Molecular targeting agents associated with transarterial chemoembolization or radiofrequency ablation in hepatocarcinoma treatment

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common cause of cancer in the world. According to Barcelona Clinic Liver Cancer modified criteria, patients with early stage disease are candidate to radiofrequency ablation (RFA), while patients with intermediate stage HCC are usually treated by transarterial chemoembolization (TACE). TACE and RFA induce a transient devascularisation effect followed by strong neo-angiogenic stimulus. In fact, after these procedures, it has been demonstrated an up-regulation of pro-angiogenic and growth factors such as vascular endothelial growth factor-A, which might contribute to accelerated progression in patients with incomplete response. Several studies have demonstrated that MAP-kinase and AKT pathways, in addition to neo-angiogenesis, have an important role in the development of HCC. In advanced HCC, anti-angiogenic therapy and tyrosine kinases inhibitors showed potential clinical benefit. Actually, a number of clinical studies are ongoing testing these agents in combination with TACE or RFA. In this paper, we have reviewed the most recent preclinical and clinical results of such trials.

Key words: Hepatocellular carcinoma; Molecular targeting agents; Angiogenesis; Chemoembolization therapeutic; Radiofrequency treatment; Sorafenib

Core tip: The outcome of patients (with early or intermediate stage according to Barcelona Clinic Liver Cancer) treated with loco-regional approach alone [radiofrequency ablation (RFA) or transarterial chemoembolization (TACE)] is disappointing because the rebound of vascular endothelia growth factors induced by tissue hypoxia. On this basis there is a strong preclinical background to associate TACE or RFA with anti-angiogenic agents. We summarized the crucial role of angiogenesis and the pathways involved in hepatocellular cancer progression, underscorong the consequences of pro- and anti-angiogenic factors produced after loco-regional therapy. We explored preclinical and clinical results of trials combining molecular targeting agents plus TACE or RFA.
INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cause of cancer in the world. To treat this tumor, it’s necessary a multidisciplinary approach due to the underlying cirrhosis.

According to the Barcelona-Clinic Liver Cancer Classification (BCLCC), subset of patients in the very early and early stage could be treated with radiofrequency ablation (RFA). Patients with intermediate stage could be considered for transarterial chemoembolization (TACE).

In classical TACE the blood supply to the tumor is blocked from chemotherapeutic agent (doxorubicin, mitomycin) plus lipiodol. Chemoembolization with drug-eluting beads (DEB-TACE) combines the drug with the embolization device by using microsphere. It’s important to underline that ischemia, induced by TACE, strongly stimulates the expression of angiogenic factors, in particular vascular endothelial growth factor-A (VEGF-A). Consequently, HCC angiogenesis and progression are stimulated in patients with an incomplete response. Also, insufficient or incomplete RFA could promote angiogenesis in residual HCC, inducing a rapid tumor regrowth.

Here, we have reviewed the pathways involved in HCC development, underscoring the angiogenic rebound following loco-regional therapy, and clinical trials employing the association between loco-regional therapy and molecular targeting agents.

MAIN PATHWAYS INVOLVED IN THE DEVELOPMENT OF HCC

Fundamental pathways involved in HCC proliferation are the RAS-mitogen-activated protein kinase (MAPK) and the phosphoinositide-3-kinase (PI3K) (Figure 1).

The RAS-MAPK pathway is the most important cascade leading to tumor cell progression. Vascular endothelial growth factor receptor (VEGFR) and platelet derived growth factor receptor (PDGFR) are transmembrane tyrosine-kinase receptors that, binding several growth factors, activate the RAS GTPase proteins. The increased activation of this pathway may be due to: (1) RAS proteins mutation; (2) overexpression of intracellular RTKs; and (3) a number of growth factors, such as VEGF or PDGF. The main RAS effector pathway is the RAF-MAPK/extracellular signal-regulated kinase (ERK) kinase. Once RAF proteins are activated and phosphorylated by different protein kinases, they phosphorylate MEK that, in turn, activates ERK1 and ERK2 that modulates gene expression via the phosphorylation of transcription factors, which have profound effects on tumorigenesis. RAF activation is crucial in HCC progression; in fact overexpression of RAF is very frequent. Interestingly, it was reported that hepatitis B and C virus infection are able to activate the RAS-MAPK pathway in HCC. Therefore, this pathway represents the ideal molecular target of several anti-angiogenic therapies, such as sorafenib. In addition, the signaling inhibition mediated by these receptors can be realized by various monoclonal antibodies (cetuximab, trastuzumab, ramucirumab, bevacizumab), and by “small” molecules (erlotinib, lapatinib).

