Abstract. Hepatocellular carcinoma (HCC) is an aggressive tumor and the sixth most common form of cancer worldwide. Surgery is the gold-standard treatment for local disease and often complemented by radiofrequency ablation or transarterial chemoembolization. In advanced disease, therapy options are limited and relapse and metastasis are common. Systemic therapy with cytotoxic drugs such as doxorubicin and cisplatin achieves low objective response rates (typically <10%) and even sorafenib, an orally administered tyrosine kinase inhibitor considered a breakthrough when introduced, prolongs median survival by little more than a year. Sorafenib blocks platelet-derived growth factor, vascular endothelial growth factor, c-KIT and rapidly accelerated fibrosarcoma signaling, and belongs to a new class of targeted drugs. It has become standard treatment for advanced-stage HCC in recent years. To date, no other agent has been shown to be more effective than sorafenib in the clinical setting, which highlights the need for ongoing research to address this important clinical challenge. The current review focuses on recent advances in molecular targeted therapy for HCC. We explore the current status of evidence, identify areas of pressing experimental need, and provide an outline of promising future therapeutic options.

Hepatocellular carcinoma (HCC) represents an important public health issue. It is the sixth most common type of cancer overall and in males it is the third most common cause of cancer-related death worldwide (1). The majority of HCC cases result from chronic viral hepatic infections, in particular hepatitis B and C (2). In Europe and the United States, hepatitis C virus is the dominant pathogen, whereas in Asia and Africa hepatitis B virus predominates (3). Other non-viral factors are also associated with an increased risk of developing HCC. These include alcoholic and non-alcoholic cirrhosis, hereditary hemochromatosis, Wilson's disease, α-1 antitrypsin deficiency, primary biliary cirrhosis and exposure to aflatoxin, a product of the fungus Aspergillus (4-6). In Western countries, hereditary and lifestyle/behavioural factors contribute roughly equally to the overall incidence of HCC (7).

Despite recent progress in our understanding of the pathogenesis of HCC and screening programs for high-risk patients, the rate of cure remains low (8, 9). This may be explained, in part, by the limited therapeutic options available and an ongoing tendency for late diagnosis, highlighting the importance of research to identify novel prognostic and therapeutic targets in HCC.

In the absence of advanced fibrosis and provided that R0 resection is feasible, surgery is the recommended course of action for the majority of patients with HCC (10). Resection is effective and safe, with a postoperative mortality of less than 5% in patients with good performance status and a single identifiable liver lesion (11). Nevertheless, tumor recurrence is common and affects 50% to 70% of patients following surgery with curative intent, either due to the presence of occult intrahepatic metastases or due to de novo tumor formation within the liver remnant (termed 'field...
effect’ due to the same pathophysiological trigger in the whole organ) (12).

Several other local therapeutic strategies such as radiofrequency ablation (RFA), microwave ablation, and ethanol or acetic acid ablation are available for HCC as an adjuvant or alternative to surgery. For lesions less than 2 cm in diameter, complete response with equivalent long-term outcomes can be achieved with each local ablation technique, whereas for HCCs larger than 2 cm, RFA is considered superior (13-15). Transarterial chemoembolization (TACE) is another therapeutic option that combines targeted injection of antineoplastic agents with selective obstruction/embolization of tumour-feeding vessels. Furthermore, patients with a solitary lesion <5 cm or three lesions each <3 cm that are not suitable for resection, may be considered for liver transplantation, a treatment option associated with greater than 65% long-term survival (16).

Despite this expanding repertoire of therapeutic options for HCC, no adjuvant chemotherapeutic agents are available that significantly improve patient survival: Doxorubicin monotherapy is associated with a response rate of less than 20% and provides no additional survival benefit but is associated with frequent adverse events (17). Moreover, a large proportion of patients with HCC have comorbidities which preclude the use of conventional cytotoxic agents (particularly liver cirrhosis thrombocytopenia, liver dysfunction or impaired coagulation). Therefore, the need for new targeted drugs which are both more effective and associated with fewer side-effects is becoming increasingly apparent.

