Phase I and Pharmacokinetic Study of Tomudex Combined with 5-Fluorouracil Plus Levofolinic Acid in Advanced Head and Neck Cancer and Colorectal Cancer

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ABSTRACT

In vitro studies have shown a schedule-dependent synergism between Tomudex and 5-fluorouracil (5-FU). Incubation of different types of head and neck and colorectal cancer cells with levofolinic acid (LFA) plus 5-FU for 4 or 24 h, after 24-h incubation with Tomudex, produces a clear synergism. The purpose of this study was to evaluate the tolerability and activity of a combination of Tomudex, LFA, and 5-FU in advanced head and neck and colorectal cancer. Furthermore, the potential for 5-FU pharmacomodulation by Tomudex was also evaluated through an intrapatient assessment of dihydropyrimidine dehydrogenase (DPD) activity and 5-FU AUC with and without pretreatment with Tomudex. Eligible patients were treated with Tomudex at the starting dose of 1.5 mg/m2 on day 1, LFA at a fixed dose of 250 mg/m2 on day 2, immediately followed by bolus 5-FU at the starting dose of 600 mg/m2. Tomudex and 5-FU doses were alternately escalated. Courses were repeated every 2 weeks. In the second course, LFA and 5-FU were administered on day 1 and Tomudex on day 2; further treatment was given according to the sequence used in the first course. Plasma 5-FU concentrations were analyzed on courses 1 and 2 using a high-performance liquid chromatography assay with UV detection. DPD activity was measured in peripheral blood mononuclear cells on courses 1 and 2 using incubation of cytostol with [14C]FU and quantitation of metabolite formation. Fifty-eight patients were enrolled in the study. Dose escalation was stopped at step 8, because of the occurrence of dose-limiting toxicity in two of three patients. The dose level immediately before (3 mg/m2; Tomudex, 1050 mg/m2 5-FU) was selected for further evaluation. Tomudex and 5-FU mean dose intensities actually delivered at the seventh step were 1.32 and 462 mg/m2/week, respectively. Six of 40 patients with metastatic colorectal cancer obtained an objective response (15%; 95% confidence interval, 6–30%). In particular, three complete responses and three partial responses were observed. Six of 17 patients with locally advanced or metastatic head and neck cancer obtained an objective response (1 complete response + 5 partial responses; 35%; 95% confidence interval, 14–62%). Median duration of response in colorectal cancer patients was 12 months. 5-FU AUC was not significantly different between the two courses (median intrapatient difference, 9.3%; P = 0.28). DPD activity in course 1 was significantly higher than course 2 (P = 0.041) in the 16 patients in which values were evaluable. The combination of Tomudex, LFA, and 5-FU is well tolerated and active in colorectal and head and neck cancer. The Tomudex mean dose intensity actually delivered is higher than usually achieved in monotherapy. The absence of a clear pharmacokinetic interaction suggests that the synergism of Tomudex and 5-FU might occur at the cellular level.

INTRODUCTION

5-FU2 is among the most extensively used anticancer drugs. Over the last 20 years, 5-FU has remained the mainstay of treatment in metastatic colorectal cancer, with a response rate that does not exceed 10–15% (1). Furthermore, 5-FU is part of most protocols used in the treatment of other neoplasms, such as head and neck cancer and breast cancer. 5-FU exerts part of its anticancer effects through inhibition of TS, the enzyme that catalyzes the reductive methylation of dUMP to thymidylate, the rate-limiting step in the de novo synthesis of TTP, which is the only nucleotide specifically required for DNA synthesis. However, some of the activity and side effects of 5-FU is attributable

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2 The abbreviations used are: 5-FU, 5-fluorouracil; TS, thymidylate synthase; LFA, levofolinic acid; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; DPD, dihydropyrimidine dehydrogenase; G-CSF, granulocyte-colony stimulating factor; CR, complete response; PR, partial response; PD, progressive disease.
to the incorporation of its metabolite, FUTP, into RNA. The main efforts to increase the activity of 5-FU have been made in the field of biochemical modulation; in particular, LFA has been extensively evaluated in this setting. LFA acts as a source of reduced folates, which optimize the inhibition of TS by increasing the formation of the stabilized ternary complex among TS, 5-dUMP, and reduced folate.