The RAS-MAPK pathway is a regulator of the phosphoinositide-3-kinase PI3K/AKT/mammalian target of rapamycin (mTOR) pathway. This pathway stimulates the Janus kinase/signal transducer and the pathway, which represent a central regulator of proliferation. The PI3K/AKT/mTOR pathway is activated in a subset of HCC and its blockade by rapamycin and/or everolimus, demonstrated to inhibit the growth of HCC cell lines.

PETENTIAL BENEFITS OF THE ASSOCIATION OF MOLECULAR TARGETING AGENTS PLUS TACE

TACE is used in the treatment of: (1) single lesion (> 3 cm); (2) multifocal HCC; and (3) awaiting liver transplantation. HCC receives prolonged contact time with chemotherapeutic agent, by its infusion directly into vessels supplying the tumor, and subsequently obstructing these vessels with an embolization material. Therefore, chemoembolization induces necrosis, prolonging the exposition between cancer cells and chemotherapy. However, ischemia correlates with expression of angiogenic factors, such as VEGF-A and angiopoietin, and stimulates angiogenesis (resulting in the formation of a rich vascular bed in residual tumors), which may allow the surviving cancer cells to proliferate. In fact, after TACE, the tumor microenvironment becomes deranged with increased hypoxia, leading to an up-regulation in hypoxia inducible factor-1 (HIF-1), which in turn up-regulates VEGF and also PDGFR, further increasing tumor angiogenesis. During tumor angiogenesis, cancer and endothelial cells secrete PDGF-B, which acts stimulating pericytes to express VEGF.

In patients in whom TACE failed, due to incomplete embolization or partial recanalization, the secretion of pro-angiogenic factors by residual tumor or surrounding tissue might contribute to HCC progression. In fact, TACE was associated with a high rate of disease recurrence, with 67% of post-therapy deaths due to tumor progression. High serum VEGF levels are frequently expressed in HCC and increased serum VEGF levels are correlated with liver function, tumor size, tumor number, microscopic venous invasion, distant metastasis, reduced overall survival (OS), and recurrence of HCC after treatment.
Preclinical and clinical data suggested that a monitoring of VEGF-A (determined by ELISA) one day before and 7 d after TACE might be used to predict HCC growth and that an increase of serum VEGF levels from the first to the third day post-TACE could be related to poor prognosis[3,16-18].

According to these evidences, the treatment with multikinase inhibitors pre/after TACE could be both anti-proliferative, anti-angiogenic and, at the same time, may prolong time to recurrence, improve survival, and target lesions distal to the TACE site. In fact, preclinical models combining bland transarterial embolization with anti-angiogenic agents have observed a reduction in tumor volume and vessel density, as well as a prolongation in survival compared with transarterial embolization alone[19].

POTENTIAL BENEFITS OF THE ASSOCIATION OF MOLECULAR TARGETING AGENTS PLUS RFA

The mechanisms of rapid growth of residual HCC after RFA or the mediators are still poorly understood. It was hypothesized that insufficient RFA, due to a not sufficiently high local ablative temperature, could promote proliferation and angiogenesis of residual HCC[4]. Moreover, after RFA in the residual tumor ablative HIF-1α levels are increased and, consequently, the tumor might exhibit an aggressive phenotype with unfavourable prognosis[4].

TACE WITH MOLECULAR TARGETING AGENTS

All clinical studies that analyzed molecular targeting drugs (Figure 1) with predominantly anti-angiogenic effect in combination with TACE are summarized in Table 1.

Sorafenib
Sorafenib is an oral multtargeted receptor TKI, blocking RAF/MEK/ERK pathway, VEGFR-2/3 and PDGFR-β, stem cell factor receptor, fms-like tyrosine kinase 3, and rearranged during transfection[22]. Sorafenib (at dose 800 mg/d) was approved from the United States Food and Drugs Administration (FDA) for the treatment of advanced HCC since November 2007[23,24].

