*Anti-Cancer Drugs 2000,* **11,** pp. 579–582

## Preclinical report

# Synergistic antitumoral activity of combined UFT, folinic acid and oxaliplatin against human colorectal HT29 cell xenografts in athymic nude mice

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**This study was designed to asses the inhibition of tumor growth by oxaliplatin combined with UFT and folinic acid (FA). Growth inhibition was studied in nude mice transplanted with human colorectal HT29 tumor cell xenografts and treated for 28 days with oral UFT (20 mg/kg/day) and FA (4 mg/kg/day), i.p. oxaliplatin (10 mg/kg on day 1) or a combination of oxaliplatin, UFT and FA, or else not treated (controls). Tumor surface area and weight were recorded twice a week, and mice were sacrificed at day 28. Two separate experiments were performed for each group of 25 mice. At day 28, mean tumor weights (g) were 2.89 ± 0.22 (controls), 2.03 ± 0.14 (oxaliplatin), 2.02 ± 0.21 (UFT/FA) and 1.23 ± 0.17 (oxaliplatin+UFT/FA). For the three treatment groups, tumor weight decreases were 30.1% ($p < 0.05$), 29.9% ($p < 0.05$) and 57.5% ($p < 0.001$), respectively. Combined treatment (UFT/FA+oxaliplatin) reduced tumor weight by 39% compared to oxaliplatin alone ($p < 0.05$) or UFT/FA ($p < 0.05$). These results demonstrate the synergistic effect of the combination of oxaliplatin, UFT and FA in this HT29 cell xenograft model, and warrant further investigations in patients with metastatic colorectal cancer. [© 2000 Lippincott Williams & Wilkins.]**

*Key words*: Folinic acid, human colorectal cancer HT29 cell xenograft, oxaliplatin, UFT.

## Introduction

The oral thymidylate synthase inhibitor UFT (a mixture of tegafur and uracil, at a molar ratio of 1:4) has been shown to be clinically active against colorectal, gastric and breast adenocarcinomas.[1,2] Subsequent animal studies demonstrated that UFT is more active than tegafur alone and that its activity is enhanced by folinic acid.[3,4] Two recent clinical studies in patients with metastatic colorectal cancer showed that a combination of UFT and folinic acid (FA), given orally, was as effective as the combination of 5-fluorouracil (5-FU) and FA, given as an i.v. bolus days 1–5 every month.[5,6]

Oxaliplatin is a recently synthesized diaminocyclohexane platinum compound, which like cisplatin causes DNA-adduct formation, resulting in damage such as intrastrand cross-links covalently binding the platinum compound to guanine radicals,[7] DNA interstrand cross-links, DNA–protein cross-links and DNA strand breaks.[8] When oxaliplatin is used alone, it is active against various malignancies, including some which are usually resistant to cisplatin, but it was mainly developed for colorectal cancer. We previously demonstrated that 5-FU and FA enhanced oxaliplatin activity, both in preclinical studies[9] and clinical studies.[10,11] The present investigation was designed to characterize the antitumoral activity of UFT, FA and oxaliplatin against xenografts of human colonic HT29 cells in nude mice, regarded as a model of highly aggressive tumor progression.

## Materials and methods

### Chemicals

UFT and oxaliplatin were generous gifts from Bristol-Myers Squibb France (Paris, France) and Sanofi Recherche (Paris, France). FA was purchased from Sigma (St Louis, MO). All other reagents were of the purest grade available.

This work was supported by INSERM and a Research Grant from Bristol-Myers Squibb France.

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### Cells and culture conditions

Human colorectal cancer HT29 cells were obtained from Dr J Fogh (Sloan Kettering Institute for Cancer Research, NY) and cultured in Dulbecco's modified Eagle's medium (DMEM; Eurobio, Paris, France), supplemented with 10% heat-decomplemented fetal bovine serum (Boehringer, Mannheim, Germany), 100 U/ml penicillin, 100 $\mu$g/ml streptomycin and 8 nM glutamine. Cells were grown at 37°C in a humidified atmosphere containing 5% $CO_2$. The medium was renewed every 2 days and the cells were passaged twice a week by trypsin–EDTA to maintain their exponential growth.

### Animals and xenografts

Female BALB/c nude mice (*nu*+/*nu*+) aged 6 weeks were maintained in a pathogen-free state. Human colorectal HT29 cell xenografts ($1 \times 10^6$ cells) were transplanted s.c. into the hind limb of each animal. Treatment began 14 days later, when tumors were palpable and measurable (mean tumor surface area=33 $mm^2$).

