Randomized Trial of Leuprorelin and Flutamide in Male Patients With Hepatocellular Carcinoma Treated With Tamoxifen

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The growth of hepatocellular carcinoma (HCC) is thought to be dependent on androgens, as androgen receptors are present in most of these tumors. The aim of this multicenter trial was to assess the effect of antiandrogens in patients who have advanced HCC. Male patients with advanced HCC were randomized into 2 groups treated with (1) leuprorelin (3.75 mg/mo subcutaneously), flutamide (750 mg/d orally), and tamoxifen (30 mg/d orally) or (2) tamoxifen alone (30 mg/d orally) administered until death. Survival was the main end point (log-rank test). The required sample size was 375 patients (alpha, 5%; beta, 10%; 1-year survival, 45% in treated group and 30% in controls). Between February 1994 and January 1998, 376 male patients (mean age, 66 years; treated group, n = 192; control group, n = 184) were included. No baseline imbalance was found between the groups. At the reference date (January 1, 2003), 183 deaths (95.3%) were observed in the treated group and 177 deaths (96.2%) were observed in controls. Thirteen patients were lost to follow-up. Median survival time was estimated to be 135.5 days (95% CI, 112-189) and 176 days (95% CI, 141-227) in treated and control groups, respectively ($P = .21$). Crude and adjusted relative risks of death in the treated group were estimated at 1.14 (95% CI, 0.93-1.40) and 1.08 (95% CI, 0.87-1.33; $P = .48$) respectively. Premature interruption of treatment was more frequent in the treated group (n = 45) than in controls (n = 22; $P = .0045$), mainly because of digestive side effects. In conclusion, no benefit in survival was found with antiandrogenic treatment in male patients with advanced HCC. (HEPATOLOGY 2004;40:1361–1369.)

Treatment of patients who have symptomatic or advanced hepatocellular carcinoma (HCC) is still a challenge, because the mean survival is only a few months.[1] As curative treatments such as surgery or percutaneous ablation are excluded, arterial chemoembolization is the preferred palliative option; however, improved survival is only observed in a small percentage of patients with good liver function.[2] No medical treatment has been proven effective.[2]

The influence of sex hormones on the growth of HCC has been suspected for a long time.[3] On the basis of experimental studies and findings showing estrogen receptors in tumor cells, treatment with tamoxifen, an antiestrogenic drug effective in breast cancer,[4] has been tested in patients who have unresectable HCC.[5,6] The positive influence of androgens on HCC growth is also supported by other results. First, HCC mainly occurs in males (with a male/female ratio between 5 and 9[1]), with a poorer outcome in males than in females.[7] Second, occurrence of HCC has been reported in patients treated with androgens[8] or in bodybuilders.[9] Third, serum testosterone has been found to be a predictive factor of HCC occurrence in patients with hepatitis C virus and cirrhosis[10] and in hepatitis B virus carriers.[11] Fourth, androgen receptors have been found in normal livers and in livers with cirrhosis[12] as well as HCC.[13] In tumor cells, androgen receptors seem to be present more frequently and in

Abbreviations: HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein; CT, computed tomography.

The members of Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire and their respective affiliations are listed in the Appendix.

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greater concentrations than estrogen receptors.[14] Furthermore, experimental studies have suggested a promoter effect of androgens on tumor growth,[15] which may be suppressed via antiandrogen treatment[16] or castration.[17] Some clinical studies have also suggested that the presence of androgen receptors has a negative influence on survival or HCC recurrence after surgery,[18] although these results remain controversial.[19,20]

Several uncontrolled studies testing the effect of antiandrogens in HCC patients have failed to demonstrate a significant effect on tumor size of either peripheral antiandrogens (*e.g.*, flutamide,[21] ketoconazole,[22] and cyproterone acetate[23]) or agonists of the gonadostimulin-releasing hormone (*e.g.*, triptorelin[24] and buserelin[25]). However, these nonrandomized trials show neither a decrease nor a stabilization of tumor growth. The aim of our randomized trial was to assess the effect of antiandrogen treatment on survival in European male patients who have advanced HCC. We chose to only include male patients because the carcinogenetic influence of androgens could be different between males and females.[26] To obtain complete androgen blockade as recommended in prostate cancer (a tumor with high concentrations of androgen receptors), we used an association of leuprorelin, a luteinizing hormone–releasing hormone agonist, and flutamide, a nonsteroid peripheral antagonist that is supposed to be more effective than steroid antagonists.[27] Because two randomized trials suggested that tamoxifen might be effective at the time our trial began,[5,6] tamoxifen was administered in both groups to specifically test the antiandrogenic effect of leuprorelin, which also depresses estrogen secretion. However, further randomized studies including larger numbers of patients[2] showed no favorable (and no deleterious) effect on survival, which supports the use of tamoxifen as a placebo in our trial.