In recent times, the most effective targeted drug introduced for HCC has been sorafenib. Sorafenib, a multi-tyrosine kinase inhibitor (TKI), remains the only systemic targeted tumor therapy in use for advanced HCC (18-21). Although sorafenib was considered a key change in the systemic treatment of HCC when it was introduced, this perhaps further reflects the lack of effective alternatives as the median survival in HCC has been extended by little more than a year with the use of sorafenib.

In the current review, we examine the impact of sorafenib in the context of new developments in molecular-targeted therapy for HCC; we discuss promising areas of active research and highlight future prospects for the treatment of patients in this challenging clinical field.

Molecular Targeted Agents – Progress and Current Status

In recent years, improved understanding over the molecular mechanisms which underpin tumour initiation and progression have provided opportunities for developing novel pharmacological interventions. In HCC specifically, the development of sorafenib was considered a breakthrough. Sorafenib is a multi-TKI known to convey an antitumoural effect by blocking vascular endothelial growth factor (VEGF) receptor, platelet-derived growth factor (PDGF) receptor, rapidly accelerated fibrosarcoma (RAF)- and FMS-related tyrosine kinase (22). However, to date, despite numerous attempts to identify the molecular targets of sorafenib, the main mechanism responsible for its therapeutic effect in HCC has not been fully elucidated.

Tai et al. found that signal transducer and activator of transcription 3 (STAT3) is one target of sorafenib (23). STAT3 target genes are involved in tumor proliferation, self-renewal, invasion and angiogenesis and STAT3 is activated in about 70% of solid and haematological cancer (24-26). STAT3 plays a key role in the cellular response to cytokines and growth factors, including, amongst others interferons, EGF, interleukins 5 and 6. It also drives tumour-induced immunosuppression and is a point of convergence for several oncogenic signalling pathways (27). Recently, Wei et al. have showed that sorafenib inhibits HCC growth and invasion by inhibiting matrix metalloproteinase-2 and Ki-67 expression through simultaneous up-regulation of P53 and suppression of forkhead box M1 (FOXM1) (28). FOXM1 is a transcription factor. It has been shown to play an important role in the pathogenesis and progression of HCC. Suppression of FOXM1 is followed by mitotic spindle defects, chromosome disaggregation, and cell-cycle arrest (29, 30). In mouse models, the depletion of FOXM1 reduces cancer cell proliferation and tumour growth in solid tumours (31).

Sprinzl et al. studied the influence of sorafenib on tumor-associated macrophages, and identified significant alterations in growth factor secretion. The group went on to show that sorafenib affects macrophage-induced tumour cell growth and reduces insulin-like growth factor I1GF1-dependent cancer growth in vitro and in vivo (32).

Although the mechanisms underlying sorafenib function have not fully been described, trial evidence does suggest that it has an impact in vivo on humans with HCC. Two large randomized controlled trials, the SHARP trial and Asia-Pacific trial, both showed a significant improvement of overall survival (OS) in advanced HCC associated with the use of sorafenib.

The SHARP trial showed significantly improved OS in patients treated with sorafenib compared with patients treated with a placebo control (10.7 vs. 7.9 months, hazard ratio=0.69, 95% confidence interval (CI)=0.55-0.87; p<0.001). The Asia-Pacific study also reported a significant survival benefit with sorafenib vs. placebo (6.5 vs. 4.2 months, hazard ratio=0.68, 95% CI=0.50-0.93; p=0.014). Furthermore, time to progression (TTP) was significantly prolonged in both trials (SHARP: 5.5 months in the sorafenib arm vs. 2.8 months in the placebo arm, p<0.001; Asia-Pacific trial 2.8 months in the sorafenib arm vs. 1.4 months in the placebo arm, p<0.001) (19, 20). It is not clear
why survival in the Asia-Pacific trial and the SHARP trial cohorts differed to such an extent, given the similarities in treatment protocols; however, this might be explained by differences in performance status, tumor localization and aetiology identified between the patient cohorts.

