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A phase II trial of oral eniluracil/5-fluorouracil in patients with inoperable hepatocellular carcinoma

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Abstract

Only a minority of patients with hepatocellular carcinoma (HCC) may benefit from curative treatments, whereas there is no standard therapy for the remaining patients. The objective of this multicentre, open label phase II study was to estimate the objective tumour response rate of a 28-day regimen of oral eniluracil/5-fluorouracil (5-FU) in patients with chemotherapy-naïve, or anthracycline-refractory, inoperable HCC. 45 patients received courses of twice daily oral 5-FU (1.0 mg/m^2) and eniluracil (10 mg/m^2) for the first 28 days of each 5-week course. Patients were assessed at regular intervals to determine the tumour response and to evaluate toxicity. Patients were followed-up for a minimum of 6 months. No patient showed a partial or complete tumour response, and 18 patients (40%) had a best response of stable disease (95% confidence interval (CI) 25%, 55%). The median duration of progression-free survival (PFS) was 13.7 weeks (95% CI 10.0–20.0 weeks), and the median duration of overall survival (OS) was 50.3 weeks (range 1.1–64.1+ weeks). The combination of eniluracil/5-FU was well tolerated and had an acceptable safety profile. Only 7 patients (16%) reported at least one adverse event (AE) of grade 3 or 4 intensity considered reasonably attributable to the study medication. In conclusion, oral eniluracil/5-FU had minimal, if any, activity in patients with inoperable HCC, but the safety profile was acceptable.

Keywords: Inoperable hepatocellular carcinoma; Eniluracil; 5-Fluorouracil; Chemotherapy

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common tumours in the world and the leading cause of death among cirrhotic patients [1,2]. The outcome of patients presenting with HCC varies according to the stage of the disease and to the treatment applied [3,4]. The natural course of HCC can only be changed in the early stages by applying radical treatments such as surgical resection, orthotopic liver transplantation or percutaneous treatments [3–5]. Unfortunately, only a minority of patients with HCC diagnosed nowadays may benefit from these approaches and, therefore, several alternative treatments have been assessed for more advanced stages of the disease. Randomised controlled trials (RCTs) comparing loco-regional (embolisation, chemoembolisation) or systemic treatments (hormonal therapy, chemotherapy, interferon) versus conservative management have shown the lack of efficacy of these treatments in terms of survival [6–10]. Therefore, there is a need to develop additional treatments for patients with advanced HCC.

Conventional chemotherapy is ineffective in patients with inoperable HCC. The most widely used single agent is doxorubicin, which produces a median response rate of 17% (range 0–79%) [11]. This low efficacy may be due to the expression of the multidrug resistance gene and *TP53* gene mutations, which are usually found in advanced HCC.

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Eniluracil is an effective inactivator of dihydropyrimidine dehydrogenase (DPD), the first enzyme in the degradative pathway of 5-fluorouracil (5-FU) [12]. There is high enzyme activity in normal liver tissue, making it the major site of 5-FU catabolism [13,14]. High levels of the enzyme are also found in HCC. By inhibiting DPD, eniluracil increases the oral bioavailability of 5-FU to approximately 100% and reduces its pharmacokinetic variability resulting in plasma levels of 5-FU comparable to those achieved with continuous intravenous (i.v.) administration [15,16].

Although published historical data indicate that i.v. bolus 5-FU has minimal activity in HCC (response rate 0–28%) [17–20], the current study (FUMB2008) was done believing that inhibition of the high DPD activity in the liver by eniluracil would improve the efficacy of 5-FU in patients with HCC. The primary objective was to estimate the objective tumour response rate of a 28-day regimen of oral eniluracil/5-FU in patients with chemotherapy-naïve, or anthracycline-refractory, inoperable HCC. The secondary objectives were to evaluate the duration of response, the duration of progression-free survival (PFS), the duration of overall survival (OS) and the toxicity profile.