Patients and Methods

The study was a prospective multicenter, randomized, open-label trial. The protocol was approved by the Ethics Committee (CCPPRB) in Aulnay-sous-Bois (Seine-Saint-Denis, France). Informed written consent was obtained from each patient. The study was designed according to the CONSORT statement.[28]

Selection of Patients. Patients over 18 years of age were included consecutively in the study. Diagnosis of HCC was based either on histology or cytology or the association of cirrhosis, liver tumor, and serum alpha-fetoprotein (AFP) levels of 250 ng/mL or higher. Patients were not included when curative treatment (*e.g.*, surgery or percutaneous ablative treatment) was indicated, in case of: previous treatment of HCC; severe hepatic disease (defined by one of the following criteria: encephalopathy, clinical ascites, or serum bilirubin $\geq$ 50 μmol/L); serum creatinine level of 120 μmol/L or higher; contraindications to the administration of tamoxifen, flutamide, or leuprorelin; estimated life expectancy below 3 months; Karnofsky index of less than 30%; or refusal.

Randomization. Patient inclusion was determined after the patient had given written informed consent and if no contraindications were detected. Centralized randomization stratified by center was done via telephone. Patients were assigned via computer-generated allocation to one of two regimens: (1) in the treated group, leuprorelin (3.75 mg/mo subcutaneously), flutamide (750 mg/d orally), and tamoxifen (30 mg/d orally), and (2) in the control group, tamoxifen (30 mg/d orally). Drugs were administered from inclusion in the trial until death. To avoid subcutaneous injection of placebo of leuprorelin in the control group, we did not use a double-blind design.

Follow-up. The patients were observed every month. Serum AFP levels were checked and computed tomography (CT) scans were performed at inclusion and every 3 months until death. All CT examinations were reviewed by two radiologists who were unaware of the clinical data (D.M. and T.D.). Changes in tumor size were assessed by measuring the average diameter of the largest nodule and were expressed as the percentage of change compared with the diameter before randomization. Changes in serum AFP levels and liver function tests were expressed as the percentage of change compared with levels at inclusion.

Sample Size. Sample size was computed based on overall survival as the main end point. The 1-year survival was expected to be 30% in the control group. It was computed that the study had a power of 0.90 to detect—on the basis of a two-sided test—a minimum difference of 15% in 1-year survival of the experimental group (*i.e.*, at least a 45% 1-year survival) based on 375 patients.

Statistical Analysis. The primary criterion was survival; secondary criteria were tumor growth and tolerance. Statistical analysis was based on an intention-to-treat basis. Comparison of randomized groups at baseline and that of 3-month variations in tumor size, serum AFP levels, and liver function tests as measures of tumor growth were based on the nonparametric Wilcoxon test or the exact Fisher test.

Failure time data were analyzed using a reference date of January 1, 2003. Survival distribution from randomization was estimated using the Kaplan-Meier method in each group,[29] then compared between groups using the log-rank test.[30] The semiparametric Cox model was used to estimate the hazard ratio of death in patients in the treated group compared with the control group, either adjusted for baseline imbalance or prognostic factors, or

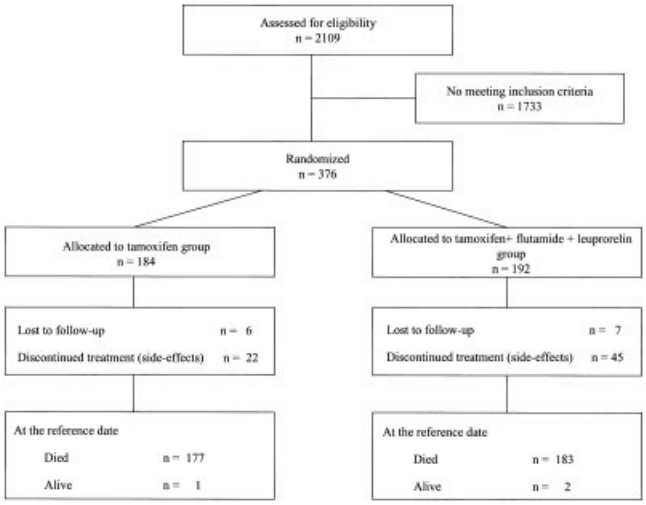

Fig. 1. Flow diagram of the patients throughout the trial according to CONSORT recommendations.