A direct and specific TS inhibition, which does not require modulation and is devoid of nonspecific effects on RNA, has represented an attractive research target. Raltitrexed (Tomudex) has been the first of these drugs to undergo extensive clinical evaluation. Raltitrexed is transported into cells via a reduced folate carrier and is then extensively polyglutamated by the enzyme polyglutamate synthase. The polyglutamated forms are up to 100 times more potent than the parent compound (2) and are retained intracellularly, allowing an intermittent dosing schedule. Phase I studies of raltitrexed were undertaken in Europe (3) and North America (4). In the European study, the MTD was 3.5 mg/m², at which the DLT of severe malaise occurred in five of nine patients. From the results of this study, a dose of 3 mg/m² every 3 weeks was chosen to take forward into the Phase II study. The North American study looked at doses of raltitrexed ranging from 0.6 to 4.5 mg/m², which was acknowledged as the MTD at which DLT (asthenia and/or neutropenia) occurred in five of nine patients. On completion of the North American study, it appeared that some patients were able to tolerate higher doses of raltitrexed, so that the Phase III North American study, which immediately followed the Phase I, included arms for both the 3 and 4 mg/m² doses.

A Phase II study (5) was carried out in Europe in 176 patients with advanced colorectal cancer treated with raltitrexed at the dose of 3 mg/m² every 21 days. A 26% objective response rate was achieved in this study with a median survival of 11.2 months; these results closely resemble those achieved with standard 5-FU + LFA and have prompted three large randomized studies, which have accrued 1361 patients. In these Phase III studies, 3 mg/m² raltitrexed every 3 weeks has been compared with the Mayo regimen of 425 mg/m² 5-FU plus 20 mg/m² leucovorin daily for 5 days every 28–35 days (5, 6) and with the Machover regimen of 400 mg/m² 5-FU plus high-dose leucovorin (200 mg/m²) daily for 5 days every 4 weeks (7). Overall, objective response rates (14–19%) were similar between treatments within each study. Median survival was similar in the two treatment arms in two of three studies, 10.1 versus 10.2 months (6) and 10.9 versus 12.3 months (8), respectively. In the North American study (7), in which the 4 mg/m² arm was stopped prematurely because of toxic deaths, median survival was higher in the 5-FU + leucovorin arm compared with Tomudex (12.7 versus 9.7 months; \( P = 0.01 \)).

Because raltitrexed and 5-FU have proved effective as single agents in the treatment of advanced colorectal cancer and their modalities of action are somewhat different, the combination of the two drugs is a promising approach. Evidence from preclinical studies supports the combined use of raltitrexed and 5-FU. In fact, Longo et al. (9) showed that the cytotoxicity of the Tomudex/5-FU combination was schedule dependent in HCT-8 cells, because synergy was obtained when cells were exposed for 24 h to Tomudex, followed by 5-FU for 4 h. Concomitant drug exposure or the opposite sequence did not produce synergistic effects. Such synergy might occur by an effect of Tomudex on 5-FU pharmacokinetics. In fact, Yan et al. (10) have shown that in rat hepatocytes, Tomudex is able to reduce the activity of DPD, the rate-limiting step in the catabolism of 5-FU, to 12% of control.

Although the concomitant administration of LFA results in in vitro interference with Tomudex uptake and polyglutamation, 4-h rescue of Tomudex growth inhibition with folic acid is much less effective in its protective effect, and this is consistent with substantial amounts of polyglutamates already being formed in the 4-h preincubation period (2). Furthermore, we have demonstrated that incubation of different types of head and neck and colon cancer cells with LFA and/or 5-FU for 4 or 24 h, after 24-h incubation with Tomudex, produces a synergism that is strictly dependent on the three drugs administered in combination, because no clear synergism is observable when only 5-FU or LFA is administered 24 h after Tomudex. Reverse sequence does not produce synergism, thus lending support to the idea of its schedule dependency (11).

On the basis of the above observations, we started a Phase I and pharmacokinetic study of Tomudex followed 24 h later by LFA + 5-FU in advanced head and neck and colorectal cancer to obtain information on the toxicity profile of the combination and the MTD of the two cytotoxic drugs when used in combination. Treatment was recycled every 2 weeks to exploit the potential advantage of a more frequent administration of two phase-specific drugs and possibly to achieve higher dose intensity. The drugs were administered with the reverse sequence in the second cycle, so that an intrapatient assessment of 5-FU pharmacokinetics and DPD activity with and without pretreatment with Tomudex could be performed.

**PATIENTS AND METHODS**

**Patient Selection.** Eligibility criteria for study entry included histologically or cytologically confirmed locally advanced or metastatic squamous cell carcinoma of the head and neck or colorectal carcinoma; no more than one line of previous chemotherapy; an Eastern Cooperative Oncology Group performance status of 0–2; adequate baseline organ function defined as a WBC of ≥3,000/µl, a platelet count of ≥100,000/µl; a bilirubin level of <1.5 mg/dl; serum transaminase levels of <2 times the upper limit of normal; a creatinine clearance ≤60 ml/min; and life expectancy of at least 3 months. Written informed consent was obtained from each patient.