Although only relatively few data were available from patients with impaired liver function in both trials, sorafenib was generally well-tolerated. In contrast, a large phase IV study known as the GIDEON trial assessed the safety and efficacy of sorafenib in patients with Child-Pugh A and with Child-Pugh B HCC and found that in accordance with both the Asia-Pacific and SHARP trials, the majority of drug-related adverse events were of a low grade. The nature of adverse events was also similar in all trials and included hand-foot skin reactions, rash/desquamation fatigue and gastrointestinal upset (19, 20, 33). As a result, sorafenib has now become standard therapy for advanced HCC (15, 34).

Sub-group analyses from the Asia-Pacific study and the SHARP trial showed sorafenib was effective in patients with advanced HCC, irrespective of baseline performance status, tumour burden, tumour stage, prior therapy, and disease aetiology (35, 36). However, several investigations showed a lower median OS for patients in the Child-Pugh B group compared with those in the Child-Pugh A group. Moreover, in patients with Child-Pugh B HCC, the serum aspartate aminotransferase level seemed to be a significant predictor for OS (37-40). In accordance with these findings, the grade of toxicity was also higher in patients with Child-Pugh B or C than in the Child-Pugh A group (41). Thus, the available data suggest that the Child-Pugh status strongly predicts OS for patients with unresectable HCC treated with sorafenib.

The efficacy of sorafenib in combination with doxorubicin has also been assessed in comparison with doxorubicin monotherapy. Compared to doxorubicin alone, the median TTP and OS in the dual-therapy arm was 6.4 (95% CI=4.8-9.2 vs. 2.8 (95% CI=1.6-5) months and 13.7 vs. 6.5 months, respectively (42). This promising initial data stimulated significant interest and recruitment to a phase III trial which is ongoing. (ClinicalTrials.gov; NCT01015833, Sorafenib Tosylate With or Without Doxorubicin Hydrochloride in Treating Patients With Locally Advanced or Metastatic Liver Cancer). The role of sorafenib as an adjuvant therapy following surgical resection or local ablation has also recently been assessed. However, the STORM (Sorafenib as Adjuvant Treatment in the Prevention Of Recurrence of Hepatocellular Carcinoma) trial did not identify any significant benefit associated with sorafenib compared with surgery alone (43).

Despite these recent advances, patients with metastatic HCC often do not survive longer than one year (44). Resistance to sorafenib is a particular clinical challenge and has triggered extensive research to identify the molecular mechanisms through which this effect is mediated. Although several studies provide insight into potential drug-resistance pathways, the exact pathophysiological mechanism remains unclear. Negri et al. recently showed that elevated expression of phosphorylated extracellular signal regulated kinase in tumor tissue was associated with poor survival in advanced HCC treated with sorafenib [hazard ratio (HR)=2.09, 95% CI=1.13-3.86; p=0.019] and VEGFR2 (HR=2.28, 95% CI=1.13-4.61; p=0.021) (45). Zhao et al. showed that sorafenib induced hypoxia-inducible factor (HIF2α) up-regulation leads to lower expression of VEGF, cyclin D1, HIF2α and transforming growth factor α, and inhibition of epidermal growth factor receptor (EGFR), thereby inhibiting proliferation and promoting HCC cell apoptosis in vitro (46). Su et al., demonstrated that the inactivation of regulatory factor X/cytosolic tyrosine phosphatase induced by continuous treatment with sorafenib triggers sorafenib resistance in HCC cells through pSTAT3 activation (47). Although mechanisms of sorafenib resistance are yet not fully understood, this important field of research offers opportunities to deliver therapy for patients with HCC in a more personalized manner, producing clinical effects which are at the same time more potent and less likely to cause unwarranted side-effects.

Molecular Targeting – Alternative Therapeutic Approaches

A number of other molecular targeting agents have been tested in the treatment of HCC. For several of these, promising anti-tumoural effects identified in phase II trials have failed to be replicated in phase III studies, and others are still being explored in large clinical trials (48-50).

