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Review Article

Therapeutic approaches to hepatocellular carcinoma

Beatrice Borgia, Dario Neri*
*Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland. *Correspondence: dario.neri@pharma.ethz.ch*

CONTENTS

Abstract

Hepatocellular carcinoma (HCC) is still an incurable disease and one of the most common malignant cancers worldwide, often associated with an extremely poor prognosis. Many staging systems have been proposed in order to stratify patients according to prognostic factors and to guide therapeutic strategies, but none has yet received universal acceptance. Therapeutic options for HCC are limited and mainly fall into two broad categories: surgical and nonsurgical treatments. Surgical ablation therapies (liver transplantation and resection) are the only potentially curative treatments, but are only applicable to a limited number of patients with early HCC. Among nonsurgical therapies, percutaneous ablation approaches and transcatheter arterial embolization are the most widely performed and have provided good results in selected groups of patients, but are unable to achieve outcomes comparable to those for surgical treatments. Drugs either alone or in combination have shown minimal or no efficacy for the treatment of HCC. However, recent data suggest that the broad-spectrum tyrosine kinase inhibitor sorafenib may prolong survival time in HCC patients. Antibody-based therapy has provided some interesting results, particularly in the radioimmunotherapy setting, and further investigation is needed in order to establish the therapeutic potential of this new group of promising biopharmaceuticals.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and one of the most malignant. It causes more than 500,000 deaths per year, making it the third leading cause of cancer-related death. The incidence of HCC follows a typical geographical pattern; 82% of cases are found in developing countries, with 55% encountered in China alone (1). The increasing interest in HCC prevention and treatment in developed countries, such as in Europe (2) and the USA (3), is due to a rising incidence in the past decade, which is predicted to continue over the next two decades (4, 5), probably due to hepatitis infection.

These geographical differences in incidence mirror the great heterogeneity in the risk factors associated with liver cancer development, with any agent that may lead to chronic liver injury, and eventually cirrhosis, being a potential oncogenic factor. Liver cirrhosis represents the major clinical risk factor for the development of HCC, for which the most established risk factors are hepatitis B virus (HBV), HCV and chronic alcohol abuse (6). HBV accounts for most of the HCC cases in Africa and some regions of Asia, where the virus is normally acquired at birth or early in life, and HCC commonly affects patients between 20 and 40 years of age. In these areas, an important role is also played by exposure to aflatoxin B1 (AFB1), a potent hepatic oncogenic factor, from contaminated food (7). In Western countries, the major causes of HCC are believed to be HCV infection and alcohol intake (8). In general, HCC is more common in men than in women, with a 3:1 ratio, and the risk increases with age (5).

Early detection of HCC is of paramount importance, representing the most effective strategy to decrease cancer-related mortality and allow more successful therapies. In this respect, improvements have been achieved in the past two decades with the development of ultrasound, which allows detection at an asymptomatic stage of the disease, and the introduction of imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI), which aid in accurately defining tumor burden (9).

Several staging systems have been proposed for the outcome prediction of patients diagnosed with HCC. They consider mainly tumor mass, underlying liver function (since HCC often develops in a cirrhotic environment) and the physical status of the patient, and serve as guidelines for identifying the optimal treatment. The first classi-

fication for determining liver function was the Child-Pugh scoring system, which takes five parameters into account: albumin, bilirubin, prothrombin time, ascites and encephalopathy. Each parameter contributes from 1 to 3 points and the final score leads to a grade A, B or C classification, which in turn defines the severity of liver cirrhosis (C being the poorest value) (10). The main limitation of this system for outcome prediction in HCC (although liver function plays a pivotal role) is the lack of parameters directly correlated with the tumor itself. To remedy this situation, the Okuda system was then developed and has been extensively used (11). However, despite linking the Child-Pugh classification to parameters associated with the extent of cancer, the Okuda staging system does not distinguish between early and advanced cancer. Several other groups have attempted to overcome these limitations by proposing new classification systems, such as the Barcelona-Clinic Liver Cancer (BCLC) (12), the Cancer of the Liver Italian Program (CLIP) (13), the Chinese University Prognostic Index (CUPI) (14) and the Japan Integrated Staging (JIS) (15, 16), but none has yet received universal acceptance.