without adjustment.[31] Nonproportionality of treatment effect over time was checked using the Cox model allowing time-varying effects of randomized groups,[32] while interactions between treatment effect and baseline covariates were assessed using the Gail and Simon test.[33] *P* values were two-sided, with values of .05 or less indicating statistical significance. Analysis was performed using the SAS software package (SAS Inc., Cary, NC).

Results

Population. Thirty-nine centers participated in the trial. The inclusion period was from February 1, 1994, to January 31, 1998. During this time, 2,109 patients presented with HCC, and 1,733 patients (82%) were excluded because they did not fit inclusion criteria. Finally, 376 patients were randomized: 192 in the treated group and 184 in the control group as shown in the CONSORT flow chart[28] (Fig. 1).

Baseline Characteristics of Patients. A diagnosis of HCC was made via histology or cytology in 255 patients and through an association of cirrhosis, liver tumor with imaging features typical of HCC (*i.e.*, hypervascularization at early arterial phase on CT scan and/or magnetic resonance imaging), and serum AFP level higher than 250 ng/mL in 121 patients. Baseline characteristics were well-matched between the two groups (Table 1). Cirrhosis was proven via histology in 333 patients (89%): 217 (65.2%), 109 (32.7%), and 7 (2.1%) patients belonging to Child-Turcotte-Pugh classes A, B, and C, respectively (112, 50, and 2 in the treated group, respectively, and 105, 59, and 5 in the control group, respectively; $P = .34$). Patients were classified as stage I (46.3%), II (50.5%), or III (3.2%) of Okuda's classification (81, 107, and 4 in the treated group, respectively, and 93, 83, and 8 in the control group, respectively; $P = .08$), and as class A (22.9%), B (66.7%), or C (10.4%) of the GRETCH prognostic classification[34] (40, 132, and 20 in the treated group, respectively, and 46, 119, and 19 in the control group, respectively; $P = .62$).

Survival. At the reference date (December 31, 2002), 13 patients were lost to follow-up and 360 deaths (96%) were observed, 177 (96.2%) in the control group and 183 (95.3%) in the treated group (see Fig. 1). Median survival time was estimated at 176 days (95% CI, 141-227) in the control group and 135.5 days (95% CI, 112-189) in the treated group ($P = .21$ according to log-rank test). One-year survival was estimated at 28.3% (95% CI, 21.7-34.8) in the control group and 23.4% (95% CI, 17.4-29.4) in the treated group (Fig. 2). Crude hazard ratio of death was estimated at 1.14 (95% CI, 0.93-1.40) in the treated group compared with the control group; when it was adjusted for baseline prognostic indexes (namely Okuda, Child-Turcotte-Pugh, and GRETCH classes) it was estimated at 1.08 (95% C, 0.87-1.33; $P = .48$). As reported previously,[34] our prognostic index yielded the following prognostic information: group A, 80 deaths in 86 cases (median survival, 364 days); group B, 241 deaths in 251 cases (median survival, 138 days); group C, 39 deaths in 39 cases (median survival, 53 days). Introducing each group as a continuous covariate in a Cox model, this reached an estimated hazard ratio of death of 2.15 (95% CI, 1.75-2.64; $P = .0001$) (Fig. 3). Finally, no statistically significant treatment based on score interaction was found ($P = .08$), although survival was slightly increased in GRETCH class A patients compared with controls and the opposite tendency was observed in class B and C patients. Causes of death are reported in Table 2.

Tumor Growth. A total of 121 patients died within the 3 months after randomization (53 in the control group and 68 in the treated group). Among the 255 patients who survived more than 3 months (68%), tumor growth—which was assessed via CT scan measurements of tumor size and serum levels of AFP—differed between the two groups, with a greater increase in the treated group than in the control group (Table 3).

