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TOPIC HIGHLIGHT

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Is human hepatocellular carcinoma a hormone-responsive tumor?

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Abstract

Before the positive results recently obtained with multitarget tyrosine kinase inhibitor sorafenib, there was no standard systemic treatment for patients with advanced hepatocellular carcinoma (HCC). Sex hormones receptors are expressed in a significant proportion of HCC samples. Following preclinical and epidemiological studies supporting a relationship between sex hormones and HCC tumorigenesis, several randomized controlled trials (RCTs) tested the efficacy of the anti-estrogen tamoxifen as systemic treatment. Largest among these trials showed no survival advantage from the administration of tamoxifen, and the recent Cochrane systematic review produced a completely negative result. This questions the relevance of estrogen receptor-mediated pathways in HCC. However, a possible explanation for these disappointing results is the lack of proper patients selection according to sex hormones receptors expression, but unfortunately the interaction between this expression and efficacy of tamoxifen has not been studied adequately. It has been also proposed that negative results might be explained if tamoxifen acts in HCC *via* an estrogen receptor-independent pathway, that requires higher doses than those usually administered, but an Asian RCT conducted to assess dose-response effect was completely negative. Interesting, preliminary results have been obtained when hormonal treatment (tamoxifen or megestrol) has been selected according to the presence of wild-type or variant estrogen receptors respectively, but no large RCTs are available to support this strategy. Negative results have been obtained also with anti-androgen therapy. In conclusion, there is no robust evidence to consider HCC a hormone-responsive tumor. Hormonal treatments should not be part of the current management of HCC.

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Key words: Hepatocellular carcinoma; Sex hormones; Hormonal treatment; Tamoxifen

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide in terms of incidence (626 000 new cases per year, representing 5.7% of new cancer cases). Due to its very poor prognosis, the number of deaths is almost the same (598 000), representing the third most common cause of death from cancer[1]. The overall sex ratio (male:female) is around 2,4. HCC causes more deaths in men (416 882 deaths in 2002, ranking third cause of cancer death) than in women (181 439 deaths, ranking sixth)[1].

Treatment options and prognosis of patients diagnosed with HCC largely depend not only on tumor characteristics, but also on the severity of the underlying chronic hepatic

disease, which affects most of the patients[2]. Prognosis is relatively better for the subset of patients eligible for surgical treatments (tumor resection, liver transplantation) or other loco-regional treatments with potentially curative aim (e.g., percutaneous ethanol injection, radiofrequency ablation). A worse outcome is expected in those patients who can be treated only with palliative loco-regional treatments (e.g., transarterial chemo-embolization) or who are not suitable for any of the above options.

Recently, sorafenib, a multi-target tyrosine kinase inhibitor targeting both the tumor cell and the tumor vasculature, has shown significant efficacy in the treatment of advanced HCC[3]. Before the publication of these encouraging results, there was no systemic treatment that could be considered standard for advanced HCC[2,4]. Cytotoxic drugs does not play a significant role in these patients: HCC cells show intrinsic resistance to chemotherapy, and treatment with these drugs is often associated with unacceptable toxicity, due to the often compromised liver function.

Normal human liver is morphologically and functionally modulated by sex hormones. Epidemiological studies in humans suggest that long-term use of oral contraceptives and anabolic androgenic steroids can induce both benign (hemangioma, adenoma, and focal nodular hyperplasia) and malignant (HCC) hepatocellular tumors[5]. Animal models of experimental liver carcinogenesis suggest a relationship between exposition to sex hormones and development of HCC, with some evidence that these hormones may play a relevant role as inducer and promoter in the process of liver carcinogenesis[6]. In typical animal models of hepatic tumor initiation and promotion following repeated estrogen stimulation, the estrogen induction of microsomally activated catechols by aryl hydrocarbon hydroxylase and estrogen 2-/4-hydroxylase causes excess free radicals and unrepaired DNA adducts and strand breaks, that produce a mutagenic and irreversible DNA damage. Several months after this tumor-initiating DNA damage, steroid receptors - estrogen receptor (ER), progesterone receptor (PgR), androgen receptor (AR) - increase well above normal levels, suggesting the relevance of sex hormones mediated pathways in cell growth and proliferation.