### Treatments

After tumor occurrence, animals were allocated to four groups: control (group 1), oxaliplatin (group 2), UFT+folinic acid (group 3) and oxaliplatin+UFT+folinic acid (group 4). UFT was administered to mice via the drinking water at a concentration that would deliver 20 mg/kg/day from days 1 to 28. Likewise, FA was administered via the drinking water at a concentration delivering 4 mg/kg/day from days 1 to 28. Oxaliplatin was administered by i.p. injection on day 1 at a dosage corresponding to 10 mg/kg.

### Tumor and toxicity measurements

The longest axis of each tumor and the axis perpendicular to the longest axis were measured by a caliper. Tumor size (i.e. surface aera) and body weight were recorded twice a week. Successive tumor measurements were normalized in relation to the initial (baseline) measurement per animal in order to establish growth curves for each group. For each experiment, all measurement were made by the same observer.

Animals were sacrificed 28 days after treatment initiation, and tumors were excised and weighed. A specimen tumor from each group was sent for pathological examination. Tumor weight (g) per group is expressed as the mean $\pm$ SEM.

Toxicity was evaluated in terms of mortality and the body weight ratio $W_n/W_0$, where $W_n$ is the body weight $n$ days after the start of treatment and $W_0$ is the weight at the start of treatment.

### Statistical analysis

As two consecutive experiments gave similar data, their results were combined and represent the average of all data for a total of 25 animals per group. Means for each group were compared by one-way analysis of variances. Barlett's test was used to verify the homogeneity of the variances and the Tukey-Kramer multiple comparison test was used for inter-group comparisons. Instat and Prism software (GraphPad, San Diego, CA) were used for statistical analyses and graphs.

## Results

No adverse effect was observed in any of the three treatment groups: all the mice were alive at day 28 and mean body weight was similar in the four experimental groups (Table 1). Compared to the controls (group 1), tumor growth was slightly inhibited by UFT+FA (group 3) and oxaliplatin (group 2), with decreases in tumor size of 19 and 24%, respectively, by day 28 (Figure 1). Tumor growth inhibition by the combined treatment (group 4) at day 28 was 54% versus the controls, 45% versus UFT+FA (group 3) and 40% versus oxaliplatin (group 2).

At day 28, mean tumor weights were $2.89 \pm 0.22$ g in the control group, $2.03 \pm 0.14$ g in the oxaliplatin group, $2.02 \pm 0.21$ g in the UFT+FA group and $1.23 \pm 0.17$ g in the combined treatment group (Figure 2). Analysis of variance showed that the differences between mean tumor weights were significant ($F$=12,536; 99 d.f.; $p < 0.0001$). The Barlett test

**Table 1.** Side-effects of UFT plus FA, oxaliplatin and the three combined

| Group | *N* | Body weight ratio (mean $\pm$ SD) | Mortality |
|---|---|---|---|
| Control | 25 | $1.01 \pm 0.08$ | 0 |
| UFT+FA | 25 | $1.02 \pm 0.07$ | 0 |
| Oxaliplatin | 25 | $1.05 \pm 0.09$ | 0 |
| Combined treatment | 25 | $1.01 \pm 0.08$ | 0 |

Side effects were evaluated in terms of body weight ratio ($W_n/W_0$) and mortality, where $W_n$ is the body weight measured $n$ days after the initial administration and $W_0$ is the body weight at the start of treatment. Animals were weighed twice a week. The table indicates the body weight ratio before sacrifice at day 28.

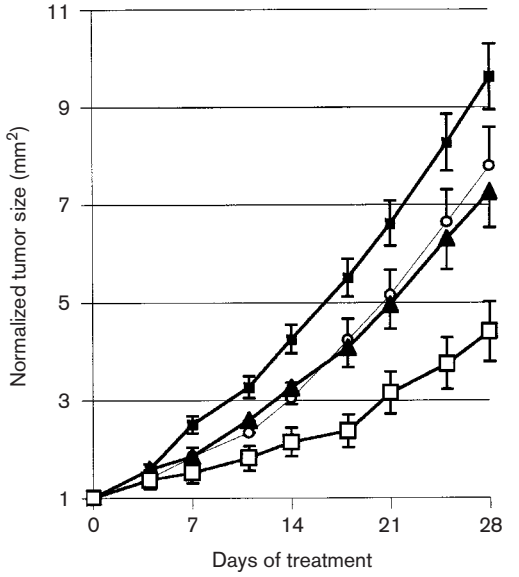


**Figure 1.** Growth curves of HT29 cell tumors in nude mice for the three treatment groups: UFT+FA (○), oxaliplatin (▲), or UFT+FA+oxaliplatin (□), versus the control group (■). Treatment began 14 days after HT29 cell xenografts. Measurements of tumor size, i.e. surface area, were normalized according to tumor size treatment initiation (day 0). Two separate experiments gave comparable results. This figure summarizes tumor growth in 25 animals per group.