Therapies for HCC can be mainly divided into surgical and nonsurgical treatments which are applied depending on the stage of disease. Potentially curative therapies currently available are represented by ablative treatments such as tumor resection and liver transplantation, which may result in a complete response for early tumors in patients with well-preserved liver function. If surgery is precluded, local nonsurgical therapies are applied. Although providing good results, these treatments do not achieve the response rates associated with surgical treatments. So far, chemotherapeutics have proven to have poor efficacy, with no definite survival advantages and with well-known treatment-related complications. Several new therapeutic strategies have been developed and some are now being tested in clinical settings.

Surgical ablation therapy

Liver resection

Liver resection is considered the first-line treatment for HCC in patients with noncirrhotic livers (40% in Asia, 5% in Western countries) or with Child-Pugh class A cirrhosis. The remaining hepatic reserve should, in fact, support adequate liver function after surgery in order to avoid liver failure complications. While this does not represent a limitation in cases where HCC develops in a normal liver, extreme caution must be taken in patients with underlying cirrhosis. Several groups have proposed selection strategies to identify those patients with the best postoperative outcomes by taking into account parameters linked to liver function reserve other than the Child-Pugh classification. In Japan this is mainly assessed by the indocyanine green (ICG) clearance test, the safety limit of which is considered to be 14% of retention time at 15 min (17). In Europe and the USA, the most reliable predictive factor is considered to be the portal hepatic pressure value, which when > 10 mmHg is associated with an increased risk of liver failure (18). Attention to the presence of co-morbid illnesses, which tend to be prevalent in the elderly, is also of paramount importance for the evaluation of the risks of hepatectomy. By carefully selecting patients according to these parameters, the overall surgical mortality is around 5% and the 5-year survival rates can be as high as 70%, a percentage that is greatly reduced if selection is not performed (19, 20).

However, the documented disease-free survival rates are low, being around 30% at 5 years compared to 14% following liver transplantation. This situation reflects the presence of intrahepatic metastases at the time of surgery or *de novo* tumor establishment from the cirrhotic liver which is already in a precancerous state (19). The two types of recurrence have different timings: whereas intrahepatic metastases normally appear within the first 2 years after surgery, new tumors tend more often to emerge later on during follow-up (21).

Various strategies, which depend on the type of relapse, have been exploited to reduce recurrence rates. To eradicate undetectable intrahepatic metastases, adaptive immunotherapy (22) and internal radiotherapy with lipiodol-^{131}I (23) were associated with a significant increase in disease-free and overall survival. In contrast, neither systemic or local chemotherapy nor preoperative chemoembolization proved to have significant efficacy (24). For preventing the development of a new primary hepatoma in the oncogenic liver, good results have been obtained with the oral administration of polyprenoic acid, an acyclic retinoid, which led to significant improvements compared to the placebo group (25). Interferon therapy also gave substantial benefit in selected patients with chronic HCV-associated HCC (26). Despite such promising results, wider clinical trials are needed before including any of these adjuvant treatments in clinical practice. Some authors have also proposed to enlist patients with a high risk of recurrence for liver transplantation, since it is associated with lower recurrence rates and a similar chance of survival (9).

Liver transplantation

Liver transplantation is commonly accepted as the intervention of choice for patients with early HCC and compromised liver function (Child-Pugh class B or C), having the added advantage of replacing the cirrhotic liver while removing the entire cancer mass. Such patients with poor liver function could not, in fact, undergo hepatic resection, mainly due to mortality and recurrence. The first attempts to cure HCC with liver transplantation in the 1980s led to poor outcomes (5-year survival < 40%) and high recurrence rates (32-54%) due to the extremely advanced state of hepatic cancer and to the presence of factors such as vascular invasion, extrahepatic dissemination and lymph node involvement, which are adverse prognostic features (27, 28).