Liver Function Tests and Possible Treatment-Related Side Effects. Liver function tests, based on measurements of biological parameters in the 255 patients who survived more than 3 months, were not different at 3 months between the two groups (Table 3). Nausea and vomiting were significantly higher in the treated group (26 patients) than in controls (8 patients, $P = .002$) (Table 4). Treatment was more frequently stopped in the treated group (45 patients) than in the control group (22 patients, $P = .0045$) (Fig. 1), mainly because of severe

Table 1. Baseline Characteristics of Patients According to Randomization

Variables	Tamoxifen Group n = 184	Tamoxifen + Flutamide + Leuproreline Group n = 192	*P* Value
Age (yr)	66 (34–87)	65 (27–88)	.46
Karnofsky index (%)	90 (30–100)	90 (30–100)	.29
Ascites*	48 (26%)	60 (31%)	.30
Platelets (number/mm^3)	169 (28–700)	157 (38–767)	.30
Serum bilirubin (μmol/L)	19 (3–91)	20 (5–96)	.43
Serum ALT (ULN)			
0–2	152 (87%)	148 (80%)	.21
2–5	21 (12%)	33 (18%)	
≥5	2 (1%)	4 (2%)	
Serum AST (ULN)			
0–2	102 (58%)	99 (54%)	.54
2–5	63 (36%)	71 (38%)	
≥5	10 (6%)	15 (8%)	
Serum alkaline phosphatase (ULN)	1.37 (0.33–10.83)	1.41 (0.36–9.80)	.48
Serum albumin (g/L)	35 (19–54)	34 (21–51)	.44
Prothrombin activity (%)	78 (22–122)	79.5 (7–111)	.82
Serum AFP (ng/mL)	180 (2–3,400.10^3)	274 (2–181.10^3)	.64
US tumor type			
Uninodular	56 (33%)	51 (32%)	.31
Multinodular	71 (42%)	70 (44%)	
Diffuse	33 (20%)	35 (22%)	
Infiltrative	8 (5%)	2 (1%)	
CT portal obstruction	27 (15%)	41 (21%)	.21
Tumor volume >50%	42 (35%)	50 (42%)	.46
Cirrhosis	164 (93%)	169 (90%)	.46
Main cause of cirrhosis†			
Alcohol use	121 (74%)	121 (72%)	.50
HCV	21 (13%)	33 (19%)	
HBV	17 (10%)	5 (3%)	
Other	5 (3%)	10 (6%)	
Child-Turcotte-Pugh classification‡			
Class A	112 (68%)	105 (62%)	.34
Class B	50 (31%)	59 (35%)	
Class C	2 (1%)	5 (3%)	
Okuda classification			
Stage I	93 (50.5%)	81 (42.2%)	.08
Stage II	83 (45.1%)	107 (55.7%)	
Stage III	8 (4.4%)	4 (2.1%)	
GRETCH classification[34]			
Class A	46 (25.0%)	40 (20.8%)	.62
Class B	119 (64.7%)	132 (68.8%)	
Class C	19 (10.3%)	20 (10.4%)	

NOTE. For continuous variables, median values are given followed by a range in parentheses; for discrete or nominal variables, the number is given followed by the percentage in parentheses.

Abbreviations: ALT, alanine aminotransferase; ULN, upper limit of normal; AST, aspartate aminotransferase; US, ultrasonography; HCV, hepatitis C virus; HBV, hepatitis B virus.

*Only detectable via US or CT scan.

†More than one cause in some patients.

‡Only in patients with cirrhosis (n = 333).

deterioration in physical status, which was probably related to HCC, and because of digestive complaints, which were probably related to flutamide (Table 4).

Discussion

The results of this trial do not show that treatment with antiandrogenic drugs had a beneficial effect on survival in patients with advanced HCC. Indeed, a reduced survival rate that was not statistically significant was observed in the treated group. Moreover, no favorable effect was observed on tumor growth after 3 months of treatment. Tumor growth that was assessed via either CT scan or serum AFP levels was even greater in treated patients, a surprising finding that could not be explained by a difference in mortality between both groups at 3 months. Whether this phenomenon is due to a paradoxical en-

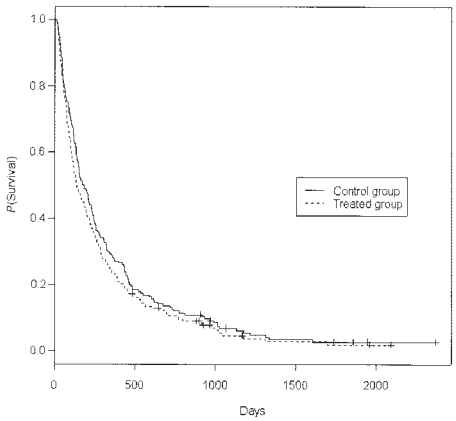

Fig. 2. Survival in the 376 patients included in the trial according to randomization ($P = .21$ according to log-rank test). One-year survival was estimated at 28.3% (95% CI, 21.7-34.8) in the control group and 23.4% (95% CI, 17.4-29.4) in the treated group.