2. Patients and methods

2.1. Patients

Patients aged 18 or over were eligible for inclusion if they had a histologically or cytologically confirmed diagnosis of inoperable HCC, other than the fibrolamellar variant, with at least one bidimensionally measurable lesion. Patients had to have a Karnofsky Performance Status score (KPS) of $\geqslant 60$ and were either chemotherapy-naïve (i.e., no prior systemic or regional chemotherapy) or were refractory to an anthracycline. All patients gave written informed consent before entering the study.

Patients were excluded from the study if they had a history of additional malignancy, known central nervous system metastases, or signs or symptoms of encephalopathy. Patients were not allowed to enter the study if they had recently undergone major surgery (4 weeks before the first dose of study drug) or received chemotherapy, biological (3 weeks before) or hormonal therapy (1 week before). Patients were also excluded if: haemoglobin <90 g/l granulocyte count $<1.5\times10^9$/l; platelet count $<75\times10^9$/l, estimated creatinine clearance <50 ml/min; total bilirubin >3 times the upper limit of normal; international normalised ratio (INR) >2 for patients not receiving anticoagulation therapy; unstable prothrombin times or a change in the dose of anticoagulant in the 2 weeks before the first dose of the study drug if receiving anticoagulation therapy.

2.2. Study design

This was an international multicentre, open-label phase II study conducted at 10 centres in Australia (1 centre), Finland (1), Greece (2), South Africa (2), Spain (3) and the UK (1). The study started in June 1997 and early termination occurred in February 1998. The study protocol was approved by an Ethics Committee at each institution, and was performed according to the latest version of the Declaration of Helsinki.

2.3. Treatment schedules

Patients received 10 mg/m^2 eniluracil and 1 mg/m^2 5-FU (10:1 ratio of eniluracil/5-FU) orally twice daily for the first 28 days of a 5-week treatment cycle, followed by a 7-day period during which no study medication was taken. Patients were told to fast for 1 h before and after each dose. Patients continued treatment courses until disease progression or unmanageable toxicity occurred. Medication was supplied by Glaxo Wellcome Inc, USA. Eniluracil was supplied as 2.5 and 10 mg tablets; 5-FU was supplied as 0.25 and 1 mg tablets.

Dose modifications were made as previously described based on drug-related toxicities and the patient's current creatinine clearance [21]. Patients received full supportive care during the study, including transfusion of blood and blood products, as well as treatment with antibiotics, antiemetics, antidiarrhoeals and analgesics when appropriate. Patients requiring other anticancer therapy were not allowed to continue to receive treatment with eniluracil/5-FU. Other anticancer therapy could not begin until at least 28 days after the final dose of eniluracil/5-FU. Subsequent therapy with 5-FU or other fluoropyrimidines was not allowed until the plasma uracil concentration had returned to normal (indicating the return of normal DPD activity).

2.4. Efficacy and safety assessments

The primary measure of efficacy was the objective tumour response rate. The response definitions were adapted from criteria established by the Southwest Oncology Group (SWOG) [22]. Measurable disease, evaluable disease and non-evaluable disease were classified according to standard SWOG definitions [22].

Patients were assessed for baseline disease within 2 weeks of starting the first dose of study medication with a chest X-ray, abdominal computerised tomography (CT) or magnetic resonance imaging (MRI) scan. All other known areas of disease were assessed by X-ray, scan, endoscopy or physical examination at baseline. All sites of disease were monitored approximately every 10 weeks, using the same method, until disease progression or treatment discontinuation.

The secondary measures of efficacy were the duration of response, duration of PFS and OS. After discontinuation of the study medication, quarterly follow-up assessments were done for all patients until death to determine their survival status and whether any subsequent anticancer therapy had been initiated.

Adverse events (AEs) were graded according to modified SWOG toxicity criteria [22]. Standard haematology and clinical chemistry tests, creatinine clearance, urinalysis, prothrombin time and/or INR were assessed regularly throughout the study.