Besides demonstrating the feasibility of liver transplantation, these early studies also served to define cer-

tain characteristics for a better classification of patients undergoing surgery in order to avoid such an expensive and invasive treatment in those individuals who would not benefit from it. The findings suggested that good results were obtained only in those patients with small and less aggressive lesions (27), and in this case liver transplantation was more effective than resection. In 1996, Mozzaferro *et al*. (29) reported excellent 4-year survival rates of 75% and recurrence rates of 14% for selected candidates who had one nodule of 5 cm or less or up to three nodules of 3 cm or less. Such parameters, the so-called Milan criteria, subsequently become universally accepted for the selection of patients to be enrolled for transplantation. Extremely good results were then reported in many other studies, showing outcomes similar to liver transplantation for nonmalignant pathologies (30) and an overall improvement in survival rates compared to those obtained without previous selection (31).

At present, we face a controversial situation. On the one hand, the candidates for liver transplantation far exceed the number of donors, leading to an extremely long waiting period, which in turn increases the number of dropouts. On the other hand, there are compelling studies which advocate the feasibility of expanding the Milan criteria without adversely affecting the survival rates (32), thereby enlarging even more the potential number of candidates for liver transplantation. Several strategies have been proposed to address this scenario, where the availability of organs is limited and the number of patients enlisted for grafting is increasing. Major efforts are centering on blocking tumor development prior to transplantation and on increasing the number of organs by living donor liver transplantation (LDLT). To reduce the number of dropouts due to tumor progression, nonsurgical therapies such as transarterial chemoembolization (TACE), percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA) are commonly used. Although the role of such interventional bridge therapies prior to transplantation is not fully established, it seems that RFA results in better tumor responses than PEI and TACE, although there is concern for needle track seeding of the tumor leading to higher recurrence rates (33).

To relieve the shortage of cadaveric donor grafts, some authors advocate the use of LDLT, which can be performed immediately without long waiting periods. The role of LDLT in the therapy of HCC has not yet been established and results are still quite controversial. While some studies suggest that LDLT is associated with higher recurrence rates compared to cadaveric donor liver transplantation (34), others indicate comparable disease-free survival rates in the two groups (35), making it difficult to assess the efficacy of such treatment with respect to others. However, despite the feasibility of this therapeutic option, the main concern stems from the potential morbidity or mortality of donors (36). This raises obvious major ethical issues of whether it is worth risking a healthy donor when there are alternative options with comparable survival rates.

Nonsurgical treatments

Percutaneous ablation therapies

Percutaneous ablation therapies are minimally invasive procedures which represent the best nonsurgical option for HCC. Tumor ablation is achieved by local injection of chemical substances (*i.e.,* ethanol, acetic acid) or modification of tissue temperature (radiofrequency, cryoablation, microwave and laser). A concern with these percutaneous therapeutic approaches arises from the risk of needle track seeding, although the degree of the risk is still unknown (37).

1. Percutaneous ethanol injection (PEI)

PEI is a well-established technique and one of the most widely used for nonsurgical tumor ablation in HCC (38). It is safe, easy to perform and can be associated with high tumor response rates. Absolute ethanol is injected via noncutting needles precisely placed in the lesion through ultrasound guidance, normally under local anesthesia. Tumor necrosis is induced by means of cellular dehydration and protein denaturation, resulting in occlusion of tumor vessels and fibrosis. Once injected, ethanol diffuses easily and selectively through the soft HCC nodules which are surrounded by the firm cirrhotic liver. It has been shown to be highly effective in small HCC (< 3 cm), leading to complete tumor shrinkage in > 90% of the cases (39), a percentage that is greatly reduced for bigger nodules.

The limitation of PEI in the treatment of large tumors results from the presence of intratumoral fibrous septa, which prevent ethanol from being evenly distributed into the tumor mass. Also, PEI is unable to generate safety margins in the liver parenchyma adjacent to the tumor mass, resulting in local recurrence as satellite nodules are often located in this region (40). Indeed, recurrence represents the main limitation of this technique. Reported 1-, 3- and 5-year recurrence rates are 26-32%, 51-82% and 60-83%, respectively (38, 39). Despite such limitations, several studies have reported long-term outcomes for PEI-treated patients comparable to those obtained with liver resection, with 5-year survival rates ranging from 35% to 60% (38, 39).