Table 2. Causes (Possibly Multiple) of Death According to Randomization

	Tamoxifen Group n = 184	Tamoxifen + Flutamide + Leuproreline Group n = 192
Number of deaths (%)	177 (96)	183 (95)
Determination of causes of death*	154	145
Liver failure	89	88
Gastrointestinal hemorrhage	24	26
Renal failure	22	17
Spontaneous bacterial peritonitis	4	5
Other causes†	35	32

*More than one cause in 43 patients (20 in control group and 23 in treated group).

†Mainly severe infections (except spontaneous bacterial peritonitis).

hancement of antiandrogens on tumor growth or chance cannot be confirmed. As expected, tolerance to treatment was significantly lower in the treated group than in controls, resulting in a significantly higher number of treatment withdrawals. Treated patients experienced more digestive side effects (well-known with flutamide[35]) but apparently no liver toxicity. It should be noted that the trial design is in accordance with the recommendations of the international Barcelona conference on HCC.[1]

Although some preliminary nonrandomized trials suggest that antiandrogenic blockade is effective in HCC, our trial did not demonstrate any benefits on survival. This lack of benefit is in accordance with the results of two smaller trials: a preliminary randomized trial using triptorelin and flutamide in a reduced number of patients[36] and a larger trial by Grimaldi et al.[37] In the latter trial, 244 patients were randomized into 4 groups that received either peripheral antiandrogen (nilutamide), luteinizing hormone–releasing hormone agonists, both, or placebo. No benefit was observed with antihormonal treatment.[37] The explanation for this lack of beneficial effect from antiandrogenic treatment is unclear. The androgenic blockade regimen was certainly effective in our trial, as demonstrated by results from prostate cancer treatment[27] and by a previous study showing a marked decrease in serum testosterone levels in patients with cirrhosis who are treated with a luteinizing hormone–releasing hormone agonist.[24] There are several possible explanations for these results. Androgen receptors that are frequently found in small HCCs are less frequent in large tumors,[38] which were present in most of the patients in our study. It has been suggested that androgen receptor status is variable in HCC and could influence the response to antiandrogens both in animals[39,40] and in humans.[41] Androgen receptors could mutate and become insensitive to antiandrogens,[39] as has been shown with estrogen receptors, which could be permanently activated and insensitive to tamoxifen in males.[42] Moreover, certain studies have suggested that malignant hepatocytes could rapidly convert androgens into less active metabolites.[14] Although this could explain the lack of efficacy, it does not explain the enhancement of tumor growth. An explanation suggested recently by Chen et al.[43] would be the switch from an antagonist to an agonist effect of antiandrogens due to an increase in androgen receptor levels at an advanced stage of tumors, similarly to prostate cancer.

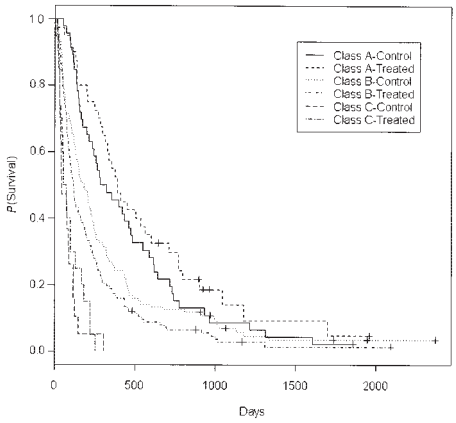

Fig. 3. Survival in the 376 patients included in the trial according to randomization and GRETCH classification at baseline. This classification yielded the following prognostic information: group A, 80 deaths in 86 cases (median survival 364 days); group B, 241 deaths in 251 cases (median survival 138 days); group C, 39 deaths in 39 cases (median survival 53 days).