Sunitinib. In an open-label phase III trial conducted by Cheng et al., OS with sunitinib, a selective inhibitor of multiple receptor tyrosine kinases, was significantly inferior to that with sorafenib (7.9 vs. 10.2 months; HR=1.30, 95% CI: 1.13-1.50; p=0.0014). Although TTP was similar across patient cohorts, 4.1 months vs. 3.8 months; HR=1.13; p=0.3082), sunitinib-treated patients suffered significantly more frequent and intense side-effects (51).

Brivanib. In another phase III trial, the BRISK-FL study, Johnson et al. compared brivanib, a VEGF and fibroblast growth factor inhibitor, with sorafenib in patients with advanced HCC (52). Although OS was similar in the brivanib and sorafenib arms (9.9 months vs. 9.5 months, respectively; HR=1.06; 95% CI=0.93 to 1.22; p=0.3116), brivanib was less well tolerated by patients than sorafenib.

Erlotinib. More recently, a multicentre, multinational, randomized, phase III trial comparing sorafenib with sorafenib/erlotinib combination therapy was performed.
Erlotinib targets EGFR tyrosine kinase activity by reversibly competing with ATP for binding in the kinase domain. It is routinely used in lung and pancreatic cancer treatment regimens.

Known as the SEARCH trial, patients with advanced HCC were offered sorafenib plus either erlotinib or placebo. The addition of erlotinib did not produce substantial clinical gains, as neither OS (9.5 in the sorafenib plus erlotinib arm vs. 8.5 months in the sorafenib plus placebo group; HR=0.929; p=0.408), nor TTP (3.2 in the sorafenib plus erlotinib arm vs. 4.0 months in the sorafenib plus placebo group; HR=1.135; p=0.18) were significantly improved (53).

Linifanib. In another phase III trial, Cainap et al. investigated the efficacy of linifanib (ABT-869) vs. sorafenib in patients with advanced HCC without prior systemic therapy (54). Linifanib is a receptor tyrosine kinase inhibitor, which is an inhibitor of members of the VEGF and PDGF receptor families (55). Median OS was similar in both treatment arms (9.1 months on the linifanib arm vs. 9.8 months on the sorafenib arm: HR=1.046, 95% CI=0.896 to 1.221). Although the median TTP was slightly more favorable with linifanib [5.4 months (95% CI=4.2 to 5.6 months) vs. 4.0 months (95% CI=2.8 to 4.2) with a hazard ratio of 0.759, (95% CI=0.643 to 0.895) p=0.001] the side-effect profile was once again substantially better with sorafenib treatment (56).

Lenavatinib, apatinib, tivozanib, regorafenib and ramucirumab. A number of clinical trials are currently underway to assess the efficacy of other promising targeted therapeutic agents. These include lenavatinib, a multi-tyrosine kinase inhibitor inhibiting VEGFR (www.clinicaltrials.gov; NCT01761266); apatinib (www.clinical-trials.gov; NCT02329860); ADI-PEG 20, an arginine-degrading enzyme (www.clinical-trials.gov; NCT01287585); tivozanib, a MET proto-oncogene inhibitor (www.clinicaltrials.gov; NCT01755767); ARQ 197 (tivozanib) (www.clinicaltrials.gov; NCT02029157); and regorafenib, a multikinase inhibitor (www.clinicaltrials.gov; NCT01774344).

Recruitment is also underway for REACH, a multicenter, randomized, double-blind, placebo-controlled trial, examining the efficacy of ramucirumab vs. placebo as a second-line agent following failure of sorafenib therapy in HCC. (www.clinicaltrials.gov; NCT01140347).

Mammalian target of rapamycin (mTOR) inhibitors. Although mTOR signalling pathway aberrations are common in cancer, mTOR inhibitors such as everolimus or temsirolimus have failed in the successful treatment of patients with HCC. Recently Knox et al. published a phase II trial comprising 26 patients receiving temsirolimus and bevacizumab as first-line treatment in advanced HCC (57). However, drug-related toxicity, as well as dose reductions and drug discontinuation, were common.