2.5. Statistical analyses

It was planned that approximately 67 chemotherapy-naïve patients, and 25 patients with anthracycline-refractory disease, were to be enrolled into separate strata. A sample size of 67 chemotherapy-naïve patients was sufficient to estimate a 95% confidence interval (CI) of the true response rate with a maximum width of 24%. A sample size of 25 anthracycline-refractory patients was sufficient to estimate a 95% CI of the true response rate with a maximum width of 39%.

A two-stage design was used to determine whether there was sufficient activity to warrant complete enrolment [23]. If there were no objective tumour responses among the first 19 evaluable patients in either stratum, the probability of a response rate greater than or equal to 15% was less than 5% and the study was to be discontinued. Alternatively, one or more responses in either stratum would have indicated that continuation was warranted in that stratum to better estimate efficacy.

All patients in the intent-to-treat population (ITT) were evaluated for safety and efficacy. The response rate was also evaluated in a 'per-protocol' (PP) population, defined as patients who received $\geqslant 75\%$ of the total prescribed amount of study medication during course 1 (or a total of 21 days on which any amount of study medication was taken), and who had a baseline disease assessment and at least one assessment at a later time-point. Time to event parameters were summarised using Kaplan–Meier product limit estimates.

3. Results

46 patients were enrolled into the study. One patient was enrolled, but did not receive study medication because of a decrease in creatinine clearance before the planned start of study medication. Therefore, 45 patients received the study drugs. As only one patient was anthracycline-resistant (having previously taken doxorubicin), and the remainder were chemotherapy-naïve, all patients were grouped together for all analyses. Patients were followed-up for a minimum of 6 months. No complete or partial tumour responses were reported by investigators in the first 19 chemotherapy-naïve patients enrolled, and so enrolment was terminated according to the early stopping rule. At the time of data reporting, 22 patients (49%) had left the study, the majority due to death (19 patients). 2 patients stopped treatment because of an AE, and these patients were alive at the time of discharge from the study. One patient was lost to follow-up.

3.1. Patient characteristics

The characteristics of the patients at baseline are shown in Table 1. Most patients were male and white, with only one site of disease. 39 patients (87%) had chronic liver disease at baseline (73% cirrhosis). Previous therapies for HCC (except for the patient who received doxorubicin) were embolisation (10 patients, 22%), percutaneous alcohol injection (7 patients, 16%), surgery (4 patients, 9%), hormonal therapy (4 patients, 9%), and radiotherapy (1 patient, 2%).

Table 1
Characteristics of the patients at baseline ($n = 45$)

Characteristic	Number of patients
Age (years) Median (range)	64 (28–77)
	n (%)
Sex	
Male/female	35 (78)/10 (22)
Race	
White	35 (78)
Black	9 (20)
Asian	1 (2)
Karnofsky performance status	
100	12 (27)
90	8 (18)
80	15 (33)
70	5 (11)
60	5 (11)
Number of disease sites	
1	32 (71)
2 or more	13 (29)
Location of extrahepatic disease	
Lymph nodes	5 (11)
Lung	4 (9)
Portal vein invasion by tumour	10 (22)
Causes of chronic liver disease	
Hepatitis B	11 (24)
Hepatitis C	16 (36)
Alcohol abuse	1 (2)
Cryptogenic cirrhosis	1 (2)
Alcohol abuse and hepatitis B	3 (7)
Alcohol abuse and hepatitis C	6 (13)
Hepatitis C and haemachromatosis	1 (2)

Table 2
Summary of objective tumour response rate

Population	Best response	Response rate Number of patients *n* (%)	95% confidence intervals (CI)
ITT ($n=45$)	Stable disease	18 (40)	25%, 55%
	Progression	14 (31)	NC
	Unknown	13 (29)	NC
PP ($n=31$)	Stable disease	18 (58)	39%, 77%
	Progression	12 (39)	NC
	Unknown	1 (3)	NC

NC, not calculated; ITT, intention-to-treat analysis; PP, per-protocol analysis.