In the last decades, moving from this epidemiological and preclinical evidence, several clinical trials have tested the efficacy of hormonal treatment in patients with HCC. In this review, we summarize the evidence about the use of hormonal treatment in HCC, trying to understand if, in the era of target-oriented therapies, this disease can still be considered a potentially hormone-responsive tumor.

HORMONAL TREATMENT SHOULD BE STUDIED AS A TARGET-ORIENTED APPROACH

In addition to oncogenes, tumor-suppressor genes, and other genetic factors, a number of growth factors involved in cell signaling pathways have been shown to play a role in liver carcinogenesis[7,8]. Angiogenic stimuli are also required for the growth of HCC, which is usually a hypervascular tumor.

Sex steroids are known to be able to stimulate cell growth directly in several cancer types. When the cellular mechanisms underlying autonomous tissue growth are linked to the growth-promoting effects of sex steroids, the clinical result is a *hormone-responsive* (also called *hormone-sensitive*) tumor. Hormonal treatment plays an established role in several solid tumors, like breast cancer and endometrial cancer in women and prostate cancer in men. As a matter of fact, hormonal treatment represents the first form of target-oriented cancer therapy, acting by disruption of growth factor (in this case, sex hormones) - receptor interactions. For example, binding of estrogen to ER induces activation of the receptor. In fact, ER, that resides in the cytosol, upon occupation by estradiol, dissociates from heat shock proteins and undergoes conformational changes, dimerization and phosphorylation[9]. The activated ER is transported to the nucleus, where it binds to estrogen response elements that are located upstream of estrogen-regulated genes, including those encoding molecules involved in cell proliferation.

The potential role of a target-oriented approach in the treatment of a human tumor can be adequately evaluated only if three relevant points are properly considered: (1) the molecular epidemiology of the target in the proposed study population; (2) the role of the target in patho-physiology of the tumor; (3) the effectiveness of the available target-oriented drug on target inhibition. If we consider hormonal treatment of HCC as a target-oriented treatment, the three relevant points to be taken into account are: (1) presence of sex hormones receptors expression in HCC cells; (2) relevance of stimulation by sex hormones in human HCC proliferation; (3) effectiveness of available drugs on inhibiting hormonal receptors activation.

As for the last point, there is no doubt that very effective drugs are available. In the last decades, the anti-estrogen tamoxifen has been a mile-stone treatment for patients with hormone-sensitive breast cancer. Tamoxifen inhibits the growth of tumor cells by competitive antagonism of estrogen at its receptor site, and levels of estrogen receptor expression are the best predictor of benefit from tamoxifen[10]. The real problem is that tamoxifen has been tested in patients with HCC, based on suggestive preclinical and epidemiological evidence, without an adequate evaluation of the first two points, that are even more important than the third one to enable the success of a target-oriented strategy: the target expression in HCC cells and the relevance of the sex hormones mediated pathways in HCC cell growth and proliferation mechanisms.

Expression of sex hormones receptors (ER, PgR and AR) - can be detected in a variable proportion of HCC[11-19]. Table 1 shows the proportion of ER+, PgR+ and AR+ HCC in the studies analysing the expression of these receptors by different techniques, mostly enzyme immunoassay or immunohistochemistry. In the recent study by Vizoso *et al*, sex hormones receptors expression was determined in 31 HCC patients by immunohistochemistry using tissue micro-arrays[19]. Their results demonstrate a wide variability in the immunohistochemical values for steroid receptors among HCCs: 67.7% of tumors stained positively for AR, 51.6%

for ER and 83.8% for PgR, but, among the positive cases, immunostaining score values for each protein were largely variable.