2. Percutaneous acetic acid injection (PAI)

Highly concentrated acetic acid is able to readily penetrate cells, easily dissolving collagen and lipids. PAI under ultrasound guidance for the treatment of HCC was first reported in 1994 by Ohnishi *et al*. (41), who subsequently performed a randomized trial to compare PAI and PEI. In this study, PAI was shown to be superior to PEI, with 1- and 2-year survival rates of 100% and 92%, respectively, for the former and 83% and 63%, respectively, for the latter (42). Later, Liang *et al.* proved the feasibility of reducing the number of injections by increasing the dose of injected acetic acid without affecting efficacy and safety (43). This represents the main advantage of PAI *versus* PEI; although other studies showed comparable therapeutic effects, PAI can be performed with

markedly fewer treatment session. Although PAI appears promising, further large randomized trials are required in order to ascertain its role in the treatment of HCC.

3. Percutaneous radiofrequency thermal ablation (RFTA)

RFTA is currently the most widely used local method of heat ablation. It works by inducing thermal injury to the tissue through electromagnetic energy deposition. A high-frequency alteration current (100-500 kHz) passes from the electrode tip (placed in the tumor mass) to the surrounding tissue, causing ionic vibrations as the ions attempt to follow the changes in direction of a rapidly alternating electric current. This agitation leads to frictional heating of the tissue. The degree of the damage depends on the temperature achieved and on the duration of heating. The optimal situation for ablative purposes is to maintain a cytotoxic temperature (between 55 and 100 °C) throughout the entire target volume for at least 4-6 min. On the other hand, careful control of the temperature should be implemented in order to avoid temperatures higher than 100 °C, which would lead to tissue carbonization around the probe (44).

RFTA can be achieved mainly via three types of electrodes: expandable electrodes, internally cooled electrodes and saline-enhanced electrodes, among which the first two types are the most widely used and appear to have equivalent efficacy in the treatment of small HCCs (45).

The role of RFTA in the management of HCC should be evaluated with extreme caution, however, as controversial results have been reported. Many studies suggest RFTA to be superior to both PEI and PAI in terms of local recurrence, overall survival and disease-free survival (46, 47), making it the putative treatment of choice for nonresectable small HCCs. However, some recent studies have reported an unexpected rapid disease progression, with the appearance of large intra- or extrahepatic nodules in 25% of RFTA-treated patients (48).

RFTA requires fewer procedures compared to PEI to induce complete tumor necrosis, but it has the disadvantage that its applicability depends on tumor location. If the treated lesion is located in the proximity of large blood vessels, the heat produced by radiofrequency will be dissipated by blood flow, thus reducing its efficacy. If it is located close to certain organs, such as the diaphragm, gallbladder and stomach, the heat produced by RFTA may seriously injure these organs (47).

4. Percutaneous microwave coagulation therapy (PMCT)

PMCT is another form of thermal ablation for HCC. It was first introduced in 1994 by Seki *et al.* (49) and has been used mainly in Japan and China. A microwave coagulator generates and transmits microwave energy to a monopolar-type needle electrode, which is placed into the tumor via a 24-gauge needle under CT or ultrasound guidance. This energy induces molecular vibrations which in turn cause an increase in tissue temperature and thermal coagulation around the electrode. PMCT, however, has similar limitations to RFA. Mainly, the heating efficacy is reduced in lesions close to large blood vessels, a situation which can be circumvented either by increasing power output or by occluding the large vessels before performing PMCT (50). Studies comparing RFA and PMCT suggest that the two techniques are equally effective when HCCs of various sizes are taken into account (51). However, when comparative analysis is restricted to small HCCs (< 3 cm), RFA appears to be superior in terms of local recurrence rates, survival rates and number of sessions needed (52). Reported 1-, 2-, 3- and 4-year survival rates are 82-92%, 61-82%, 50-73% and 37-66%, respectively (50-52).