**Treatment Plan.** Treatment consisted of Tomudex given on day 1 by 15-min i.v. infusion in 250 ml of normal saline solution and 5-FU given as an i.v. bolus injection on day 2, immediately preceded by LFA, which was administered in 500 ml of normal saline solution over 2 h. Standard antiemetic prophylaxis was used on both days. The Tomudex starting dose was 1.5 mg/m²; 5-FU starting dose was 600 mg/m², and the LFA dose was fixed at 250 mg/m². Tomudex and 5-FU doses were subsequently escalated alternately. Doses were assigned at registration, and no dose escalation was permitted in individual patients. In the second course, LFA and 5-FU were administered on day 1 and Tomudex on day 2 to facilitate pharmacology studies; further treatment after the second course was administered according to the sequence used in the first course. Treat-
Tomudex, 5-FU, and LFA in Head and Neck and Colorectal Cancer

was recycled every 14 days and withheld for 1 week (until day 22) if the neutrophil count was <1,500/µL, platelet count was <100,000/µL, or hemoglobin <9.5 mg/dL at the time of chemotherapy recycling. If treatment could not be administered on day 22 because of persistent toxicity, it was postponed to day 29; in this case, a 25% dose reduction was applied to Tomudex and 5-FU. If hematological recovery did not occur within 2 weeks, treatment was discontinued. In case of grade 4 bone marrow toxicity or febrile neutropenia occurring at nadir, even after full recovery, Tomudex and 5-FU were administered with a reduction to 75% of the planned dose over subsequent courses. If further grade 4 neutropenia occurred also with this dose reduction, an additional 25% dose reduction was applied for both drugs. In case of grade 3 reduction, an additional 25% dose reduction was applied for both drugs. In case of grade 3 or 4 neutropenia with bleeding, grade 3 nonhematological toxicity (except alopecia, nausea, and vomiting) occurring at day 15, the drug administration was delayed until grade 1 up to day 29. The use of G-CSF was allowed in the presence of grade 4 neutropenia or febrile neutropenia. Cohorts of at least three patients were treated at each dose level. Dose escalation was performed if no patients had DLT after the first cycle. If one of three patients had DLT, three more patients were enrolled at that level. Dose escalation was stopped if more than one-third of patients of a given cohort had DLT, which was defined as grade 4 neutropenia or thrombocytopenia, grade 3 febrile neutropenia, grade 3 thrombocytopenia with bleeding, grade 3 nonhematological toxicity (except for nausea and alopecia), or >2-week delay in recycling. MTD was defined as the dose level causing DLT in more than one-third of patients.

**Patient Evaluation.** A complete history, physical examination, recording of performance status, CBC count with differential, serum biochemistry, urinalysis, electrocardiogram, and chest x-ray were obtained at baseline for each patient. Disease extension was evaluated by thoracic and cervical computed tomographic scan or magnetic resonance imaging for patients with head and neck cancer and by abdominal computed tomographic scan or ultrasonography for patients with colorectal cancer. Other exams were performed only in the presence of a clinical indication. Patients were monitored weekly throughout treatment by physical examination and by recording of toxic effects; complete blood count count was performed twice a week during the first treatment cycle and weekly thereafter, along with a complete chemistry profile. Evaluation of tumor response was performed every four cycles of chemotherapy with repetition of all tests that were abnormal at baseline. A CR was defined as the complete disappearance of all signs and symptoms of disease. A PR was defined as a >50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions; both CRs and PRs were required to persist for at least 4 weeks. Stable disease was defined as a <50% reduction or a <25% increase in the sum of the products of two perpendicular diameters of all measured lesions; both CRs and PRs were required to persist for at least 4 weeks. Stable disease was defined as a <50% reduction or a <25% increase in the sum of the products of two perpendicular diameters of all measured lesions; both CRs and PRs were required to persist for at least 4 weeks. Stable disease was defined as a <50% reduction or a <25% increase in the sum of the products of two perpendicular diameters of all measured lesions; both CRs and PRs were required to persist for at least 4 weeks. Stable disease was defined as a <50% reduction or a <25% increase in the sum of the products of two perpendicular diameters of all measured lesions; both CRs and PRs were required to persist for at least 4 weeks. Stable disease was defined as a <50% reduction or a <25% increase in the sum of the products of two perpendicular diameters of all measured lesions; both CRs and PRs were required to persist for at least 4 weeks. Stable disease was defined as a <50% reduction or a <25% increase in the sum of the products of two perpendicular diameters of all measured lesions; both CRs and PRs were required to persist for at least 4 weeks. Stable disease was defined as a <50% reduction or a <25% increase in the sum of the products of two perpendicular diameters of all measured lesions; both CRs and PRs were required to persist for at least 4 weeks.