As for ERs, normal liver expresses almost exclusively wild-type ERs derived from the full-length transcript of the gene. Actually, there are two different ERs, ER-alpha and ER-beta, that are produced by distinct genes. During progression of liver disease to HCC, variant forms of ERs have been demonstrated[20]. Peritumoral cirrhotic tissue of patients with hepatocellular carcinoma, especially males, expresses a variant form of ER with an exon 5 deletion. This variant lacks the hormone-binding domain of the receptor but, being intact in the DNA-binding domain, maintains constitutive transcriptional activity. In HCC, variant ER largely predominates and sometimes becomes the only form expressed[20]. The occurrence of variant ER alone is limited almost exclusively to males, and this suggests that it could be one of the molecular events that eventually lead to the preferential development of hepatocellular carcinoma in males. In addition, variant ER is more frequent in patients infected with the hepatitis B virus. The growth rate of HCC in patients with variant ER is also significantly higher than that in patients with tumors expressing wild type ER. These tumors with variant ER, that are a significant percentage of HCC, are characterized by a worse prognosis, with significantly shorter survival[21].

Table 1 Expression of estrogen receptors, progesterone receptors and androgen receptors in HCC

Study	Number of cases	Ethnicity	ER (%)	PgR (%)	AR (%)
Nagasue[11]	30	Japanese	40	NA	NA
Ohnishi[12]	8	Japanese	14	NA	50
Hamazaki[13]	22	Japanese	23	NA	19
Nagasue[14]	19	Japanese women	37	NA	37
Boix[15]	26	Western	15	0	54
Ng[16]	71	Chinese	24	14	NA
Jonas[17]	33	Western	39	18	NA
Liu[18]	66	Chinese	27	30	NA
Vizoso[19]	31	Western	52	84	68

ER: Estrogen receptors; PgR: Progesterone receptors; AR: Androgen receptors; NA: Not available.

EVIDENCE-BASED SUMMARY OF TAMOXIFEN EFFICACY IN HCC: RANDOMIZED CLINICAL TRIALS AND META-ANALYSES

Although only a limited percentage of HCC are ER+ and there is no robust evidence about the relevance of sex hormone-dependent pathways in HCC proliferation, in the last decades there has been great interest in the potential usefulness of tamoxifen for patients with HCC. Tamoxifen is characterized by a favourable tolerability profile, that, together with the easy oral administration, makes this drug an interesting candidate for treatment of solid tumors potentially responding to hormonal manipulation.

A number of randomized controlled trials have tested the efficacy of tamoxifen, with conflicting results (Table 2). Many of these studies were characterized by several methodological drawbacks, and by a really small sample size. Median survival in the control group was very variable, emphasizing the extreme variability in prognosis of patients with HCC.

With the aim of clarifying the benefit associated with tamoxifen and with other treatment strategies producing conflicting results in HCC, several systematic reviews have been conducted and published. Systematic reviews of health-care interventions are an attempt to collate information from all relevant studies and, if deemed appropriate, combine their results using meta-analysis. There have been four systematic reviews with meta-analysis of randomized clinical trials of tamoxifen in HCC[34-37].

The two earlier reviews[34,35] were conducted and published about ten years ago, when only small-randomized trials were available. Both reviews showed a marginal increase in overall survival with the use of tamoxifen in advanced HCC, suggesting that the preclinical rationale supporting the use of hormonal therapy in HCC patients could translate in to some clinical benefit.

The systematic review by Simonetti *et al* identified and considered seven trials: two trials[22,24] evaluated the addition of tamoxifen to chemotherapy, and the other five trials[23,25-27,29] were designed to compare tamoxifen versus no treatment or placebo. In these latter studies, pooled odds ratio of surviving at 1-year for patients receiving tamoxifen was 2.0, with 95% confidence intervals (CI) 1.1-3.6. This means a statistically significant result favouring tamoxifen. However, considering the limited quality of the evidence, the authors of the meta-analysis suggested a note of caution in considering these results definitive, calling for a large randomized controlled trial to definitely address the issue of the efficacy of tamoxifen in HCC.

Similarly, also the authors of the other review noted that further large, well-designed trials were needed to definitely answer this question, because controversy persisted about tamoxifen efficacy[35]. In fact, their meta-analysis, considering seven trials, showed a borderline survival benefit, but, in sensitivity analysis, the survival benefit of tamoxifen was no longer significant.

