Published in final edited form as:

Gynecol Oncol. 2007 March ; 104(3): 572–579.

PHASE II CLINICAL TRIAL OF CAPECITABINE IN THE TREATMENT OF ADVANCED, PERSISTENT OR RECURRENT SQUAMOUS CELL CARCINOMA OF THE CERVIX WITH TRANSLATIONAL RESEARCH: A GYNECOLOGIC ONCOLOGY GROUP STUDY

Agustin A. Garcia, M.D.¹, John A. Blessing, Ph.D.², Kathleen M. Darcy, Ph.D.³, Heinz Josef Lenz, M.D.⁴, Wu Zhang, M.D.⁵, Ed Hannigan, M.D.^{6,*}, and David H. Moore, M.D.⁷

¹ Director, Breast Cancer Program, Women's Cancer Research Institute, Cedars Sinai Medical Center, Los Angeles, CA 90048

² Executive Director, Gynecologic Oncology Group Statistical and Data Center, Roswell Park Cancer Institute, Buffalo, NY 14263

³ Translational Research Scientist, Gynecologic Oncology Group Statistical and Data Center, Roswell Park Cancer Institute, Buffalo, NY 14263

⁴ Associate Professor of Medicine, University of Southern California Keck School of Medicine Norris Comprehensive Cancer Center, Los Angeles, CA 90089

⁵ Research Associate, University of Southern California Keck School of Medicine Norris Comprehensive Cancer Center, Los Angeles, CA 90089

⁶ Director, Division of Gynecologic Oncology, University of Texas, Galveston Medical Branch, Galveston, TX 77555

⁷ Mary Fendrich Hulman Professor, Chief of Gynecologic Oncology, Indiana University School of Medicine, Indianapolis, IN 46202

Abstract

Purpose— To evaluate the anti-tumor activity and adverse events of capecitabine in advanced, persistent or recurrent squamous cell carcinoma of the cervix, and to explore biomarkers with the potential to predict capecitabine response and toxicity.

Corresponding Author: Agustin A. Garcia, MD, Premiere Oncology, 2020 Santa Monica Blvd., Santa Monica, CA 90404, Phone: 310-633-8400; Fax: 310-633-8419, Email: agarcia@premiereoncology.com.

* Affiliate of the University of Mississippi

For Reprints: Ms. Denise Mackey, Gynecologic Oncology Group, Four Penn Center, 1600 JFK Blvd. - Suite 1020, Philadelphia, PA 19103, Phone: 215-854-0770, Email: dmackey@gog.org

This study was supported by National Cancer Institute grants to the Gynecologic Oncology Group Administrative Office (CA 27469) and the Gynecologic Oncology Group Statistical Office (CA 37517).

The following member institutions participated in this study: University of Mississippi Medical Center, Indiana University School of Medicine, Wake Forest University School of Medicine, University of California Medical Center at Irvine, University of Texas-Galveston.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Experimental Design—Eligible, consenting patients were treated with a starting dose of 2500 mg/m²/day or 1800 mg/m²/day (divided into two doses given every 12 hours) for 14 days of each 21-day cycle. Prior chemotherapy was allowed only in the context of radiation “sensitization”. Genotyping in the 5’ and 3’ ends of thymidylate synthase (TS) was performed in DNA from pre-treatment blood. Relative gene expression of TS, dihydropyrimidine dehydrogenase (DPD) and thymidine phosphorylase (TP) was quantified in RNA extracted from paraffin-embedded tumor.

Results—All patients had prior radiotherapy and 22 received a radiation sensitizer. A partial response was observed in 4 of 26 (15%) evaluable patients. An additional 35% of patients achieved stable disease while 42% experienced increasing disease. The most common serious non hematological toxicities were gastrointestinal and dermatologic. Exploratory analyses suggested that: a germline polymorphism in the 3’ or the 5’ end of TS was not associated with TS gene expression, relative tumor expression of TS, DPD and TP were not correlated, and relative tumor expression of TP may predict severe anemia.