The addition of sorafenib to TACE compared to TACE alone (Figure 2A and B) in patients with advanced or intermediate unresectable HCC and good liver function is under clinical investigation.

In regard to sequential timing of anti-angiogenic and loco-regional therapy, Strebel et al[25] proposed three different schedules: sequential, interrupted, continuous of combining TACE with sorafenib. In the first approach sorafenib was administered after chemoembolization. In a second model, sorafenib was given throughout, and it
Table 1  Clinical studies that analyzed targeted therapy in combination with transarterial chemoembolization

| Author, Ref. or No. of clinical trials | Phase | Drug | Patients (n) | Type of TACE | Timing | Primary endpoint |
|---------------------------------------|-------|------|--------------|--------------|--------|-----------------|
| Dufour et al[^5^](#)                  | I     | Sorafenib (from 400 to 800 mg/d) | 14            | Classical (doxorubicin, mytomycin-C) | 7 d before TACE and continuously | Safety |
| NCT01042041                           | I     | Sorafenib (800 mg/d)             | 18 TR         | Classical (cisplatin, doxorubicin, and mitomycin-C) | 2 wk before TACE and continuously | Dose adjustment |
| Pawlik et al[^3^](#)                  | II    | Sorafenib (800 mg/d)             | 35            | DEB-TACE (doxorubicin) | 1 wk before TACE and continuously | Safety |
| Sieghart et al[^2^](#)                | Pilot study | Sorafenib (800 mg/d) | 15            | Classical (bilirubin-adjusted doxorubicin doses) | 2 wk before TACE and continuously | Safety |
| Chung et al[^2][^3]                   | II    | Sorafenib (800 mg/d)             | 165           | Classical (doxorubicin) | After TACE (interrupted schedule) | Safety |
| START study                           | II    | Sorafenib (800 mg/d)             | 50            | Classical (doxorubicin) | 3 d after TACE for up to 24 wk | Safety |
| Sansonno et al[^2][^3]                | RCT   | Sorafenib (800 mg/d)             | 62            | Classical (doxorubicin and mytomycin C) | 30 d after TACE (sequential schedule) | TTP |
| ECOG 1208 trial NCT01004978           | II    | Sorafenib (800 mg/d)             | 40 TR         | Classical (doxorubicin) | 1 wk after TACE (sequential schedule) | TTP |
| Kudo et al[^2][^3]                    | III   | Sorafenib (800 mg/d)             | 458           | Classical (epirubicin, cisplatin, doxorubicin, mitomycin-C) | After TACE (sequential schedule) | TTP |
| Lencioni et al[^5]                    | II    | Sorafenib (800 mg/d)             | 307           | DEB-TACE (doxorubicin) | 3-7 d before TACE and continuously | TTP |
| SPACE trial                           | II    | Sorafenib (from 400 to 800 mg/d) | TR not specified | Classical (drugs not specified) | Before TACE (interrupted schedule) | TTUP |
| TATICS trial NCT01217034              | II    | Sorafenib (800 mg/d)             | TR not specified | Classic (doxorubicin, mitomycin-C, cisplatin) or DEB-TACE (doxorubicin) | 2 wk before TACE (interrupted schedule) | PF5 |
| Meyer et al[^2][^3]                   | III   | Sorafenib (800 mg/d)             | 412 TR        | DEB-TACE with doxorubicin | Together with TACE and continuously | PF5 |
| Hoffmann et al[^2][^3]                | III   | Sorafenib (800 mg/d)             | 208 (waiting LT) | Classical (carboplatin) | Together with TACE and continuously | TTP |
| Britten et al[^2][^3]                 | Pilot trial | Bevacizumab (10 mg/kg) | 23            | DEB-TACE (doxorubicin, cisplatin, mitomycin-C) | 1 wk before TACE beyond week 16 | Neovessel by angiography |
| AVATACE-1 NCT00280007                 | II    | Bevacizumab (5 mg/kg in every 14 d) | 32            | Classical (drugs not specified) | After TACE for 52 wk | Effectiveness |
| NCT00049322                           | II    | Bevacizumab (10 mg/kg every 14 d) | 31            | Classical (doxorubicin, cisplatin, mitomycin-C) | Before TACE continuously | Neovessel formation by angiography |
| NCT0035829                            | II    | Bevacizumab (dose not specified) | 26            | Classical (drugs not specified) | Before TACE in weeks 1, 3, 5 (up to a maximum of 5 courses) | Median PF5 |
| NCT00318557                           | II    | Recombinant human endostatin     | 60 TR         | Classical (epirubicin) | During TACE (via hepatic artery) | Safety, tolerability, mortality |
| Hao et al[^6]                         | RCT   | Thalidomide (200 mg/d)           | 108           | Classical (gemcitabine, oxaliplatin, flouxuridine) | Before TACE and continuously for 3-6 mo | Feasibility, potential activity of thalidomide |
| NCT000906016                          | II    | Thalidomide (dose not specified) | 75 TR         | Classical (doxorubicin) | Before TACE (interrupted schedule) | OS |
| NCT00921531                           | II    | Thalidomide (from 200 mg/d to 400 mg/d) | 200 TR       | Classical (5-fluorouracil, oxaliplatin, mitomycin-C) | After TACE continuously | OS |
| NCT01009801                           | I - II| Everolimus (10 mg/d)             | 98 TR         | DEB-TACE (doxorubicin) | Before TACE for up to 12 mo | Dose-limiting toxicity |
| TRACER study NCT01379521              | II    | Everolimus (dose not specified)  | 80            | Classical (drugs not specified) | Together with TACE continuously | PF5 |
| SATURNE trial NCT01164202             | II - III | Sunitinib (50 mg/d on days 1-28 before TACE) | 190 TR     | Classical (drugs not specified) | 7-10 d before TACE and after TACE (every 6 wk for 1 year) | Unacceptable bleeding or hepatic failure, OS |