5. Percutaneous cryoablation

Cryoablation destroys liver tumors by *in situ* freezing. It is normally performed by circulating argon or liquid nitrogen through metallic probes placed into the lesion core. The cytotoxic effects of cryoablation are mainly due to intra- and extracellular ice formation and subsequent thawing. Ice crystal formation causes damage to intracellular structures, membrane rupture, osmotic dehydration and anoxia, which finally lead to cell death (53). The degree of the cytotoxic effect depends on the vicinity to the probe; it will be maximal for cells closer to the probe, while cells at the periphery may survive, especially when the tumor is surrounded by large intrahepatic blood vessels. Typically, ablation is obtained by freezing at temperatures below –160 °C using two freeze/thaw cycles of 10-15 min each, with the goal of achieving a safety margin in the hepatic parenchyma surrounding the tumor (54, 55). Control of the freezing zone can be monitored with real-time visualization by intraoperative ultrasonography, which represents the major advantage of cryoablation over RFTA and PMCT. Reported clinical results for cryoablation are difficult to interpret because this therapeutic modality has been used as the sole treatment for HCC in most studies.

6. High-intensity focused ultrasound (HIFU)

HIFU is a novel thermal ablation therapy for HCC which has emerged in recent years. It is a noninvasive local technique that relies on the use of focal ultrasonic energy to induce a well-defined volume of coagulation in deep tissues through intact skin by a thermal effect and cavitation (56). The main advantages are represented by the possibility to execute HIFU extracorporeally without surgical intervention and to achieve ablation of large tumor volumes under the guidance of real-time imaging techniques. It is normally performed under epidural or general anesthesia, depending on ablation time, patient position, tumor location and therapeutic ultrasound exposure (57). Although considered a safe procedure, it has some common side effects, such as skin or subcutaneous burns, trauma in adjacent organs and fever (57, 58). Interestingly, HIFU has been used in the treatment of patients with large HCCs (average size = 8.2 ± 3.4 cm), for whom reported survival rates at 6, 12 and 18 months are 86%, 61.5% and 35.3%, respectively (57). Also, good results have been obtained for the TACE/HIFU combina-

tion in the ablation of advanced HCCs, for which there are not many therapeutic options at present, but large-scale randomized trials are necessary for confirmation (59).

Transcatheter arterial chemoembolization (TACE)/ transarterial embolization (TAE)

TACE is a regional therapy for HCC in which targeted chemotherapy and arterial embolization are combined. It consists of the injection of anticancer drugs, with or without lipiodol, into the hepatic artery, followed by the administration of embolizing agents. Transarterial embolization (TAE), which is also used in the treatment of HCC, refers only to the last process without previous administration of chemotherapeutics. The most commonly used anticancer drugs in TACE are doxorubicin, epirubicin, cisplatin and mitomycin C administered either alone or in combination (60). Lipiodol (iodized oil), is used as a vehicle to carry and keep the drug longer inside the tumor, because it is thought to preferentially accumulate in the nodule due to arterial hypervascularization and the absence of Kupfer cells in cancer tissue (61).

Embolization is normally commenced after drug injection and aims at hepatic arterial obstruction leading to ischemic damage and necrosis of HCC nodules. Since HCCs mainly receive arterial blood, while healthy liver tissue mostly relies on a portal vein blood supply, arterial embolization preferentially affects tumor cells. The most commonly used embolizing agents are gelatin sponges of various dimensions, which occlude the hepatic artery temporarily for about 2 weeks, but recent data suggest that novel drug-eluting beads (DEBs) might be even more valuable for TACE technology (62). The DEBs consist of polyvinyl alcohol (PVA) beads (500-700 μm) loaded with a chemotherapeutic agent that is slowly released upon injection, which may therefore increase the intensity and duration of ischemia while also enhancing drug delivery to the tumor (62) and reducing drug-related side effects.