Two years before the publication of the first systematic review, in 1995, the Cancer of the Liver Italian Program (CLIP) Investigators started the CLIP-1 large randomized trial, with the aim of verifying whether earlier interesting but conflicting data on tamoxifen effect were confirmed in a larger study[31]. A pragmatic approach was chosen for the conduction of the trial. Eligibility criteria were broad, and all HCC patients with a life expectancy longer than 3 mo were eligible. Overall survival was the only endpoint of the intent-to-treat analysis, no placebo was used in the control arm and follow-up was conducted according to the usual clinical practice of participating centers. Patients assigned to the experimental arm received oral tamoxifen, 40 mg daily, until death or inability to assume the drug. Overall, 496 patients were randomized. Patients were predominantly males, with underlying viral cirrhosis. About half of them had a well compensated liver function. The results of the trial, published in 1998, showed no

Table 2 Randomized clinical trials testing the efficacy of tamoxifen in HCC

Study characteristics						Enclosed in meta-analysis			
Reference	Patients	Tamoxifen arm		Comparator		Simonetti	Mathurin	Llovet	Cochrane
		Treatment	Overall survival	Treatment	Overall survival				
Melia, 1987[22]	59	Adriamycin 60 mg/m^2 + tam 20 mg/d	Median: 6 wk	Adriamycin 60 mg/m^2	Median: 8 wk	X	X		X
Farinati, 1990[23]	38	Tam 30 mg/d	Median: 36 wk	No treatment	Median: 8 wk	X			
Uchino, 1993[24]	26	Cisplatin, adriamycin, 5-fluorouracil + Tam 25 mg/m^2 per d + MPA 400 mg/m^2 per d	1-yr: 44.5%	Cisplatin, adriamycin, 5-fluorouracil	1-yr: 33.0%	X	X		
Elba, 1994[25]	22	Tam 60 mg/d	Median: 74 wk	Placebo	Median: 52 wk	X	X	X	X
Martinez Cerezo, 1994[26]	36	Tam 20 mg/d	Median: 261 d	Symptomatic treatment	Median: 172 d	X	X	X	X
Castells, 1995[27]	120	Tam 20 mg/d	1-yr: 51%	Placebo	1-yr: 43%	X	X	X	X
Coll, 1995[28]	61	Tam 20 mg/d	1-yr: 24%	Placebo	1-yr: 25%		X		X
Manesis, 1995[29]	85	Tam 30 mg/d + triptorelin	Median: 282 d	Placebo	Median: 127 d	X	X	X	
				Flutamide 750 mg/d + triptorelin	Median: 112 d				
Riestra, 1998[30]	77	Tam 40 mg/d	1-yr: 30%	Placebo	1-yr: 37.8%			X	X
CLIP group, 1998[31]	496	Tam 40 mg/d	Median: 15 mo	No treatment	Median: 16 mo			X	X
Liu, 2000[18]	119	Tam 30 mg/d	Median: 44 d	Placebo	Median: 41 d			X	X
Chow, 2002[32]	329	Tam 60 mg/d Tam 120 mg/d	3 mo: 41% 3 mo: 35%	Placebo	3-mo: 44%				X
Barbare, 2005[33]	420	Tam 20 mg/d	Median: 4.8 mo	Symptomatic treatment	Median: 4.0 mo				

Tam: Tamoxifen; MPA: Medroxyprogesterone acetate.

overall survival advantage deriving from the administration of tamoxifen[31]. Median survival was 15 and 16 mo in the tamoxifen and the control arm, respectively. One-year survival probability was similar in the two arms, 55% and 56%, respectively. After adjustment for known prognostic factors, the relative hazard of death for patients receiving tamoxifen was equal to 1.07 (95% CI, 0.83-1.39). Considering that the sample size of the CLIP-1 trial was much higher than that of all the previous studies, it is not surprising that the results of this trial changed the results of the meta-analysis. The addition of the CLIP-1 data to the four previous trials considered in the review by Simonetti *et al* comparing tamoxifen alone versus no active treatment produced a pooled odds ratio of being alive at 1 year for patients receiving tamoxifen of 1.19 (95% CI, 0.88-1.61), and there was no more statistically significant advantage for tamoxifen.