**5-FU Pharmacokinetics.** Blood samples for 5-FU pharmacokinetics were collected at the end of infusion and 5, 15, and 60 min after infusion. Samples were placed in ice and centrifuged at 3000 × g for 5 min. Plasma was then stored at −20°C until transfer to Aberdeen on dry ice. Plasma 5-FU concentrations were analyzed in triplicate using a previously described high-performance liquid chromatography assay with UV detection (12). The assay had a limit of detection of 10 ng/mL, was linear from 25 to 1500 ng/mL, and had an interassay coefficient of variation of <8%. Evaluation of dilution protocols demonstrated reproducible detection of plasma concentrations up to 1 ng/mL. An iterative two-stage analysis was used to estimate 5-FU pharmacokinetic parameters in a two-compartment model with nonlinear elimination, using ADAPT II software (13). Repeated Bayesian analysis, using an updated covariance matrix and parameter values from the previous iteration, was then conducted until <1% change in mean values for all pharmacokinetic parameter estimates occurred. Twelve iterations were required to achieve the final Bayesian parameters for analysis of patient data in this study. Because incomplete data collection occurred in 6 of 20 patients in this study, the ADAPT II software was used to simulate missing data points using the individual patient Bayesian pharmacokinetic parameters. 5-FU AUC from start therapy (time zero) to 60 min after infusion was then determined using the trapezoidal rule and expressed as µg/mL/min. 5-FU systemic clearance was calculated as dose divided by AUC.

**DPD Activity.** Blood samples were obtained 24 h after the first dose of Tomudex (course 1) and 14 days after the first dose of Tomudex (prior to course 2). The samples were taken between 9 a.m. and 11 a.m. and prior to 5-FU administration to avoid the influence of circadian variation and drug effect (14, 15). Peripheral blood mononuclear cells were isolated from 20 ml of heparinized blood using a Ficoll-Hypaque centrifugation approach (16). Samples were frozen at −20°C until transfer to Aberdeen on dry ice. DPD activity was measured using incubation of cytosol with [14C]FUT and quantitation of metabolite formation, as described previously in detail (16). Activity was expressed as pmol of metabolite formed per minute of incubation and standardized per milligram of cytosolic protein (pmol/min/mg protein).

**Statistical Analysis.** The statistical significance of differences in 5-FU AUC, systemic clearance, and DPD activity between course 1 and course 2 were evaluated using the Wilcoxon signed-ranks test. The influence of Tomudex or 5-FU dosing cohort on AUC, systemic clearance, or DPD activity was assessed using the Kruskal-Wallis test.

**RESULTS**

**Patient Characteristics.** From June 1997 to March 1998, 58 patients were enrolled in this Phase I study. Median age was 58 years (range, 22 to 72); Eastern Cooperative Oncology Group performance status was 0 in 10 patients, 1 in 32 patients, and 2 in 16 patients. Seventeen patients had locally advanced or metastatic head and neck cancer, and 41 patients had metastatic colorectal cancer; the majority of the patients had received prior chemotherapy for metastatic disease, which included in all cases 5-FU. Patient characteristics are detailed in...
Dose Escalation Results. A total of eight dose levels were tested (Table 2). At the last dose level (3 mg/m² Tomudex, 1200 mg/m² 5-FU), two of three treated patients showed a DLT. In particular, in one patient a grade 4 neutropenia was observed, whereas another patient had a transient grade 3 nephrotoxicity. The third patient had a nonfebrile grade 3 neutropenia. Therefore, this dose level was defined as MTD, and the seventh dose level (3 mg/m² Tomudex, 1050 mg/m² 5-FU) was further expanded up to 16 patients (mostly chemo-naive), with the aim of obtaining a more reliable estimate of the activity of the regimen. This dose level was feasible, because only 2 of 16 patients showed a DLT after the first cycle.

Hematological Toxicity. Neutropenia was not observed at the first dose level, was observed (grade 1) in one of six patients, and was observed in two of six patients at the second and third dose levels, respectively, and was again totally absent at the fourth dose level. Grade 2 neutropenia occurred in one of seven patients at the fifth dose level, whereas at the sixth dose level, the first case of grade 4 neutropenia was observed, along with two additional cases of grade 2 neutropenia. At the eighth dose level (3 mg/m² Tomudex, 1200 mg/m² 5-FU), one of three patients had grade 4 neutropenia, and one patient had uncomplicated grade 3 neutropenia (not dose limiting). This level was selected as the MTD because a grade 3 nephrotoxicity occurred as well, and the preceding dose level (3 mg/m² Tomudex, 1050 mg/m² 5-FU) was expanded up to 16 patients. At this dose level, neutropenia occurred in six patients, reaching grade 4 in two of them; two additional patients at this dose level had grade 3 neutropenia, and two had grade 2 patients neutropenia. In all four cases of grade 4 neutropenia, none of which was febrile, G-CSF was given at the dose of 300 μg/day for 3 consecutive days, and full hematological recovery occurred in a median of 3 days (range, 2 to 4).