[^5]: Targeted therapy and loco-regional treatments in hepatocarcinoma
was interrupted only during TACE\textsuperscript{[22]} in the third model, sorafenib, started before TACE, was administered continuously\textsuperscript{[28]}.

Several phase II/III studies used a continuous schedule, according to Dufour phase I study, that confirmed sorafenib anti-angiogenic action, due to significant decrease in VEGF-plasma concentration levels in HCC patients, against a good tolerability (only 4/14 patients have interrupted treatment for toxicity\textsuperscript{[26]}).

Also, a phase II trial showed a good safe of sorafenib started before doxorubicin DEB-TACE from 1 to 5 cycles- with a median of 71 d of therapy with a disease control rate (DCR) of 95\% [according to Response Evaluation Criteria in Solid Tumors (RECIST)] with an objective response of 58\% [according to European Association for the Study of the Liver -EASL]\textsuperscript{[27]}. Conversely, a pilot study evaluating safety of TACE (with bilirubin-adjusted doxorubicin doses) plus sorafenib, administered according to continuous schedule, was terminated prematurely for toxicity evidence, such as dermatologic toxicity (47\%), and diarrhoea (47\%)\textsuperscript{[28]}.

The interim analysis of phase II START study showed in Asian patients treated with TACE (TACE cycles were repeated every 6-8 wk on demand, up to a maximum of 6 TACE cycles) plus sorafenib (400 mg bid on day 4 to day 7 after the first TACE and stopped 4 d before each next TACE), as an interrupted schedule, a DCR of 91.2\%, an overall response rate of 52.4\%, a median progression free survival (PFS) and a time to progression (TTP) of 9 mo, with an OS probability upper 90\% at 18 mo\textsuperscript{[13]}. The side effects (mainly gastrointestinal and dermatologic) were mild or moderate\textsuperscript{[13]}.

In a Korean phase II trial patients treated with sorafenib, as interrupted schedule, presented an overall median TTP of 7 mo\textsuperscript{[30]}. The 6-mo PFS rate, based on RECIST criteria, was 52\% and the median OS was 20.8 mo. Nevertheless the use of interrupted approach, it was required sorafenib dose reduction in 70\% of patients because of toxicity\textsuperscript{[30]}.