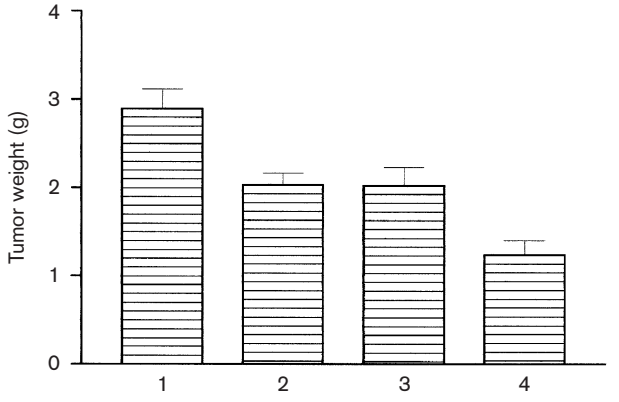


**Figure 2.** Mean tumor weight (±SEM) after 28 days of treatment for group 1 (control), group 2 (oxaliplatin), group 3 (UFT+folinic acid) and group 4 (UFT+folinic acid+oxaliplatin). Each group comprised 25 mice.

showed that the differences between the standard deviations were not significant (Barlett=6.648; $p$=0.084). Tumor weight decreases compared to the controls were 30% for UFT+FA ($q$=4.442; $p<0.05$), 30% for oxaliplatin ($q$=4.416; $p<0.05$) and 58% for the combined treatment ($q$=8.672; $p<0.001$). Comparisons of tumor weight in the UFT+FA and oxaliplatin groups showed no significant difference. Combined treatment (UFT+FA+ oxaliplatin) reduced tumor weight by 39% compared to oxaliplatin alone ($q$=4.256, $p<0.05$) or UFT+FA ($q$=4.230, $p<0.05$). Pathological examination of the tumors showed no difference between the four groups.

In this model, data concerning bidimenstional tumor size (i.e. surface area) and tumor weight were very similar. To validate the method of measurement, we assessed the relation between tumor size and tumor weight at day 28. The strong correlation between them ($r$=0.94) indicated that tumor weight can be estimated by tumor surface area, according to the following equation: weight (in g)=0.77 × Size (in $mm^2$).

## Discussion

Treatment of colorectal cancer is based on 5-FU modulated by FA. Oral prodrugs of 5-FU were recently developed in order to simplify the modality of treatment. The preliminary results of two randomized studies[5,6] indicate that oral UFT+FA is as active as, and less toxic than, the standard FUFOL i.v. regimen (5-FU and FA administered as a bolus for 5 consecutive days every 4 weeks). During the past few years, steady improvements in both the response rate and progression-free survival have been observed with 5-FU infusion, and with the development of new drugs such as oxaliplatin or CPT-11. A clear synergism between oxaliplatin and 5-FU has been demonstrated, both in preclinical studies[9] and clinical studies. Indeed, when oxaliplatin was used alone, 10 and 20% response rates were observed in second- and first-line treatments, respectively,[12] and for combined 5FU+oxaliplatin response rates reached 20–40% in second-line treatment[10,13] and up to 50% in first-line treatment.[11] Therefore, almost all ongoing studies with oxaliplatin are using it combined with 5-FU or other drugs in various regimens.[14]

As already stated, UFT is a mixture of tegafur and uracil, at a molar ratio of 1:4. Tegafur is a 5-FU prodrug, while uracil inhibits 5-FU degradation.[15] This 1:4 5-FU/uracil ratio was retained for the UFT design on the basis of the optimal tumor/serum 5-FU levels measured in rats bearing AH-130 tumors.[16] As previously observed, the antitumoral activity of UFT or 5-FU is enhanced by FA.[3]

Our study was designed to assess the expected beneficial effect of UFT combined with oxaliplatin. We chose the same animal model as we previously used to evaluate the 5-FU and oxaliplatin combination.[9] The nude mice bearing human colon cancer HT29 cell

xenografts were given UFT *per os*, at a daily dosage of 20 mg/kg, because this dosage corresponds to the dose most widely used in animal models,[15,17] although higher doses have been given in some experiments.[3] For oxaliplatin, 10 mg/kg was given i.p. on day 1 and for FA, a low dose of 4 mg/kg/day was given on the basis of our previous results.[9] The tumor growth inhibition noted with the combined treatment was significantly greater than that observed with oxaliplatin alone or UFT+FA, versus controls.

## Conclusion

This study demonstrates the synergistic activity between UFT+FA and oxaliplatin, and therefore argues strongly in favor of the clinical use of this combined treatment in colon cancer patients.

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*(Received 11 April 2000; revised form accepted 29 May 2000)*