Thrombocytopenia was an extremely mild toxic effect and never exceeded grade 1, even at the highest dosages. Anemia was, on the contrary, a more frequent than expected toxic effect, especially at lower dosages. In fact, four of six patients at the first dose level had anemia, which reached grade 3 in one case.

This unexpected finding led us to increase the number of patients to be treated at each dose level to a minimum of six since the first dose level. However, the incidence of anemia did not appear to increase significantly with the higher dosages, and only 11 additional cases of anemia were recorded, two of which reached grade 3 at dose levels 5 and 7, respectively. The three patients who had grade 3 anemia received a median number of two (range, 1–3) transfusions of RBC bags.

Nonhematological Toxicity. Nonhematological side effects were generally mild. Grade 1–2 alopecia was observed in only five patients. Nausea and vomiting were generally well controlled by 5-hydroxytryptamine, receptor blockers, occurring in eight patients, but reaching grade 3 in only one patient. Renal toxicity occurred in three patients, and in one of them reached grade 3, thus qualifying as DLT. However, in this patient, adequate i.v. hydration completely reversed the side effect within 7 days. Liver enzyme derangement was observed in five cases and was always reversible. Mucositis was quite frequent (15 of 58 patients) and reached grade 3 in three patients, leading in two cases to the prescription of parenteral nutrition up to the resolution of toxicity. In one case, a grade 3 phlebitis occurred, and heparin treatment was promptly instituted to prevent embolic complications; this toxic effect reversed in 2 weeks. Grade 1–2 Hand-Foot syndrome was observed in six patients, whereas diarrhea occurred in eight patients and reached grade 3 in only one case. Grade 1–2 fatigue occurred in only six patients and, like all other toxicities, was reversible and rapidly vanished after treatment interruption. Grade 3–4 hematological and nonhematological toxicities are detailed in Table 3.

Treatment Delays. Treatment was delayed 1 week in 56 courses (15%) because of neutropenia and/or other toxicities. Tomudex and 5-FU doses were reduced by 25% in 41 courses (11%). Tomudex and 5-FU mean dose intensities actually delivered at the seventh step were 1.32 and 462 mg/m²/week, respectively. No patients were withdrawn from the study because of failure of recovery from toxicity.

Response Evaluation. Fifty-seven patients completed at least four cycles of treatment and were evaluable for response. One patient with metastatic colorectal cancer had grade 4 neutropenia after the first cycle and withdrew from the study after the second course. The combination of Tomudex and 5-FU + LFA showed a significant degree of antitumor activity. Six of 40 patients with metastatic colorectal cancer obtained an objective response.

Table 1. A total of 359 courses of treatment were given for a median of 6 courses/patient (range, 2–12).

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<th>Age</th>
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<th>Previous (5-FU-based) chemotherapy</th>
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* Total number of patients, 58.

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<tr>
<th>Table 2 Dose escalation scheme</th>
<th>Tomudex/LFA/5-FU (mg/m²)</th>
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<th>DLT type</th>
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<td>2.0/250/600</td>
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| 8                             | 3.0/250/1200             | 3              | 2      | 1 patient, grade 4 neutropenia
|                               |                          |                |        | 1 patient, grade 3 renal toxicity |

Table 2. Level 1 patients DLT no. DLT type

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response (15%; 95% confidence interval, 6–30%). In particular, three CRs and three PRs were observed in this patient population, which mainly consisted of pretreated patients (33 of 40). Two CRs and one PR were observed in seven chemo-naive patients, for an overall response rate of 43%, whereas one CR and two PRs were recorded in pretreated patients, for an overall response rate of 9%. Six of 17 patients with locally advanced or metastatic head and neck cancer obtained an objective response (35%; 95% confidence interval, 14–62%). In particular, one CR and five PRs were observed. Patients with head and neck cancer were more frequently chemo-naive than patients with colorectal cancer (8 of 17). One PR was observed in a patient who had been treated previously with a combination of cisplatin, methotrexate, LFA, and 5-FU; the other five objective responses, i.e., those achieved at the last two dose levels, were all obtained in chemo-naive patients, for an overall response rate of 62.5% in chemo-naive patients. No responses were observed at the first two dose levels. At the third dose level, a patient with pretreated metastatic colorectal cancer achieved a PR; at the fourth dose level, surprisingly two CRs (one in a pretreated patient) were observed in metastatic colorectal cancer and one PR in a patient with pretreated advanced head and neck cancer. No objective responses were obtained at the fifth dose level, whereas at the sixth dose level, one CR was recorded in a chemo-naive patient with metastatic colorectal cancer and one PR in a pretreated patient metastatic colorectal cancer. The seventh dose level was expanded up to 16 patients and was mainly restricted to chemo-naive patients to have a more reliable estimate of the activity of the regimen. In this step, we observed one CR in a patient with chemo-naive locally advanced head and neck cancer and four PRs, among which there were three in chemo-naive patients with head and neck cancer and one in a patient with pretreated metastatic colorectal cancer. One PR in a chemo-naive patient with head and neck cancer was obtained at the eighth dose level.