Conclusions—Based on the modest response rate, this trial was closed without a second stage of accrual; single agent capecitabine was not selected for further study in advanced persistent or recurrent squamous cell carcinoma of the cervix previously treated with radiation or chemoradiation.

Keywords

Capecitabine; xeloda; cervical cancer; TS; DPD; TP; biomarkers

INTRODUCTION

Cervical cancer is the second most common cancer in women worldwide and, in developing countries, it is the leading cause of death by cancer. In the United States, the estimated numbers of new cervical cancer cases and deaths in 2005 are 10,370 and 3,710, respectively [1].

The treatment of advanced or recurrent cervical cancer has been improved by the introduction of new active chemotherapy agents of which cisplatin, ifosfamide and paclitaxel are traditionally described as the most active [2–5]. However, continued identification of more effective agents used individually or in combination is of great importance for patients with the aggressive forms of this cancer. For example, the fluoropyrimidines have been studied in this patient population. In previously untreated cervical cancer, the response rate for single agent 5-fluorouracil (5-FU) is approximately 10–20% [1,6].

Attempts have been made to increase the anti-tumor activity of the fluoropyrimidines. Based on the observation that leucovorin increases the activity of 5-FU in colorectal cancer, the Gynecologic Oncology Group (GOG) conducted two studies evaluating this combination in patients with recurrent adenocarcinoma [7] or recurrent squamous cell carcinoma [8] of the cervix. Unfortunately, the combination of 5-FU and leucovorin exhibited a response rate below 15% in both of these trials [7,8].

More recently, the GOG considered capecitabine, (N4-pentylocarbonyl-5’deoxy-fluorocytidine), an orally bioavailable fluoropyrimidine carbamate which is easily absorbed from the gastrointestinal tract and preferentially converted in malignant tissues to 5-fluorouracil [9]. Capecitabine has documented activity in breast [10,11] and colon [12] cancer. In preclinical models, it appears that capecitabine is more active than 5-FU; as in cervical cancer human xenografts, the former produces a 66–98% tumor growth inhibition, while 5-FU produces only a 2–20% inhibition [13]. An anecdotal, impressive response to capecitabine was reported [14]. Furthermore, it appears that capecitabine exhibits non-cross resistance with several other chemotherapy agents, as seen in breast cancer where capecitabine has been reported to yield a 25% response rate in chemotherapy resistant tumors [11].

Fluoropyrimidines have been shown to inhibit thymidylate synthase (TS), be detoxified by dihydropyrimidine dehydrogenase (DPD), and be activated by thymidine phosphorylase (TP), which is present in higher amounts in malignant tissue than non-neoplastic tissue [15,16]. Given the pivotal role that these enzymes play in regulating the cytotoxicity and metabolism of these agents, investigators have tested the hypothesis that these enzymes may be useful predictors of response and toxicity to a fluoropyrimidine-based therapy. For example, high gene expression of TS (determined by reverse transcriptase-polymerase chain reaction [RT-PCR]) has been shown to be associated with a low response to 5-FU (less than 5% response) in metastatic colon cancer. Although high TS expression effectively predicted non-response, low TS expression does not necessarily predict response, as a subset of non-responding patients also exhibited low TS expression [17,18]. Gene expression of TP and DPD has also been proposed to provide the additional predictive information, as tumors with low TS and TP expression were shown to exhibit a response rate of over 80%, while those with high TS and TP had a response rate of zero [19,20]. Finally, certain germline polymorphisms in TS have been shown to influence the level of TS expression [21], which supports the hypothesis that genotyping patients for TS polymorphisms may also serve as a useful predictive marker of fluoropyrimidine response and toxicity [21,22], and, unlike the determination of intratumoral gene expression which requires available tumor tissue, genotyping can be performed on blood, which is more readily available.