In our trial, the survival of patients treated with antiandrogens was lower than that of controls (≈4.5 months in the treated group, 6 months in the control group). This difference, which was also seen in the study by Grimaldi et

Table 3. Three-Month Variations in Tumor Size, Serum AFP Levels, and Liver Function Tests Measured From Baseline in 255 Patients With HCC (68%) Surviving More Than 3 Months After Randomization

	Tamoxifen Group (n = 131)	Tamoxifen + Flutamide + Leuproreline Group (n = 124)	*P* Value
Main tumor diameter (cm)*	−0.9 ± 12.9 0 (−65; +55)	2.6 ± 18.7 0 (−87; +80)	.012
Serum AFP	6,847 ± 22,971 3 (−14,424; +144,410)	24,228 ± 135,438 5 (−4,761; +1,010,002)	.014
Serum AST (ULN)	2.20 ± 12.7 0 (−1.78; +93.9)	0.21 ± 5.47 0.34 (−41.3; +8.4)	.093
Serum ALT (ULN)	1.45 ± 10.1 0 (−0.8; +74.5)	−0.23 ± 2.25 −0.07 (−16.5; +3.8)	.40
Serum alkaline phosphatase (ULN)	0.12 ± 1.62 −0.12 (−3.6; +8.7)	−0.05 ± 0.58 −0.07 (−1.6; +1.6)	.79
Serum γ-glutamyltranspeptidase (ULN)	0.97 ± 6.50 0 (−9.2; +34.8)	0.44 ± 3.23 0.3 (−15.9; +8.9)	.35
Prothrombin activity (%)	−2.25 ± 12.2 −4.0 (−25; +43)	−6.73 ± 13.46 −4.5 (−72; +13)	.22
Serum bilirubin (μmol/L)	11.20 ± 48.3 1.0 (−16; +297)	10.2 ± 21.4 5.0 (−26; +100)	.034

Data are expressed as the mean ± SD and median (range) of the difference between 3-month and baseline values (positive value, increase; negative value, decrease).

Abbreviations: AST, aspartate aminotransferase; ULN, upper limit of normal; ALT, alanine aminotransferase.

*In patients with nodular tumor.

al.,[37] was close to statistical significance and could be related to a higher rate of tumor growth, as suggested by our finding of significant increases in tumor size and serum AFP levels at 3 months in the treated group in comparison with controls (Table 2). Acute cytolytic hepatitis has been reported with flutamide[44] and an increase in serum levels of aminotransferases has been shown in approximately 10% of flutamide-treated patients with prostate cancer.[45] However, no difference in serum aminotransferase levels was observed at 3 months in our trial between treated and control patients. On the other hand, deleterious effects on liver function might be a result of androgen deprivation.[46] Testosterone is a trophic factor for the liver,[47] and androgens administered to patients with liver diseases might improve liver function.[48] Hypothetically, antiandrogenic treatment could enhance apoptosis in the nontumoral liver, as suggested in a population of patients with chronic hepatitis.[49] A subclinical proapoptotic effect of cyproterone acetate, a steroid antiandrogen, has been reported in the normal liver and is related to androgen deprivation.[50] We therefore hypothesize that antiandrogens could have a deleterious effect on the nontumoral liver with cirrhosis favoring the growth of androgen-insensitive tumoral cells. The discrepancies between treatment responses according to the severity of liver disease—which were close to significant—supports this interpretation.

In conclusion, no benefit on survival could be found after treatment with leuprorelin and flutamide in European male patients with unresectable HCC. Furthermore, our study suggests that this treatment may enhance tumor growth and may have a possible deleterious effect on the nontumorous liver. Because most patients with HCC

Table 4. Side Effects Possibly Related to Treatment in the 376 Patients Included in the Trial According to Randomization

	Tamoxifen Group n = 184	Tamoxifen + Flutamide + Leuproreline Group n = 192	*P* Value
Nausea and/or vomiting	8 (4%)	26 (14%)	.002
AST and/or ALT increase ≥10 N or ≥3 times from baseline values	9 (5%)	14 (7%)	.39
Venous thrombosis	2 (1%)	3 (1.5%)	1.00
Hot flashes	3 (1.6%)	6 (3%)	.50
Premature interruption of treatment	22 (12%)	45 (23%)	.0045
Severe deterioration in physical status	14	22	.22
Severe digestive complaints	3	13	.02
Severe liver tests abnormalities	5	10	.29

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; N, times the upper limit of normal.

have underlying chronic liver disease (most often cirrhosis), it might be important for future trials to consider the possible deleterious influence of antiproliferative compounds on the nontumorous liver, because this may counterbalance their potential antitumoral effects.

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APPENDIX

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