In an European study, in which sorafenib was administered 30 d after TACE in HCC patients with chronic hepatitis C virus infection, the TTP was 9 mo, without unexpected side effects ($P < 0.001$), in fact a similar number of patients (belonging 25\% to sorafenib and 23\% to placebo group) were withdrawn from the trial because of toxicity\textsuperscript{[31]}.

Conversely, a phase III Asian trial concluded that sorafenib, given in patients who responded to TACE, did not significantly improve median TTP (sorafenib plus TACE: 5.4 mo vs TACE: 3.7 mo), because a long time interval elapsed before giving sorafenib after TACE. In fact, 60\% of patients had a treatment lag upper 9 wk prior to randomization\textsuperscript{[32]}.

Actually, four ongoing randomized trials (SPACE, ECOG1028, TACE2 and TACTICS) are addressing the mode and timing to add sorafenib to TACE or DEB-TACE with a continuous dosing of sorafenib, as in the SPACE or TACE2 studies\textsuperscript{[33,34]} vs interrupted dosing in ECOG 1208 or TACTICS trials (ClinicalTrials.gov Identifier: NCT01217034; NCT01004978).

### Bevacizumab

Bevacizumab is a humanized monoclonal anti-VEGF-A antibody approved by the United States FDA for several metastatic tumors, such as colon cancer, non-small cell lung cancer, renal cancer, glioblastoma multiforme, and approved by the European Medicines Agency (EMA) for breast cancer\textsuperscript{[15]}

Based on preclinical evidences that demonstrated in HCC models a strong neo-angiogenesis inhibition after bevacizumab\textsuperscript{[37]} the addition of bevacizumab to TACE compared to TACE alone in patients with unresectable HCC and good liver function is under clinical investigation.

A pilot study showed in patients treated with bevacizumab (administered intravenous according to continuous schedule) plus TACE a statistically significant improvement in PFS at 16 wk [0.19 in TACE group vs 0.79 in combination arm ($P = 0.021$)]\textsuperscript{[37]}. Overall, bevacizumab was well tolerated, not showing an increased risk of bleeding. In fact, only 3 patients, who received bevacizumab, had grade 2/3 treatment-related gastrointestinal bleeding, which was not life threatening\textsuperscript{[37]}.

In conclusion, the Authors claimed that the addition of bevacizumab to TACE was safe and feasible in selected HCC patients and that this combination was able reduce neo-vessel formation (as showed by angiography), in particular, at week 14\textsuperscript{[37]}.

A phase II AVATACE-1 trial will evaluate tumor progression in patients treated with bevacizumab (after one year treatment) plus TACE measured by liver magnetic resonance imaging (MRI) and positron emission tomography-scanning and several angiogenesis markers (circulating endothelial progenitors, pro-angiogenic hematopoietic cells, HGF levels) (ClinicalTrials.gov Identifier: NCT00280007).
Endostatin

The precise mechanisms of antitumor activity have not yet been fully clarified. It’s known that endostatins had been utilized to modify 12% of the human genome to down regulate pathological angiogenesis and inhibit 65 tumor types in animal models, including HCC.

According to preclinical evidences that showed anti-angiogenic activity of endostar (a recombinant human endostatin expressed and purified in Escherichia coli with an additional nine-amino acid sequence and forming another his-tag structure) blocking VEGF-induced tyrosine phosphorylation of KDR/FLK-1 in endothelial cells, and a tumor reduction (P < 0.01) 2 wk after treatment with both TACE plus endostar (arterially administered at dose of 0.25 mg/kg) in HCC models, the addition of endostar to TACE in patients with unresectable HCC and good liver function is under investigation.

Two clinical studies are considering safety of endostatin plus TACE and endostatin haematic level as biomarker of HCC recurrence (ClinicalTrials.gov Identifiers: NCT00834028; NCT00518557).

Thalidomide

Thalidomide is an inhibitor of angiogenesis induced by fibroblast growth factor immunomodulatory and a drug able to potentiate the cytotoxic activity of immune system by several mechanisms: (1) inhibition of the production of interleukin-6 (IL-6); (2) activation of caspase 8; (3) induction of c-JUN terminal kinase (JNK)-dependent release of cytochrome-c and Smac; (4) direct activation of T cells to produce IL-2; and (5) increasing the activity of NK-dependent.