After the publication of the CLIP-1 trial, two systematic reviews with meta-analysis have been published[36,37]. Both did not show any survival benefit for patients assigned to tamoxifen.

In the systematic review conducted by the Barcelona-Clinic Liver Cancer Group, and published in 2003, seven RCT were considered eligible for meta-analysis of tamoxifen effect[36]. Tamoxifen showed no survival benefit [odds ratio (OR), 0.64; 95% CI, 0.36-1.13, $P = 0.13$], and the authors noted that only the low-quality trials showed any benefits.

Similar results are described in the Cochrane meta-analysis[37] that considered nine randomized trials (one testing two doses of tamoxifen) for a total of 1709 patients. Tamoxifen *versus* placebo/no intervention had no significant effect on overall survival [hazard ratio (HR), 1.05; 95% CI, 0.94-1.16; $P = 0.4$], without statistical heterogeneity between the trials. Trials were classified according to the adequacy or inadequacy of three methodological components: generation of the allocation sequence, allocation concealment and blinding. Subgroup analysis showed that a trend in reduction of mortality with tamoxifen was limited to trials with one or less adequate/three methodological components (HR 0.82; 95% CI 0.60-1.12; $P = 0.2$), whilst tamoxifen showed no significant effect in trials with two adequate/three methodological components (HR, 1.00; 95% CI, 0.84 to 1.18; $P = 0.98$) and tended to increase mortality in trials with three adequate/three methodological components (HR, 1.15; 95% CI, 0.99-1.34; $P = 0.06$).

ARE THERE ANY SUBGROUPS OF PATIENTS WHO RECEIVE BENEFIT FROM TAMOXIFEN?

Almost ten years ago, Mathurin *et al* discussed the conflicting results obtained with tamoxifen in their systematic review, stating that one of the main objectives in the future should have been to identify, using clinical and biological factors, the subgroups of patients responding to tamoxifen[35].

Clinical factors

As for the identification of clinical subgroups, updated results of the CLIP-1 study, published in 2002, confirmed the original negative result obtained in the overall study population, both in the subgroup of advanced patients and in those eligible for potentially curative loco-

regional treatments[38]. In a more recent RCT, conducted in France, 420 patients with HCC and a prevalence of alcohol-related liver cirrhosis were randomized to receive tamoxifen or supportive care alone[33]. Despite the negative result in the overall population, following a post-hoc unplanned subgroup analysis, French authors suggested that tamoxifen might be effective in a population of Okuda stage Ⅰ or Ⅱ, i.e. those patients without major hepatic insufficiency, and that new trials on tamoxifen are still warranted, at least in this subset of patients. It should be noted that subgroup analyses carry a relevant risk of false positive results, and their results should be always considered with great caution. However, we tried to validate the hypothesis generated by the French trial using updated data from the CLIP-1 randomised trial, but tamoxifen still resulted not effective both in patients with Okuda stage Ⅰ-Ⅱ and in patients with Okuda stage Ⅲ disease or Okuda unknown[39]. We also tested the same hypothesis in subgroups defined according to the CLIP score. CLIP score is actually the most widely accepted and validated prognostic score for HCC[40-42], and it takes into account liver function measured by Child-Pugh category, portal vein thrombosis, and level of alpha-fetoprotein and tumor size. In the patients of the CLIP-1 study, results were negative again both in patients with good CLIP score (0-1) and in those with worse or unknown CLIP score[39].

Biological factors

Hormonal treatment can be considered the first form of target-oriented cancer treatment. Greater emphasis should be probably given, when planning a clinical trial and interpreting its results, to the great impact that the molecular heterogeneity of tumors, affecting sensitivity to the experimental treatment, may have on the results of the trial[43]. This concept has been seldom taken into account in the planning and the analysis of clinical trials with cytotoxic agents, but in our opinion it should be necessarily applied in clinical trials with molecular targeted agents and, similarly, with hormonal treatment. We can imagine the whole population of potentially eligible patients as divided in to two distinct groups: one characterized by sensitivity to hormonal treatment, that will potentially produce in these patients an outcome better than the control, and the other, on the contrary, characterized by insensitivity to hormonal manipulation, that will translate in the absence of difference in efficacy between hormonal treatment and control. The higher the proportion of the latter patients in the study sample, the lower the power of the clinical trial to show a potentially positive result.