Based on this rationale, the GOG conducted a phase II clinical trial to evaluate the anti-tumor activity and adverse effects of capecitabine in patients with advanced, persistent or recurrent squamous cell carcinoma of the cervix. This protocol also incorporated translational research to explore the ability of gene expression of TS, DPD and TP in archival tumor tissue and TS genotype in a pre-treatment blood specimen to predict capecitabine response and toxicities in this patient population.

MATERIALS AND METHODS

Patients

Patients were required to have histologically-confirmed advanced, persistent or recurrent squamous cell carcinoma of the cervix with documented disease progression after local therapy with measurable disease defined as lesions that could be measured in at least one dimension by physical examination or imaging techniques. Patients must have at least one “target lesion” to be used to assess response on this protocol as defined by RECIST criteria [23]. Tumors within a previously irradiated field will be designated as “non-target” lesions. Patients were not allowed to have received prior chemotherapy, except when used as a radiosensitizer (5-FU was allowed in such cases). Any prior therapy directed to the malignant tumor had to be discontinued at least 3 weeks prior to study entry. Participating patients were required to have adequate bone marrow (absolute granulocytes $\geq 1500/\mu\text{l}$ and platelets $\geq 100,000/\mu\text{l}$), renal (estimated creatinine clearance $\geq 50 \text{ mL/min}$), and hepatic (bilirubin ≤ 1.5 times the upper limit of normal, serum glutamic oxaloacetic transaminase (SGOT) and alkaline phosphatase ≤ 2.5 times the upper limit of normal) functions. Patients were to have a GOG performance status of 0–2. Patients who received one or more doses of treatment and lived 3 weeks were considered evaluable for response and patients who received at least one dose of therapy were evaluable for toxicity. Patients provided written informed consent consistent with federal, state and local institutional requirements to participate in the clinical and translational research components of this protocol prior to receiving any protocol therapy. In addition, the protocol was approved by the GOG, the Cancer Therapy Evaluation Program of the National Cancer Institute and the institutional review board at each of the participating GOG institutions, and was performed in accordance with assurances filed with and approved by the Department of Health and Human

Services. Histologic diagnosis was confirmed for each patient enrolled on this protocol by members of the GOG Pathology Committee.

Treatment

Patients were treated with a daily starting dose of 2500 mg/m²/day (divided in two doses given approximately every 12 hours) for 14 consecutive days followed by a seven- day rest period. Each cycle was 21 days. However, due to significant toxicities observed in the first 18 patients, the dose was subsequently reduced to 1800 mg/m²/day for the next 8 patients enrolled in the protocol. Adverse events are reporting according to the National Institutes of Health (NIH) Common Toxicity Criteria v2.0.

Dose modifications

Treatment was continued without dose reductions in the event of neutropenia grade 0–3 and thrombocytopenia grade 0–2. Otherwise, treatment was interrupted and restarted with a 1-dose level reduction. The dose of capecitabine was decreased by one dose level in the event of febrile neutropenia, and/or documented grade 4 neutropenia persisting ≥ 7 days. Treatment was also to be interrupted for $>$ grade 2 diarrhea, palmar -plantar erythrodysesthesia (hand-foot syndrome), elevated liver function tests or other non-hematological toxicities $>$ grade 1. Subsequent doses were reduced by 25%. Treatment was discontinued if a third dose reduction was required.

Evaluation of Response

Measurements of the longest dimension of each lesion were made after every two cycles of treatment. The following criteria for response to treatment were employed. Complete response (CR) was defined as the disappearance of all target and non-target lesions and no evidence of new lesions. Partial response (PR) was defined as a 30% or greater reduction in the sum of longest dimensions of all target measurable lesions and no unequivocal progression of non-target lesions. Increasing disease was defined as a 20% or greater increase in any of the sums of the longest dimensions of target lesions, or the appearance of new lesions within 8 weeks of study entry. Stable disease was defined as any condition not meeting the above criteria.