United States FDA approved thalidomide in 2006 in combination with dexamethasone and in 2007 EMA approved this drug in combination with melphalan plus prednisone for the treatment of multiple myeloma.

In advanced HCC two Asian studies demonstrated that thalidomide (from 200 mg/d to maximum tolerated dose) had good safety, but only modest response.

Hao et al assessing the association of thalidomide to TACE vs TACE alone in HCC patients, showed a median OS of 28 mo (95%CI: 12-24) in the thalidomide arm and of 13 mo (95%CI: 10-16) in the control group (P < 0.05), with an OS at 2-year of 51.0% and 24.6%, respectively. Moreover, adverse events in patients taking thalidomide were mild and infrequent.

Actually, two phases II and III trials are evaluating the effectiveness of thalidomide plus TACE as adjuvant or neoadjuvant therapy with an interrupted schedule.
Evetolimus

Evetolimus (RAD-001) is an oral derivative of sirolimus that inhibits mTOR. Everolimus was approved by United States FDA in several tumors: advanced kidney cancer, subependymal giant cell astrocytoma associated with tuberous sclerosis, advanced pancreatic neuroendocrine tumors not surgically removable, and metastatic breast cancer (hormone-receptor positive, HER2-negative type) in combination with exemestane [48].

A randomized phase I-II trial is investigating on the dose limiting toxicity of everolimus given in combination to DEB-TACE (ClinicalTrials.gov Identifier: NCT01009801). The phase II multicentric TRACER trial will evaluate TTP of everolimus in patients with unresectable HCC (ClinicalTrials.gov Identifier: NCT01379521).

Sunitinib

Sunitinib is an oral multitargeted receptor TKI, approved by the United States FDA for the treatment of metastatic renal cell carcinoma, imatinib-resistant gastrointestinal stromal tumor [47] and unresectable, locally advanced or metastatic pancreatic neuroendocrine tumors [46].

A phase II-III SATURNE trial will assess safety (bleeding or hepatic failure at 10 wk post-treatment) of sunitinib (at interrupted schedule before and after TACE) in combination with TACE (ClinicalTrials.gov Identifier: NCT01164202) in about 200 patients with unresectable HCC.

A phase II study will evaluate the RR and PFS at 4 mo of patients treated with sunitinib (at interrupted schedule before and after TACE) plus TACE (ClinicalTrials.gov Identifier: NCT00524316).

RFA WITH MOLECULAR TARGETING AGENTS

Clinical studies that analyzed targeted therapy in combination with RFA are summarized in Table 2.

Sorafenib

Patients with small HCC candidate to RFA have an OS at 5 year of 50%-70% [41] (Figure 2C and D).

Considering preclinical data that demonstrated after sorafenib plus RFA a reduced neovascularisation [49], an increased time to recurrence (TTR) (inhibiting HIF-1α and VEGF-A expression) and an inhibited proliferation in HCC models [49], the combination of sorafenib to RFA in patients with unresectable HCC and good liver function is under investigation.

All clinical studies achieving the efficacy of sorafenib addition to RFA are ongoing.

A phase II trial will evaluate the anti-angiogenic properties of sorafenib in limiting tumor blood flow measured through a novel MRI technique (ClinicalTrials.gov Identifier: NCT00813293).

The phase II SORAMIC trial is considering the benefit of sorafenib addition to RFA in prolonging TTR compared to RFA plus placebo in patients who were candidates for RFA (local ablation group) (ClinicalTrials.gov Identifier: NCT01126645). However, the study will evaluate the benefit of adding radioembolization with yttrium-90 microspheres (SIRT) to sorafenib in comparison to sorafenib alone, in those patients in which RFA is not appropriate and who are not candidate for TACE (palliative group) (ClinicalTrials.gov Identifier: NCT01126645). This study will confirm the non-inferiority or superiority of Gadobitic acid-based MRI contrast agent (Primovist®-enhanced MRI) compared with contrast-enhanced computer tomography in stratifying patients to a palliative or local ablation treatment strategy (ClinicalTrials.gov Identifier: NCT01126645).