The use of temsirolimus in HCC has not been routinely recommended due to limited evidence of available data from prospective trials (58). The second currently approved mTOR inhibitor, everolimus, was explored in second-line treatment after sorafenib failure in the EVOLVE-1 trial. In this phase III trial, everolimus was tested versus placebo. However, only a minimal and statistically not significant benefit in median OS was shown (everolimus 7.6 months vs. placebo 7.3 months; HR=1.05, p=0.675) (59).

New Targeted Anticancer Agents

Currently the application of immune checkpoint inhibitors is being intensively investigated in a number of types of cancer. Importantly, these agents have already been approved for use in metastatic melanoma and very recently for lung cancer by regulatory authorities in North America and Europe (60).

Programmed death-1 (PD1) is an inhibitory receptor expressed by T cells which functions as an immune checkpoint. By blocking PD1, Topalian et al. (61) and Brahmer et al. (61, 62) demonstrated a therapeutic tumour response in non-small cell lung cancer, melanoma, and renal cell cancer. In HCC, the role of PD1 has also been described (63-66). and currently nivolumab, another PD1-blocking agent, is being assessed in a phase I study which was presented in abstract form at the American Society of Clinical Oncology conference 2015, and demonstrated very impressive (early) results (www.clinicaltrials.gov; NCT01658878).

In advanced melanoma, ipilimumab, a cytotoxic T-lymphocyte-associated antigen 4 (CTLA4)-directed antibody, has been used with great success for the treatment of advanced melanoma (60). CTLA4 is a repressive co-receptor that interacts with T-cell proliferation and activation pathways. Sangro and colleagues investigated the effect of tremelimumab, another CTLA antibody, in patients with HCC and found a partial response rate of 17.6% and a disease control rate of 76.4%. Currently, another pilot study investigating the safety and effectiveness of-remelimumab in combination with TACE or RFA in advanced HCC is under way (www.clinicaltrials.gov; NCT01853618).

Another interesting approach in the treatment of HCC is the application of JX-594. A number of interesting studies have now been published using this oncolytic vaccinia virus in a therapeutic context (67-69).

The performance of mitogen-activated protein kinase enzyme inhibitors refametinib and selumetinib, has also been tested in treatment-naïve patients with HCC, both alone and in combination with sorafenib. A number of studies, however, were stopped due to lack of efficacy in terms of survival and substantial toxicity (70-72). Recently Tarocchi et al. have investigated ACV-TP-T, which is a thymidine analogue prodrug, acycloguanosyl-5’-thymidyltriphosphate, which is
specifically activated by telomerase in HCC cells and its antitumour efficacy (73). They found ACV-TP-T activated by telomerase in HCC cells that reduces proliferation and induces apoptosis in human and murine liver cancer cells. Furthermore, telomerase activity-inhibiting substances seem to be an imaginable approach to future HCC treatment options (74). Unfortunately the IGF1R inhibitor cixutumumab did not demonstrate significant antitumoural activity in a recent phase II study (75).

**Conclusion**

The overall prognosis in advanced HCC remains poor. Sorafenib is the gold standard in treatment and highlights the potential of targeted therapeutic approaches in HCC. Although a considerable amount of research has been made for other molecular targeted therapies, none have demonstrated a statistically significant advantage over sorafenib in large prospective clinical trials. However, our understanding over the biological processes which drive HCC development and progression is improving and the new knowledge may help in the development of new anticancer therapies which are both more effective and associated with a favourable side-effect profile.

**Conflicts of Interest**

The Authors have no conflict of interest related to the manuscript.

**Acknowledgements**

Supported by Erwin-Schroedinger Scholarship of the Austrian Science Funds, No. J3389-B23.

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Received June 30, 2015
Revised August 27, 2015
Accepted September 4, 2015