Genotyping Patients for Germline Polymorphisms in the 5' and 3' Ends of TS

Genomic DNA was isolated from blood cells recovered from 10 ml of peripheral blood collected in a purple-top Vacutainer® tube with the anti-coagulant EDTA and shipped overnight at room temperature for genotyping. A 220 or 250 base pair region of the TS gene containing the polymorphic site was polymerase chain reaction (PCR) amplified. PCR products were then separated on 4% ethidium bromide stained agarose gel. The PCR products were sized relative to a known genotype and a molecular weight marker. The two types of fragments that were amplified at the 5' end of the gene were a 220 base pair fragment (2R) that contains a double tandem 28 base pair repeat downstream of the cap site and/or a 250 base pair fragment (3R) that contains a triple repeat variant. An individual patient was either homozygous short (2R/2R), homozygous long (3R/3R) or heterozygous (2R/3R) for the polymorphic site in the 5' end of TS [21]. A similar type of genotyping was conducted to examine a polymorphic site in the 3' end of the TS gene. The two types of amplified fragments were a short fragment (–6bp) that has a 6 base pair (TTAAAG) sequence deleted and/or a long fragment (+6bp) that contains this 6 base pair sequence. An individual patient was either homozygous short (–6bp/–6bp), homozygous long (+6bp/+6bp) or heterozygous (–6bp/+6bp) for the polymorphic site in the 3' end of the TS gene.

Quantification of the Tumor Expression of TS, DPD and TP Relative to β -Actin

Transcript expression of TS, DPD and TP was determined by quantitative RT-PCR in tumor cells excised by laser capture microdissection (P.A.L.M. Microlaser Technologies AG, Germany) in 10 μ m thick sections of formalin-fixed, paraffin-embedded tumor tissue stained with neutral fast red to enable visualization of histology. RNA was subsequently extracted from the formalin-fixed and paraffin-embedded tumor cells according to a proprietary procedure (US patent number 6,248,535). Following RNA isolation, cDNA was prepared from each sample as described previously [24]. The relative expression of TS, DPD and TP was quantified using Taqman assays previously established for these three enzymes [25,26]. Quantification of cDNA's and an internal reference gene (β -actin) was performed using a fluorescence based real-time detection method (ABI PRISM 7700 Sequence Detection System, Perkin Elmer Applied Biosystems, Foster City, CA).

Statistical Design and Analysis

The study employed a two-stage accrual design with an early stopping rule in the event that the treatment demonstrated insufficient activity. The study was designed to test the hypothesis that the true response rate was .40. During the first stage of accrual, approximately 28 patients were to be entered and evaluated. If at least seven responses were observed, a second phase of accrual would be initiated to increase accrual to 62 patients. The regimen would be considered active if at least seventeen responses were observed among 62 patients. If the true probability of responding was only 20%, the study design provided a 95% chance of correctly classifying the treatment as inactive. Conversely, if the true response rate was 40%, then the probability of correctly classifying the treatment as active was 95%.

Statistical analysis of the laboratory and clinical data were performed using Statistical Package for the Social Sciences (SPSS) version 10.1 (SPSS Inc., Chicago, IL) and Statistical Analysis System (SAS) version 8.2 (SAS Institute Inc., Cary NC). All tests were two-sided. As an exploratory study, the results of the laboratory correlates cannot be considered to be conclusive. The exact p-values associated with these analyses are reported as suggestive of confidence in the results and will be used to prioritize further testing. Exact logistic regression and descriptive univariate receiver operating characteristics (ROC) analyses were performed to estimate the exact odds ratio, the exact 95% confidence intervals for the odds ratios, the exact midpoint p-values and the area under the ROC curve for each of the one variable prediction models to provide an index for the predictive utility (c-statistic).

