoembolisation in Japanese and Korean patients with unresectable hepatocellular carcinoma. Eur J Cancer. 2011;47(14):2117–27.
97. Zhu AX, Rosmorduc O, Evans TR, Ross PJ, et al. SEARCH: a phase III, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with advanced hepatocellular carcinoma. J Clin Oncol. 2015;33(6):559–66.
98. Michielsen PP, Francque SM, van Dongen JL. Viral hepatitis and hepatocellular carcinoma. World J Surg Oncol. 2005;3:27.
99. Yin J, Li N, Han Y, et al. Effect of antiviral treatment with nucleotide/nucleoside analogs on postoperative prognosis of hepatitis B virus-related hepatocellular carcinoma: a two-stage longitudinal clinical study. J Clin Oncol. 2013;31(29):3647–55.
100. Weinstein IB. Cancer. Addiction to oncogenes—the Achilles heal of cancer. Science. 2002;297(5578):63–4.