In all cases but one, responses were recorded at the first disease evaluation after four cycles. One patient who had stable disease after four courses showed a PR after eight courses. Median duration of response in colorectal cancer was 12 months (range, 6–14+ months). Because responding patients with untreated locally advanced head and neck cancer underwent either radiotherapy and/or surgery after chemotherapy, a duration of response for these patients cannot be calculated.

**Pharmacokinetics.** Analysis of 5-FU pharmacokinetics was performed 24 h (course 1) and 14 days (course 2) after the first dose of Tomudex. Plasma sampling for 5-FU pharmacokinetics from both course 1 and course 2 was available for 20 patients. End of infusion plasma concentration ranged from 13.9 to 486.7 μg/ml. 5-FU pharmacokinetics were best characterized by a two-compartment model with nonlinear elimination. Simulation studies of plasma 5-FU concentrations, using actual patient pharmacokinetic parameters, demonstrated a high degree of variability in estimated concentrations at time points >1 h after the start of infusion. Therefore, 5-FU AUC was calculated for the first 60 min after the start of infusion (AUC<sub>60</sub>) to allow more definitive comparisons between results from courses 1 and 2. A 3.8- and 7.1-fold range in AUC<sub>60</sub> was observed for courses 1 and 2, respectively (Table 4). However, AUC<sub>60</sub> was not significantly different between the two courses (median intrapatient difference, 9.3%; P = 0.28; Fig. 1). Analysis of 5-FU systemic clearance demonstrated similar findings. A 5.3- and 8.8-fold range in systemic clearance was observed in courses 1 and 2, respectively (Table 4). The intrapatient median change in systemic clearance was an 8.8-fold range in systemic clearance was observed in courses 1 and 2, respectively (Table 4).

### Table 3  Grade 3–4 hematological and nonhematological toxicities in all delivered cycles<sup>a</sup>

<table>
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<th>Dose level</th>
<th>No. of patients</th>
<th>Neutropenia</th>
<th>Anemia</th>
<th>Mucositis</th>
<th>Diarrhea</th>
<th>Nausea/Vomiting</th>
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<sup>a</sup> Total number of patients, 58.

### Table 4  5-FU pharmacokinetics and DPD activity in patients after Tomudex treatment (course 1) or before Tomudex treatment (course 2)

| No. of patients | Course 1 | | Course 2 | | Difference | |
|----------------|---------|----------|----------|------------|----------|
|                | Median  | Range    | Median   | Range     | % median | P        |
|                |         |          |          |           |          |          |
| AUC (μg/ml/min) | 20      | 1917–4599| 2034     | 747–5270  | 9.3      | 0.28     |
| CL<sup>a</sup> (ml/min/m<sup>2</sup>) | 20      | 346–870  | 355      | 114–1004  | −8.5     | 0.71     |
| DPD (pmol/min/mg) | 16      | 66–626   | 137      | 54–450    | −26.4    | 0.041    |

<sup>a</sup> CL, clearance.
Mononuclear cells were obtained for DPD activity 24 h after (course 1) and before (course 2) Tomudex administration. Activity values were evaluable for both courses 1 and 2 in 16 patients. Samples that had low cytosolic protein or high erythrocyte contamination were excluded from the analysis. A 9.5- and 8.3-fold range in activity was observed for courses 1 and 2, respectively (Table 4). DPD activity in course 1 was significantly higher than course 2 (Fig. 2; $P = 0.041$). The median absolute difference in DPD activity was a decrease of 42 pmol/min/mg protein. This was a consistent finding, occurring in 11 of 16 patients (Fig. 2). The relationship between 5-FU systemic clearance and DPD activity could not be evaluated, because there were only a limited number of patients in which complete data were available for both variables. There was no relationship between the dose of either Tomudex or 5-FU and the change in DPD activity from course 1 to 2 ($P > 0.5$).

### DISCUSSION

TS is an important target for chemotherapy against colorectal cancer, head and neck cancer, and other solid tumors. Although Tomudex and 5-FU share TS as a target for their anticancer action, preclinical studies support the combined use of the two drugs. In fact, Longo et al. (9) showed that synergistic cell kill was observed when HCT-8 colon carcinoma cells were exposed to Tomudex for 24 h, followed by 4 h of exposure to 5-FU. Marginal synergy was obtained with the same sequence but with a 5-day exposure to 5-FU. The reverse sequence resulted in less than additive cell kill. In this study, the authors looked at the mechanism of synergistic effect, which was not attributable to augmented inhibition of TS but rather to either an inhibitory effect on dihydrofolate reductase or an indirect effect on purine biosynthesis, leading to a 5–6-fold increase in intracellular level of phosphoribosylpyrophosphate (9). This increase results in augmented 5-FU nucleotide formation and an ~30% increase in FUTP and FdUMP levels when compared with cells not pretreated with Tomudex. The consequent high 5-FU incorporation into RNA is probably the key event leading to enhanced cell death. These authors in a subsequent experiment added leucovorin to 5-FU and interestingly observed that leucovorin not only did not reverse the synergistic effect but possibly enhanced it (9).