3.2. Efficacy results

Overall best responses, as assessed by the sponsor, are shown in Table 2. 14 patients were excluded from the PP population (13 of these because they did not have a post-baseline disease assessment). No patient had a complete or partial tumour response. However, 40 and 58% of patients achieved stable disease (ITT and PP populations, respectively). In the ITT population, the median duration of stable disease was 21.3 weeks (95% CI 20.0–28.9 weeks), and ranged from 13.7 to 64.1+ weeks. 26 patients were alive at the time of data reporting. The median duration of survival was 50.3 weeks (range 1.1–64.1+ weeks) (Fig. 1). The median duration of PFS was 13.7 weeks (95% CI 10.0–20.0 weeks) and ranged from 1.1 to 64.1+ weeks.

3.3. Dosing results

A total of 163 courses were given to 45 patients. The median number of courses per patient was 3 (range 1–13). The median duration of treatment was 12.0 weeks (range 0.9–64.1 weeks). The median percentage of projected eniluracil/5-FU dose intensity was 94.3% (range 45.7–105.4%). Nineteen courses (12%) were not completed because of toxicities: granulocytopenia caused a dosing deviation in three courses (2%), thrombocytopenia in three courses (2%), diarrhoea in two courses (1%), and mucositis in one course (<1%). Ten courses (6%) were delayed due to toxicity. Only 8% of courses (beyond course 1) had changes in the prescribed dose.

3.4. Toxicity

The combination of eniluracil/5-FU was well tolerated. AEs considered reasonably attributable to study medication are shown in Table 3. 25 patients (56%) reported at least one AE that was considered reasonably attributable to the study medication, the most common of which was nausea (all occurrences were grade 1 or 2 in intensity). 7 patients (16%) reported at least one AE of grade 3 or 4 intensity that was considered reasonably attributable to study medication. Treatment-emergent grade 3 or 4 haematological toxicities were rare and, with the exception of hyperbilirubinemia, grade 3 or 4 clinical chemistry abnormalities were also rare. 23 patients (51%) had treatment emergent hyperbilirubinaemia (11 grade 3, 12 grade 4). Although only 2 of these patients did not have a raised bilirubin level at baseline and/or progressive disease during the study, a treatment-related effect cannot be excluded.

AEs that are commonly seen with and considered attributable to i.v. continuous 5-FU infusion were uncommon in this study. For example, the incidences of nausea, mucositis and vomiting considered reasonably attributable to study medication are shown in Table 3; all of these events were grade 1 or 2 in intensity. Hand–

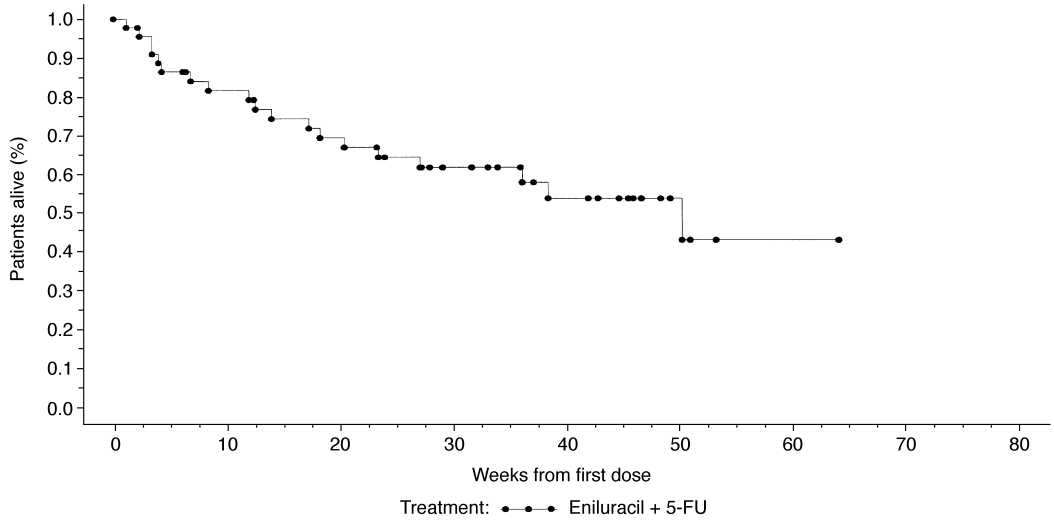

Fig. 1. Overall survival (OS) of 45 patients with inoperable hepatocellular carcinoma treated with oral eniluracil/5-fluorouracil (5-FU) (weeks after the first dose).