To achieve maximal response, TACE is often done in multiple sessions, with an interval of roughly 2 months between each course (63). TACE and TAE do not show significant differences in survival rates, which suggests that ischemic damage induced by embolization plays a major role in tumor size reduction after TACE (64). The mean survival rates reported for patients treated with chemoembolization or embolization at 1, 2, 3 and 5 years are 62 ± 20%, 42 ± 17%, 30 ± 15% and 19 ± 16%, respectively (63). The most common complication is the so called “postembolization syndrome”, which manifests with fever, local pain and vomiting. In order to avoid liver failure complications, strict candidate selection should be applied. In this respect, patients with portal vein thrombosis and insufficient liver reserve (Child C) are not good candidates for TACE and TAE therapy.

Chemotherapy and novel drugs

Although many chemotherapeutics have been tested in clinical trials for the treatment of unresectable HCC, no single agent or combination regimen was found to be particularly effective. Only recently, sorafenib (Nexavar) was found to significantly improve overall survival, progression-free survival (PFS) and time to progression (TTP). With most of the chemotherapeutic agents, response rates are low and, more importantly, no significant improvement in survival rates has been reported. The most studied chemotherapeutic agent is doxorubicin (DOX), for which two recent large phase III studies reported 4% and 10.5% response rates in the DOX-treated group and median survival times of 8 and 6.8 months (65, 66). Significant toxicities were also encountered, however.

Epirubicin, a doxorubicin derivative believed to have a more favorable toxicity profile, has also been tested in the treatment of HCC, but outcomes showed limited activity (67). Also, many well-known drugs, such as cisplatin, 5-fluorouracil (5-FU), mitoxantrone and etoposide, as well as newer chemotherapeutics, such as paclitaxel, irinotecan and capecitabine, were tested for HCC but gave disappointing results (68). Nolatrexed (Thymitaq), a novel antitumor drug that inhibits the thymidylate synthase, was recently tested in a phase III clinical trial. Although previous phase II studies suggested it to be superior to DOX treatment, the phase III trial failed to confirm the preliminary results (65). A variety of combination regimens have been studied, but none showed encouraging results. The treatment that demonstrated the highest response rate in HCC was the combination of cisplatin, interferon, doxorubicin and 5-FU (PIAF), which demonstrated a 20.9% response rate and a survival time of 8.6 months in a phase III clinical trial (66).

Some novel molecularly targeted agents have also been tested recently in phase I, II and III studies, since the mechanisms of hepatocarcinogenesis are now better understood. Erlotinib (Tarceva), a tyrosine kinase inhibitor with specificity for epidermal growth factor receptor (EGFR)/HER1, was evaluated in a phase II clinical trial. Modest benefit was seen in 32% of patients, who were progression-free at 6 months, the primary objective of this study (69). Promising results were also recently reported for sorafenib, an oral multikinase inhibitor that targets both tumor cells and tumor vasculature. Targeted kinases include Raf, vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) kinases, which are implicated in HCC angiogenesis. Although sorafenib was shown to induce modest response rates (5%) in a phase II study in HCC, the response was found to be correlated with phosphorylated extracellular signal-regulated kinase (pERK) levels in the tumor. This suggests that HCCs containing higher levels of pERK have been more responsive to sorafenib treatment (70). A pivotal phase III study evaluating 602 liver cancer patients, the Sorafenib HCC Assessment Randomized Protocol (SHARP) trial, showed a significant increase in the median overall survival time of sorafenib-treated patients *versus* placebo-treated patients (10.7 and 7.9 months, respectively) (www.viva.vita.bayerhealthcare.com). These promising data have also been

confirmed in the recent phase III Asia-Pacific Liver Cancer Trial, which was designed to evaluate the efficacy and safety of sorafenib in Asian-Pacific patient populations. Among the 226 patients enrolled, both PFS and TTP, the objectives of this study, were improved in those administered sorafenib 400 mg twice daily compared to patients receiving placebo (www.viva.vita.bayerhealthcare.com).