Two clinical studies that explore the toxicity and the activity of the combination Tomudex/5-FU are currently ongoing. Schwartz et al. (17) are running a Phase I trial of sequential administration of Tomudex on day 1 and bolus 5-FU on day 2 every 3 weeks in pretreated patients with metastatic colorectal cancer. In this study, dose-limiting febrile neutropenia has been observed at a Tomudex dose of 3.0 mg/m² and a 5-FU dose of 1350 mg/m². Four objective responses with one CR have been observed in 28 pretreated patients (14%), whereas in 10 patients, disease has remained stable. In this study, at Tomudex doses ≥2.5 mg/m², a pharmacokinetic interaction between Tomudex and 5-FU seems to be evident, translating into an increase in mean 5-FU $C_{max}$ and mean 5-FU AUC. Harstrick et al. (18) are conducting a Phase I study in chemo-naive patients with metastatic colorectal cancer, in which 5-FU is administered by 24-h infusion on a weekly schedule for 5 weeks at four different dose levels (from 1200 to 2400 mg/m²), and Tomudex is given along with 5-FU on weeks 2 and 5 at two different dose levels (2.6 and 3 mg/m²). Myelosuppression and diarrhea have represented DLT in this study, whereas MTD has been 3 mg/m² Tomudex and 2400 mg/m² 5-FU. From dose levels 3 to 6, 9 of 17 patients (53%) have achieved a PR. Also in this study, Tomudex has shown a significant impact on 5-FU pharmacokinetics, increasing mean 5-FU $C_{max}$ and AUC, and prolonging its terminal half-life. The results of the above two studies point to the Tomudex/5-FU combination as a feasible approach. In fact, the combined treatment is well tolerated and permits the achievement of the drug doses that are used in monotherapy. The activity of the regimen looks noteworthy, because in the American study, a 14% overall response rate (including one CR) has been achieved in pretreated patients, and in the German study, a 53% PR rate in chemo-naive patients treated at the highest dose levels has been observed. Furthermore, both studies suggest a

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**Fig. 1** Influence of Tomudex therapy on 5-FU AUC ($n = 20$; $P = 0.28$).

**Fig. 2** Influence of Tomudex therapy on DPD activity ($n = 16$; $P = 0.041$).
pharmacokinetic interaction between the two drugs with an increase in 5-FU $C_{\text{max}}$ and AUC induced by previous or concomitant treatment with Tomudex. These data point to a possible 5-FU pharmacokinetic modulation by Tomudex (10).

The concept of adding LFA to 5-FU when used in combination with Tomudex is much more intriguing. LFA interferes with Tomudex uptake and polyglutamation, which is required to synthesize the active form of the drug. However, some preclinical data suggest that this interference is greatly reduced when a 4-h interval elapses between Tomudex and LFA, which seems to be enough for Tomudex to undergo uptake and polyglutamation (2). We have recently completed some in vitro studies (11) in which we have evaluated the cytotoxicity of Tomudex, 5-FU, and LFA when used in several different schedules in three colon cancer cell lines (LoVo, GEO, and SW 620) and in three head and neck cancer cell lines (KB, ZA, and HOC313). The most interesting finding in our study was the clear synergism that was observed in all tested cell lines when cells were exposed for 24 h to Tomudex and then for 4 or 24 h to 5-FU and LFA, whereas only an additive effect was shown when 5-FU alone was added 24 h after Tomudex, thus indicating an important role for LFA in this schedule-dependent synergistic effect. Furthermore, even in the Longo study (9), the addition of LFA to 5-FU 24 h after Tomudex resulted in clear synergism.