Table 3
Summary of most frequently reported (>5%) adverse events reasonably attributable to study medication

Adverse event	Number of patients with AE *n* (%)	Number of patients with grade 3 or 4 AE *n* (%)
Non-haematological		
Nausea	6 (13)	0
Mucositis	4 (9)	0
Vomiting	4 (9)	0
Abnormal bilirubin levels	3 (7)	3 (7)
Diarrhoea	3 (7)	1 (2)
Gastrointestinal discomfort and pain	3 (7)	0
Hyposalivation	3 (7)	0
Haematological		
Thrombocytopenia	4 (9)	4 (9)
Anaemia	3 (7)	1 (2)

AE, adverse event.

foot syndrome, skin rashes and alopecia were experienced by $\leqslant 5\%$ of patients.

4. Discussion

The treatment of advanced HCC remains an active area of research since there is no standard therapy [3,4]. Several antineoplastic treatments such as chemoembolisation, systemic chemotherapy, interferon and hormonal blockade by tamoxifen have not been shown to provide an unequivocal benefit in terms of survival improvement [6–11]. Patients with HCC at an intermediate-advanced stage have been treated by embolisation either with or without chemotherapy resulting in objective responses in up to 50% of the cases [6,7]. However, no RCT has demonstrated a benefit in terms of survival [6,7]. Similarly, intra-arterial chemotherapy provides partial responses in a notable proportion of HCC patients in phase II studies [8,9], perhaps as a result of its ischaemic effect due to endothelitis, rather than by a truly antineoplastic effect. Anti-oestrogen (tamoxifen) therapy is ineffective for advanced HCC either in terms of response or survival. This was demonstrated in a large double-blind placebo-controlled trial; these results have also been further confirmed in other studies [10]. Finally, the effectiveness of interferon is still controversial. Therefore, new agents or combination of agents have to be assessed in the setting of prospective studies to provide effective antitumoral tools for patients with inoperable HCC.

The present phase II study evaluated a new treatment option for this neoplasm by combining oral 5-FU with eniluracil, an effective inhibitor of the degradation of 5-FU. An orally active treatment with low toxicity which could be administered at home would be particularly beneficial for HCC patients in poorly developed areas of the world which have a high incidence of this neoplasm.

Unfortunately, no complete or partial tumour responses were seen in our investigation. This justified the early termination of the study. The failure to meet the criteria to continue recruitment was a disappointing result because inhibition of DPD activity was expected to be effective at increasing the efficacy of 5-FU in HCC. Not only does the liver have high levels of DPD which make it the main site of 5-FU catabolism [13,14,24], but high levels of tumoral DPD have also been reported in HCC [25]. Only 18 patients (40%) had a best response of stable disease with a median duration of 21.3 weeks. However, since the natural course of the disease was recently reported to lead to a one year probability of tumour progression of 70%, vascular invasion of 21% and extrahepatic spread of untreated tumours of 9% [26], these results cannot necessarily be ascribed to the antitumoral effect of 5-FU.