Antibody-based therapy

Antibodies represent the fastest growing area of pharmaceutical biotechnology (71), with several products approved for the therapy of certain types of cancer. In general, antibodies can be developed as biopharmaceuticals either as intact immunoglobulins (*e.g.*, bevacizumab [Avastin™], cetuximab [Erbitux™], trastuzumab [Herceptin™], alemtuzumab [Campath™], rituximab [Rituxan™]) or as antibody derivatives (72, 73). While only two radiolabeled antibodies have been approved so far for cancer therapy applications (Zevalin™ and Bexxar™, both for B-cell lymphoma), a number of radiolabeled antibodies have been tested in clinical trials, with the aim of demonstrating a preferential accumulation at HCC sites and assessing the potential therapeutic benefit for patients.

The F19 murine monoclonal antibody specific for fibroblast activation protein (FAP), a cell-surface glycoprotein not present in most normal tissues but abundantly expressed by reactive stromal fibroblasts in cancer, has been studied in patients with liver metastases of colorectal cancer (74). Since FAP is expressed in a number of different aggressive cancer types, including HCC, it would be conceivable to use the F19 antibody (or better a humanized version) for the targeted delivery of bioactive molecules to liver cancer. Similarly, human monoclonal antibodies specific for the alternatively spliced EDA domain of fibronectin have recently been shown to preferentially localize to liver lesions in an immunocompetent mouse model of cancer (75).

Monoclonal antibodies with a proven ability to selectively recognize HCC cells in a large portion of cancer specimens include the AF-20 antibody raised against the human HCC cell line FOCUS (76) and metuximab, an antibody specific for a member of the CD147 family. Interestingly, [^{131}I]-labeled metuximab was studied in a phase II radioimmunotherapy clinical trial involving administration to 106 patients with HCC of 27.75 MBq/kg by hepatic arterial infusion (77). This dose had previously been found to be safe in a phase I dose-escalation trial. The authors observed that the radioiodinated antibody was able to preferentially localize to tumor tissues. Of the 73 patients completing 2 cycles, 6 (8.22%) had a partial response, 14 (19.18%) a minor response and 43 (58.9%) stable disease. The 21-month survival rate was 44.54%. Using a similar strategy, the mouse monoclonal antibody Hepama-1, labeled with ^{131}I, was studied in a phase I/II clinical trial evaluating hepatic artery ligation plus hepatic artery cannulation and antibody infusion (78).

The bevacizumab and cetuximab antibodies, approved for the treatment of colorectal cancer in combination with chemotherapeutic regimens, have been studied in clinical trials in HCC patients. Bevacizumab was administered in combination with gemcitabine and oxaliplatin in a phase II study. Thirty patients were assessable for efficacy, revealing a 20% response rate, and 27% of the patients had stable disease. Median overall survival was 9.6 months and median PFS was 5.3 months (79). In contrast, cetuximab was studied in a phase II monotherapy clinical trial in patients with HCC, revealing that the antibody could be safely administered at an initial dose of 400 mg/m^2, followed by weekly infusions at 250 mg/m^2. However, no antitumor activity was demonstrated in this study (80).

Summary and outlook

HCC remains an incurable disease. Surgical ablation therapy and percutaneous procedures continue to be widely used and may contribute to increased survival. Pharmacological approaches for the treatment of HCC patients have so far exhibited minimal efficacy, but the situation could be changing, with sorafenib being possibly the first drug to demonstrate a survival benefit in this disease area. The therapeutic results with monoclonal antibodies, particularly those with radiolabeled products administered using locoregional procedures, have shown promising results, but to our knowledge none of the approaches tested so far has led to complete remissions. The L19-IL2 antibody-cytokine fusion protein, developed by L. Zardi in collaboration with our lab (81-83), has shown curative therapeutic results in an orthotopic mouse model of human HCC (84). This product is currently being investigated in phase II clinical trials in patients with renal cell carcinoma and in phase Ib studies in combination with gemcitabine in patients with pancreatic cancer. So far, the product has not been studied in the HCC setting. More generally, we believe that the use of antibodies for the targeted delivery of bioactive molecules to sites of tumor angiogenesis (85, 86) may represent a promising avenue for the treatment of aggressive, incurable cancer types. Clinical trials in the HCC setting are needed in order to evaluate the therapeutic potential of this new class of biopharmaceutical agents.

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