The phase III STORM trial will assess efficacy in recurrence free survival of sorafenib after surgical resection or local ablation of HCC, as adjuvant therapy within 4 mo from potentially curative treatment (ClinicalTrials.gov Identifier: NCT00692770).

Bevacizumab

There are only two preclinical studies showing the synergistic interaction between bevacizumab and RFA to reduce pro-angiogenic effect and HCC progression, that occur after insufficient local ablation [43].

The most significant study demonstrated whether hyperthermia could induce sublines of a human hepatoma cell line (HepG2 cells) with rapid proliferation and enhanced pro-angiogenic effect through a HIF-1α/VEGF-A dependent mechanism [50]. A subline of HepG2 cells, selected for higher viability and significant heat tolerance after 47°C heat treatment, showed 18% increase in viability and enhanced pro-angiogenic effect compared with parental HepG2 cells in which it was observed an up-regulated cellular protein levels of VEGF-A, HIF-1α and P-AKT, VEGF-A mRNA and secreted VEGF-A [50]. Bevacizumab, inhibiting VEGF-A effect, reduced the difference in pro-angiogenic effect between HepG2 k and parental HepG2 [51]. Moreover, the Authors supposed that higher viability subline, hyperthermia-induced, exerted its stronger pro-angiogenic effect through overexpressed PI3K/AKT/HIF-1α/VEGF-A signaling pathway [50].

However, additional preclinical studies are required to confirm the involvement of PI3K/AKT/HIF-1α/VEGF-A pathway in the mechanism of tumor progression and more clinical studies demonstrating the efficacy of bevacizumab to prevent tumor recurrence after RFA.

Thalidomide

Actually, there is only one phase II-III trial ongoing with aims to evaluate PFS and morbidity of low-dose thalidomide after RFA for unresectable HCC (ClinicalTrials.gov Identifier: NCT00728078).

NEW AGENTS IN DEVELOPMENT

4-[3,5-bis (trimethylsilyl) benzamide] benzoic acid (TAC-101)
is an oral synthetic retinoid with a specific binding activity on retinoid acid receptor-α[15]. This interaction induces the inhibition of activated protein-1, a transcription factor, which normally activates the expression of metastasis-related genes, including urokinase-type plasminogen activator, matrix metalloproteinase-9, and VEGF[16]. Preclinical models have shown that TAC-101 (at dose established of 20 mg/d) have an antitumor activity in HCC. A recent Japanese phase I study on TAC-101, observed a disease control rate of 38.5% in patients with advanced HCC[17]. Despite of an acceptable toxicity profile, the most frequent side effects were fatigue, headache, and skin toxicity[18].

Actually, a phase II study will evaluate the time to appearance of new lesions in patients with advanced HCC treated with TAC-101 (at dose 20 mg/d) plus TACE (ClinicalTrials.gov Identifier: NCT00667628).

Brivanib (BMS-582664) is an oral drug with antiangiogenic activity, inhibiting VEGFR-1-2-3 and fibroblast growth factor receptors (FGFR1-2-3). In a subanalysis performed to evaluate the effects of brivanib (at dose of 800 mg/d), as first-line therapy between Asian and non-Asian patients, the median OS was 10.6 mo in Asian patients and 5.7 mo in non-Asian patients. In contrast, in patients receiving brivanib as second-line therapy, the median OS was comparable between Asian and non-Asian patients (9.8 mo vs 9.4 mo, respectively)[19]. A phase III BRISK TA trial will evaluate the benefits of brivanib (at dose 200 mg/d), as adjuvant therapy, and TACE association in patients with unresectable HCC (ClinicalTrials.gov Identifier: NCT00908752).

Axitinib is an oral multitargeted receptor TKI, binding VEGFR1-2-3, PDGFR, and c-KITR. Axitinib was approved by FDA in January 2012 for pretreated patients with advanced renal cell carcinoma[20]. A phase II Chinese study will achieve two-year survival rate of patients with unresectable HCC, treated with axitinib (5 mg/d for 6 cycle) plus TACE, followed by axitinib alone (ClinicalTrials.gov Identifier: NCT01352728).