HCC is known to be resistant to chemotherapy with most of the antineoplastic treatments assessed. In fact, there is some consensus that no single agent or combination of agents can lead to objective responses in over 20–25% of cases. The fact that HCC responds poorly to all types of systemic therapy [8,9,11] suggests that multiple mechanisms of drug resistance may arise during the development of the malignancy. Over-expression of *P*-glycoprotein [27] and *TP53* gene mutations [28] have both been implicated. The efficacy results of the current study suggest that HCC is also intrinsically resistant to 5-FU. This may be the result of reasons other than high DPD activity, for example increased expression of the thymidylate synthase enzyme [29]. However, drug resistance is unlikely to explain the minimal activity of eniluracil/5-FU against HCC in the current study, as all but one of the patients recruited were chemotherapy-naïve.

Oral eniluracil/5-FU had an acceptable safety profile in this patient population. The toxicity profile of 5-FU-based regimens (i.e. with leucovorin) in patients with

inoperable HCC is different with generally higher incidences of diarrhoea, mucositis, stomatitis, anaemia and granulocytopenia that are more severe in intensity [17–20]. In contrast, in the present study, there was a low incidence of these toxicities. The hyperbilirubinemia seen in this study only needs to be considered when treating HCC cirrhotic patients with eniluracil/5-FU. The low toxicity seen in this study also suggests that the patients may have been able to tolerate higher doses of eniluracil/5-FU which may in turn have led to improved efficacy.

The median survival in our study was approximately 11 months (50.3 weeks), and is similar to the outcome expected according to the natural course of this stage of the disease. However, our survival was better than that obtained in other phase II studies using 5-FU (e.g. 5-FU/leucovorin; median survival: 14–19 weeks [16–19], 5-FU alone: 32.7 weeks [30]). These discrepancies may reflect differences in the baseline characteristics of the patients recruited more than a decade ago [17,18] or inclusion of patients with a truly end-stage disease, rather than a benefit of the treatment itself. Therefore, optimistic comparisons of survival data with other data should be treated with caution.

The data from the present study show that oral eniluracil/5-FU has minimal, if any, activity in inoperable HCC.