In particular, even in the case of solid tumors that are definitely considered hormone-sensitive, or hormone-dependent, not all the patients will derive benefit from hormonal treatment. In breast cancer, the disparity in clinical response to tamoxifen between women with hormone receptor-positive disease and those with receptor-negative tumors clearly established the utility of hormone receptor status in identifying those likely and, equally important, those unlikely to benefit from endocrine therapy[10]. In fact, it is now well established that the efficacy of hormonal treatment is relevant, but it is limited to patients with tumors expressing hormonal receptors. This principle became the basis on which current clinical practice guidelines were established for the application of this treatment in breast cancer.

If we try to transfer these simple considerations to the hormonal treatment of HCC, it seems reasonable that a possible explanation of the negative results obtained with tamoxifen could stay in the lack of proper selection of the patients. In our opinion, it is really disappointing that none of the RCTs testing the efficacy of tamoxifen in HCC did select eligible patients according to hormonal receptors expression. The expression of these receptors is not so frequent in HCC, and the levels of expression in positive cases are largely variable. This might have diluted the positive effect of tamoxifen, potentially limited to a small subgroup of patients.

The only attempt of correlating target expression and efficacy of hormonal treatment in HCC patients comes from a secondary analysis of a Chinese randomized trial comparing tamoxifen versus no treatment for patients with advanced and otherwise untreatable HCC[18]. Immunohistochemical tests for ER and PgR were performed on the tumor tissues obtained from a subgroup of patients enrolled in the study. Disappointingly, in that series, the tumor expression of sex hormones receptors did not seem to affect the efficacy of tamoxifen[18]. However, it should be noted that, in that trial, (1) patients were not prospectively selected or stratified according to qualitative or quantitative hormonal receptors expression, (2) immunohistochemical determinations were performed only on a subgroup of 66 patients with adequate tissue specimen, out of 119 enrolled patients, and (3) the prognosis of the patients enrolled in that study was really dismal, with a median survival of 44 d *versus* 41 d, in the tamoxifen and control group, respectively. Adequately powered prospective phase Ⅲ trials assessing the efficacy of tamoxifen in patients selected or stratified (prospectively or retrospectively) for ER expression have never been conducted.

Another intriguing hypothesis about the possibility that tamoxifen could be effective only in a selected subgroup of patients with HCC is related to the presence of variant estrogen receptors (vER)[20]. Tamoxifen could not be effective in tumors with vER, because of its inability to bind the receptor, and this could contribute to justify tamoxifen lack of efficacy, considering that a relevant proportion of HCC patients have predominant vERs[44]. In a small experimental experience[45], anti-hormonal therapy of HCC was tailored according to the presence of wild-type or exon 5-deleted vER transcripts, limiting the administration of tamoxifen (at a daily dose of 80 mg) to patients with wild-type ER, and treating patients with vER with megestrol acetate, at the daily dose of 160 mg. Interestingly, tumor volume in all patients with wild-type ERs was halved after 9 mo of tamoxifen treatment, and the investigators concluded that choosing anti-hormonal treatment according to the presence of wild-type or variant ERs in the tumor definitely improves the response rate to tamoxifen.

Of course, in our opinion, these intriguing results are not sufficient to definitely claim the efficacy of tamoxifen in a selected subgroup of HCC patients. These preliminary

results still lack confirmation in adequately powered and designed randomized controlled trials, prospectively selecting patients with wild-type ER and randomizing them to receive tamoxifen or no treatment.

SUPPOSED MECHANISM OF ACTION OF TAMOXIFEN IN HCC: ER-DEPENDENT *VS* ER-INDEPENDENT PATHWAYS

Some years ago, it has been proposed that the positive results obtained in some of the early small trials with tamoxifen in HCC and the negative results of the majority of the other trials might be explained if activity of tamoxifen in HCC could be related to an ER-independent pathway[46]. Several mechanisms have been proposed by which tamoxifen could act on HCC cells independently from the expression of ER: the interaction of tamoxifen and 4-hydroxy-tamoxifen with membrane phospholipids, with decrease in cell membrane fluidity and inhibition of adenylate cyclase, the inhibition of Protein Kinase C activity, the inhibition of calmodulin-dependent cAMP phosphodiesterase and the increase in Transforming Growth Factor beta1 levels, that can be obtained also in ER- cells[46].