Orantinib (TSU-68) is an oral multitargeted receptor TKI, binding VEGFR-2, PDGFR, FGFR and c-KITR[21]. The Asian phase III ORIENTAL trial will assess OS in patients with unresectable HCC, treated with orantinib (200 mg/d bid) in combination with TACE (ClinicalTrials.gov Identifier: NCT01465464).

**DISCUSSION**

The combination of loco-regional treatments of HCC plus molecular targeting agents is a recent approach, and a number of questions still remain open: (1) the best sequential timing of targeted therapy and loco-regional therapy; (2) the number of TACE cycles to be performed; (3) the best criteria to evaluate the clinical outcome; (4) the best imaging technique to evaluate response; (5) the best targeted drug to use in combination with loco-regional treatment; and (6) the most correct primary endpoint of the studies.

With regard to the first point, there are three potential schedules: sequential, interrupted or continuous[22]. According to few actual evidences, we don’t really know which is the optimal schedule to administer sorafenib in addition to TACE or RFA.

Concerning the second point, there is not a magic number of TACE cycles, in fact TACE is to be repeated as many times as requested. However, it is also true, that after 5 to 6 treatments, the tumor progression usually does not allow further loco-regional approaches, and a systemic treatment alone should be preferred.

About the third point, the only agreement in literature is that RECIST criteria are inadequate[23,24], but, if these criteria should be replaced by EASL, CHOI or MASS criteria is still a matter of debate[25-27]. For what concern tumor necrosis induction, we think that its incorporation in evaluated criteria needs to await a prospective validation like the one that is part of the CALGB 80802 randomized phase III trial evaluating sorafenib plus chemotherapy with doxorubicin vs sorafenib alone (ClinicalTrials.gov Identifier: NCT01015833).

Considering fourth point, all studies considered, as imaging technique to evaluate response classical MRI and/or contrast-enhanced CT, and only few studies now are investigating on the best imaging technique to assess clinical outcome (SORAMIC, AVATACE-1).

With regard to fifth point, there are more clinical studies that assessed safety and TTP given by the
addition of sorafenib to TACE. Globally, these studies obtained an increased TTP of 7.9 mo in favour to sorafenib adjunct. Considering thalidomide, although the exciting results reported by Asian studies, the perspective of this drug in the combined HCC approach may be limited because of its low activity as single agent. Nevertheless, based on the favourable safety data, some Authors still think rationale to consider thalidomide in combination with loco-regional therapy, in order to increase outcome of HCC patients. The same considerations of thalidomide apply to bevacinumab. In fact, although this drug in combination with TACE was able to reduce VEGF serum levels, the disappointing results in terms of survival, raise some concern about the future of this anti-angiogenic drug. The sixth controversial point is the definition of efficacy. In that regard, the primary endpoints of the ECOG 1208 (ClinicalTrials.gov Identifier: NCT01004978) and SPACE studies are different although both studies try to evaluate efficacy in HCC. The SPACE study has TTP as the primary endpoint and defines failure of therapy as an inability to achieve objective response after more than two TACE procedures in the treated tumor nodule. ECOG 1208 in contrast, using DFS as the primary endpoint. It could be that the failure rate is defined as the occurrence of the liver and not just in the ablated lesion makes an evaluation more adherent to reality and patient overall health status. As a consequence of this disagreement, the optimal endpoint of the studies, actually, still remains unknown.

CONCLUSION

In early or intermediate stages, traditionally treated with RFA or TACE, the contribution of a systemic therapy may have the effect of reducing necroangiogenesis, thus prolonging time to recurrence and, possibly, survival.

It is our opinion that there should not be a long time elapsing between TACE and start of anti-angiogenic therapy, given as “adjuvant”, to promote the synergistic interaction between local and systemic treatment, in order to prevent early rebound of VEGF, and, consequently, HCC relapse.

A field of research that should be further developed is related to haemato-pro-angiogenic factors (such as VEGF) monitoring (before and after loco-regional therapy), which may predict the usefulness of the addition of targeted therapy in HCC patients.

In conclusion, the complexity of HCC and its different therapeutic strategies will require continued adjustments based on even more strict multidisciplinary approach and mainly on better knowledge of the biology of this disease.

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