The results of our dose escalation clearly demonstrated that it is possible to combine Tomudex and 5-FU at the doses used in monotherapy. This is the first study in which Tomudex is given in a biweekly schedule, and this has allowed us to achieve a dose intensity (1.32 mg/m²) at the recommended dose level, which is substantially higher than that achievable with standard every 3 weeks schedule. Such a high-dose intensity could be achieved because toxicity was moderate and noncumulative. In fact, in our study, hematological toxicities were very well manageable. No toxic deaths occurred. Only four cases of grade 4 neutropenia were recorded; febrile neutropenia never occurred, and 3-day administration of G-CSF induced prompt and complete reversal of toxicity. Myelotoxicity was not cumulative. Thrombocytopenia was recorded very rarely, whereas anemia was a frequently observed finding, even at low drug dosages, requiring RBC transfusion in three patients. Extrahematological toxicity was mild. Grade 3 nephrotoxicity was observed in one patient, but indeed it was reversible after treatment interruption and adequate i.v. hydration. Mucositis was observed in 15 patients but reached grade 3 in only three cases, in two patients demanding parenteral nutrition. Expected toxicities, such as fatigue and Hand-Foot syndrome, were actually observed but never caused particular concern. The activity shown by our combination in metastatic colorectal cancer is really noteworthy. In fact, three patients, one of whom had already undergone previous chemotherapy for metastatic disease, achieved a CR to treatment, which lasted 12, 13+, and 14+ months, respectively. Three more patients (two of whom were pretreated) achieved a PR. These are the first published final results of this drug combination and are at least as favorable as those achieved preliminarily by Schwartz et al. (17), who obtained a 14% objective response rate but only one CR in pretreated patients with metastatic colorectal cancer. Very encouraging activity was observed in advanced head and neck cancer as well, with a 35% overall response rate and a 62.5% response rate in chemo-naive patients.

DPD activity was significantly higher in samples obtained 24 h after Tomudex administration, compared with those obtained prior to administration of the second course of chemotherapy. The DPD sample was obtained prior to 5-FU therapy in both courses to minimize any influence of 5-FU on DPD activity (15). These data are consistent with induction of DPD activity by Tomudex and are not in keeping with reported preclinical data (10), which, however, have been obtained in rat hepatocytes and might be at least partly explained by DPD ex vivo down-regulation (16). Our observed DPD induction may reflect a biological feedback loop in which DPD activity is increased to accommodate an increase in uracil nucleotides resulting from inhibition of TS by Tomudex (19). The pharmacokinetic parameters observed in this study are similar to those described after other high-dose 5-FU regimens (20). 5-FU systemic clearance in patients receiving 600–1050 mg/m² over 5 min ranged from 113.8 to 1003.8 ml/min/m², without a significant difference between courses 1 and 2. There was high variability and no statistically significant change also in 5-FU AUC between course 1 and course 2 (range, 84% decrease to 157% increase). This is similar to that described after bolus administration of 600 mg/m² 5-FU to patients with breast cancer (21). These studies provide evidence that inpatient variation in 5-FU pharmacokinetics is high and may be the limiting step in approaches to optimize 5-FU therapy through use of therapeutic drug monitoring. The finding of a significant change in mononuclear cell DPD activity without altered 5-FU systemic clearance may be attributable to the high plasma concentrations of 5-FU, which were greater than the DPD $K_{m}$ (2 mg/ml) for much of the study period. Substrate inhibition of DPD has been demonstrated in vitro for DPD and would not be detected by measurement of pretherapy DPD activity alone. Alternatively, peripheral blood mononuclear cell DPD activity may be inadequate for prediction of 5-FU systemic clearance (22). The good clinical activity of the combination, expressed by both the high response rate and the duration of response, points to a potential synergism between the three drugs, despite the absence of an alteration in systemic 5-FU pharmacology. This is consistent with any potential synergism in antitumor activity occurring at the cellular level, in keeping with Longo’s study, in which cellular pharmacology studies suggested an enhancement of 5-FU RNA incorporation as a potential mechanism for in vitro synergy with Tomudex. If this is the case, Tomudex and methotrexate might partly share the mechanism of biochemical interaction with 5-FU, and this can explain the response rate in our pretreated patients, which is substantially lower than in chemo-naive patients. In fact, previous chemotherapy in colorectal cancer consisted of a regimen of methotrexate, LFA, and 5-FU (23), whereas in head and neck cancer, it included cisplatin, methotrexate, LFA, and 5-FU (24). However, we trust that a DPD modulation cannot be ruled out and are trying further to clarify this issue.

Although evaluation of antitumor activity was not the main end point of the study and the sample size is too small to draw conclusions about the efficacy of the combination, we believe that the activity shown by our regimen is encouraging enough to justify a Phase II study, which has been started in chemo-naive...
patients with metastatic colorectal cancer. With regard to head and neck cancer, taking into account both its higher chemosensitivity with respect to colorectal cancer, and preclinical evidence of absence of overlapping between tumor-inhibitory properties and mechanisms of resistance of cisplatin and Tomudex (25), we have decided to add cisplatin to our regimen, starting a Phase I–II trial of cisplatin and Tomudex on day 1, followed by LFA and 5-FU on day 2 as front-line therapy in newly diagnosed patients with locally advanced and metastatic squamous cell carcinoma of the head and neck.

REFERENCES

Phase I and Pharmacokinetic Study of Tomudex Combined with 5-Fluorouracil Plus Levofolinic Acid in Advanced Head and Neck Cancer and Colorectal Cancer

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