Interestingly, these mechanisms, potentially interfering with cellular pathways relevant to HCC proliferation, require much higher doses of tamoxifen than those used in most of the clinical trials conducted so far. In fact, tamoxifen is known to have therapeutic actions independent of ER status at higher doses (4-8 times higher than the dose established for ER-positive breast carcinoma)[46]. Thus, high-dose tamoxifen would potentially have therapeutic actions on both ER-positive and ER-negative HCC. Although correlation between sex hormone receptor expression and efficacy of tamoxifen in HCC has never been adequately studied, Tan *et al,* in 2001, called for a "paradigm shift" to dissociate the action of tamoxifen from the expression of ERs[46]. They suggested that future trials with tamoxifen in HCC should have used higher doses of tamoxifen, at least four to eight-fold that of the dose intended to be efficacious in an ER-dependent mechanism. Moving from this intriguing hypothesis, a double-blind RCT was conducted in the Asia-Pacific region with 329 HCC patients, comparing tamoxifen versus placebo[32]. Tamoxifen was given at two distinct doses (120 mg daily and 60 mg daily), in order to assess possible dose-response effect. Quite disappointingly, rather than indicating a dose-response effect in favor of tamoxifen, the analysis showed a significant detrimental effect for the higher dose of tamoxifen. Three-month survival rates were 44%, 41%, and 35%, respectively for the placebo, tamoxifen at 60 mg, and tamoxifen at 120 mg groups, with a statistically significant trend difference in survival across the 3 arms. There was a significantly higher risk of death in the tamoxifen 120 mg group compared with the placebo group (HR, 1.39; 95% CI, 1.07-1.81). The detrimental effect of tamoxifen seemed not to be related to a higher toxicity of the higher dose. The trial, indeed, was unable to identify significant toxicity due to tamoxifen, and the rate of reported treatment toxicity (3%) was extremely low, without significant differences among the arms; however, the Authors cautiously postulated that the general rapid decline of patients with inoperable HCC could make it difficult to identify treatment toxicities.

Although the mechanism by which higher doses of tamoxifen seems to have a negative impact on the prognosis of HCC patients is not completely clear, the unexpected findings of the Asian trial are confirmed by the results of the Cochrane meta-analysis[37]. In fact, with increasing dose of tamoxifen, there was an overall survival trend favouring the arm without tamoxifen. Namely, the HR for overall survival was lowest for trials of tamoxifen given at 20 mg daily (HR 0.88; 95% CI, 0.69-1.44; $P = 0.71$), higher in trials of tamoxifen given at 40 mg daily (HR 1.00; 95% CI 0.85-1.19; $P = 1.0$), even higher in trials of tamoxifen given at 60 mg daily (HR, 1.03; 95% CI, 0.81-1.31; $P = 0.8$), and highest in the single trial of tamoxifen given at 120 mg daily (HR, 1.29; 95% CI, 1.04-1.6; $P = 0.02$).

According to these results, we believe that, unfortunately, neither further trials are warranted with tamoxifen in HCC, nor any use in clinical practice should be considered because of its clear lack of efficacy.