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References

1. Bosch X, Ribes J, Borràs J. Epidemiology of primary liver cancer. *Semin Liver Dis* 1999, **19**, 271–285.
2. Fattovich G, Giustina G, Degos F, *et al*. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997, **112**, 463–472.
3. Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999, **19**, 329–339.
4. Bruix J. Treatment of hepatocellular carcinoma. *Hepatology* 1997, **25**, 259–262.
5. Llovet JM, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999, **30**, 1434–1440.
6. Group d'Etude et de Traitement de Carcinome Hepatocellulaire. A comparison of lipiodol chemoembolization and conservative treatment for unresectable hepatocellular carcinoma. *New Engl J Med* 1995, **332**, 1256–1261.
7. Bruix J, Llovet JM, Castells A, *et al*. Transarterial embolization *versus* symptomatic treatment in patients with advanced hepatocellular carcinoma. Results of a randomized controlled trial in a single institution. *Hepatology* 1998, **27**, 1578–1583.
8. Mathurin P, Rixe O, Carbonell N, *et al*. Overview of medical treatments in unresectable hepatocellular carcinoma — an impossible meta-analysis? *Alimen Pharmacol Therap* 1998, **12**, 111–126.
9. Simonetti RG, Liberati A, Angiolini C, Pagliaro L. Treatment of hepatocellular carcinoma: a systematic review of randomized controlled trials. *Ann Oncol* 1997, **8**, 117–136.
10. Castells A, Bruix J, Brú C, *et al*. Treatment of hepatocellular carcinoma with tamoxifen: a double-blind placebo-controlled trial in 120 patients. *Gastroenterology* 1995, **109**, 917–922.
11. Nerenstone SR, Ihde DC, Friedman MA. Clinical trials in primary hepatocellular carcinoma: current status and future directions. *Cancer Treat Rev* 1988, **15**, 1–31.
12. Spector T, Harrington JA, Porter DJT. 5-Ethynyluracil (776C85): inactivation of dihydropyrimidine dehydrogenase *in vivo*. *Biochem Pharmacol* 1993, **46**, 2243–2248.
13. Naguib FNM, el Kouni MH, Cha S. Enzymes of uracil catabolism in normal and neoplastic tissues. *Cancer Res* 1985, **45**, 5405–5412.
14. Ho DH, Townsend L, Luna MA, Bodey GP. Distribution and inhibition of dihydrouracil dehydrogenase activities in human tissues using 5-fluorouracil as a substrate. *Anticancer Res* 1986, **6**, 781–784.
15. Baker SD, Khor SP, Adjei AA, *et al*. Pharmacokinetic, oral bioavailability, and safety study of fluorouracil in patients treated with 776C85, an inactivator of dihydropyrimidine dehydrogenase. *J Clin Oncol* 1996, **14**, 3085–3096.
16. Baker SD, Diasio RB, O'Reilly S, *et al*. Phase I and pharmacologic study of oral fluorouracil on a chronic daily schedule in combination with the dihydropyrimidine dehydrogenase inactivator eniluracil. *J Clin Oncol* 2000, **18**, 915–926.
17. Zaniboni A, Simoncini E, Marpicati P, Marini G. Phase II study of 5-fluorouracil (5-FU) and high dose folinic acid (HDFA) in hepatocellular carcinoma. *Br J Cancer* 1988, **57**, 319.
18. van Eeden H, Falkson G, Burger W, Ansell SM. 5-fluorouracil and leucovorin in hepatocellular carcinoma. *Ann Oncol* 1992, **3**, 404–405.
19. Tetef M, Doroshow J, Akman S, *et al*. 5-Fluorouracil and high-dose calcium leucovorin for hepatocellular carcinoma: a phase II trial. *Cancer Invest* 1995, **13**, 460–463.
20. Porta C, Moroni M, Nastasi G, Arcangeli G. 5-Fluorouracil and d,l-leucovorin calcium are active to treat unresectable hepatocellular carcinoma patients: preliminary results of a phase II study. *Oncology* 1995, **52**, 487–491.
21. Smith IE, Johnston SRD, O'Brien MER, *et al*. Low dose oral 5-fluorouracil with eniluracil (776C85) as first-line chemotherapy against advanced breast cancer. *J Clin Oncol* 2000, **18**, 2378–2384.
22. Green S, Weiss GR. Southwest oncology group standard response criteria, endpoint definitions and toxicity criteria. *Invest New Drugs* 1992, **10**, 239–253.
23. Gehan EA. The determination of the number of patients required in a preliminary and follow-up trial of a new chemotherapeutic agent. *J Chron Dis* 1961, **13**, 346–353.

24. Lu Z, Zhang R, Diasio RB. Population characteristics of hepatic dihydropyrimidine dehydrogenase activity, a key metabolic enzyme in 5-fluorouracil chemotherapy. *Clin Pharm Therap* 1995, **58**, 512–522.
25. Jiang W, Lu Z, He Y, Diasio RB. Dihydropyrimidine dehydrogenase activity in hepatocellular carcinoma: implication in 5-fluorouracil-based chemotherapy. *Clin Cancer Res* 1997, **3**, 395–399.
26. Llovet JM, Bustamante J, Castells A, *et al*. Natural history of untreated nonsurgical untreated hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999, **29**, 62–67.
27. Huang C, Wu M, Xu GW, *et al*. Overexpression of the MDR1 gene and p-glycoprotein in human hepatocellular carcinoma. *J Natl Cancer Inst* 1992, **84**, 262–264.
28. Chang F, Syrjanen S, Syrjanen K. Implications of the p53 tumour-suppressor gene in clinical oncology. *J Clin Oncol* 1995, **13**, 1009–1022.
29. Peters GJ, van der Wilt CL, van Triest B, *et al*. Thymidylate synthase and drug resistance. *Eur J Cancer* 1995, **31A**, 1299–1305.
30. Benson AB, Mitchell E, Abramson N, *et al*. A mulicenter phase II trial of oral eniluracil plus 5-FU in patients with inoperable hepatocellular carcinoma. *Proc Am Soc Clin Oncol* 1999, **18**, 256a.