EVIDENCE WITH OTHER HORMONAL TREATMENTS: MEGESTROL ACETATE AND ANTI-ANDROGENS

Megestrol acetate exerts its action on ER-pathways at post-receptorial level. Efficacy of megestrol acetate has been tested in HCC with vER, according to the hypothesis that in these tumors a progestin drug might work better than tamoxifen[45,47]. A small prospective, randomized study assigned patients with advanced HCC characterized by variant liver ER to receive megestrol or placebo[47]. Out of 133 patients diagnosed with HCC and screened for eligibility, 45 patients (33.3%) had variant ER transcripts demonstrated in the tumor and were enrolled in the study. Twenty-four patients were randomized to no treatment and 21 to megestrol at the daily dose of 160 mg. Median survival in untreated patients was 7 mo (95% CI, 3.01 mo -10.99 mo) versus 18 mo (95% CI, 13.47 mo-22.53 mo) in patients treated with megestrol ($P = 0.009$). According to the comment of the investigators, megestrol was associated with a remarkable increase in overall survival in patients with HCC characterized by variant ERs, who usually show a rapidly progressive disease, making a trial with this drug more than warranted. We believe that no firm conclusions on the effectiveness of megestrol acetate in that selected subgroup of HCC patients should be drawn, in the absence of adequately powered randomized trials. Such trials should select patients according to the presence of variant ER, randomizing patients to receive megestrol or no treatment.

Similarly to estrogens, there is some preclinical evidence supporting a positive influence of androgens on HCC growth, with a potential role of treatment with anti-androgens for patients with HCC. In tumor cells, androgen receptors seem to be present more frequently and in greater concentrations than estrogen receptors[48]. Furthermore, experimental studies have suggested a

promoter effect of androgens on tumor growth[49], which may be suppressed via anti-androgen treatment[50] or castration[51].

A randomized trial conducted in unresectable HCC by the European Organization for Research and Treatment of Cancer tested the efficacy of anti-androgen therapy[52]. The trial was conducted according to a factorial two-by-two design: patients were randomized to receive pure anti-androgen (nilutamide 300 mg daily for 1 mo, then 150 mg daily), luteinizing hormone-releasing hormone (LHRH) agonist (goserelin acetate at 3.6 mg or triptorelin at 3.75 mg administered monthly by subcutaneous injection), both treatments or control. Unfortunately, neither pure anti-androgen nor LHRH agonist showed significant efficacy in terms of survival. Another randomized phase Ⅲ trial, designed with the aim of assessing the effect of anti-androgens in patients with advanced HCC, was conducted by the French collaborative group GRETCH[53]. Male patients with advanced HCC were randomized into 2 arms. Patients assigned to the experimental arm received leuprorelin (3.75 mg/mo subcutaneously), flutamide (750 mg orally daily), and tamoxifen (30 mg orally daily). Patients assigned to control arm received tamoxifen alone, considered as a standard treatment at the time of study planning. Between February 1994 and January 1998, 376 male patients were included. Median survival time was 135.5 d and 176 d in combination and tamoxifen groups, respectively ($P = 0.21$). Adjusted relative risk of death in the treated group was estimated 1.08 (95% CI, 0.87-1.33). In conclusion, no benefit in survival was found with anti-androgenic treatment in male patients with advanced HCC.

MOVING TOWARD OTHER SYSTEMIC TREATMENTS

In the last decades, although supported by weak and conflicting results, use of tamoxifen in patients with advanced HCC has been probably encouraged by the absence of other active systemic options.

In recent years, with the development of new drugs targeting growth factor receptor pathways or other cellular pathways potentially relevant to tumor cell proliferation, research efforts have been focused on targeted therapies. Recently, sorafenib, that is a small molecule tyrosine kinase inhibitor acting against Raf kinase, VEGF, PDGFR-β, c-KIT and Flt has shown efficacy compared to placebo in a randomized controlled study conducted in 602 patients with advanced HCC, in the setting of Child-Pugh A cirrhosis[3]. A planned interim analysis concluded that the trial met its primary end point, demonstrating a statistically significant and clinically relevant better survival in the sorafenib arm, without striking difference in the incidence of serious adverse events. Most recently, a planned interim analysis found similar results favouring sorafenib in an Asia-Pacific regional Phase Ⅲ trial of patients with advanced HCC, enrolling 226 patients from sites in China, Korea and Taiwan. Based on these results, sorafenib has been the first drug approved for treatment of HCC, by both US Food and Drug Administration and European Medicines Agency.

On the contrary, disappointing results of clinical trials that have tested the efficacy of hormonal treatment in HCC raise serious doubts about the real relevance of sex hormones mediated pathways in the clinical course of HCC. Hormonal treatments should not be part of the current standard management of patients affected by hepatocellular carcinoma.

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