RESULTS

The study was activated in April 2001 and was closed to accrual in June 2003. Thirty patients were enrolled. Two patients were not eligible, one because of wrong histology, one because of prior chemotherapy. Two patients were inevaluable since they never received treatment. Table 1 presents characteristics of the 26 eligible and evaluable patients. Of these patients, the first 18 were treated with a starting dose of 2500 mg/m²/day and the next 8 treated with a starting dose of 1800 mg/m²/day for 14 days of each 21-day cycle. The starting dose was changed based on the significant toxicities observed in the first 18 patients.

Tumor Response

Of the 26 patients, four (15.4%) achieved an objective response rate, all of them PR. Of the patients who experienced a PR, three were treated with a starting dose of 2500 mg/m²/day and one was treated with a starting dose of 1800 mg/m²/day. Responses were seen in 3 patients with extrapelvic disease and one with both pelvic and extrapelvic disease. Nine patients (34.6%) had stable disease, and eleven patients (42.3%) had increasing disease. Response could not be assessed in two patients (7.7%). The median time to progression was 2.9 months while

median survival time was 5.9 months. Based on the modest response rate, the study was closed after the first stage of accrual.

Adverse Events

Table 2 indicates the adverse events by starting capecitabine dose. Among the 18 patients treated with a starting dose of 2500 mg/m²/day for 14 days of each 21-day cycle, anemia was the most common and severe hematological toxicity, with 4 cases of grade 3 and one of grade 4. Of the 5 patients with grade 3 or 4 anemia, grade 1 anemia was observed in two patients and grade 2 anemia was seen in three patients prior to capecitabine administration. Neutropenia and thrombocytopenia were uncommon. However, there was one case of grade 4 neutropenia, one grade 4 leukopenia and one grade 4 thrombocytopenia. Only one of the eight patients treated with the a starting dose of 1800 mg/m²/day for 14 of each 21-day cycles experienced a severe hematological toxicity (grade 4 neutropenia).

The most common serious nonhematological toxicities attributable to capecitabine were gastrointestinal and dermatological. Five patients treated with a starting dose of 2500 mg/m²/day and four women treated with a starting dose of 1800 mg/m²/day experienced grade 3 gastrointestinal toxicity (6 diarrhea, 4 nausea and/or vomiting, 2 dehydration, 1 each of stomatitis, gastritis and bowel obstruction). Six patients treated with 2500 mg/m²/day and one women treated with 1800 mg/m²/day experienced grade 3 dermatologic toxicity (hand and foot syndrome). Other adverse events for women treated with 2500 mg/m²/day included, one case of grade 3 and one case of grade 4 coagulation abnormality and four cases of grade 3 venous thrombosis whereas there was one case of grade 4 embolism, fatigue (constitutional) or fever among the women treated with 1800 mg/m²/day. There were no treatment-related deaths observed with either starting dose.

Laboratory Correlates

Table 3 provides a case summary of the specimen submissions, laboratory testing results, tumor response and the most common serious toxicities observed in this phase II study of capecitabine. Pre-treatment blood was submitted for 25 of the eligible patients and genotyping was performed in DNA extracted from each of these specimens to define the germline polymorphisms in the 5' and 3' end of the TS gene using PCR amplification coupled with gel electrophoresis. Among the 25 cases that were evaluable for TS genotype, the frequencies of genotypes observed in the 5' end of TS were as follows: homozygous for the triple repeat variant (3R/3R) in 10 (40%) eligible patients, heterozygous for the repeat variant (2R/3R) in 13 (52%) patients and homozygous for the double repeat variant (2R/2R) in two (8%) patients. The frequencies of genotypes seen in the 3' end of TS were the following: 12 (48%) patients were homozygous for the insertion/insertion variant (+6bp/+6bp) and 13 (52%) patients were heterozygous for the deletion/insertion variant (-6bp/+6bp). None of the patients were homozygous for deletion/deletion variant (-6bp/-6bp).

The relative expression of TS, DPD and TP was quantified in 19, 19 and 18 of the eligible patients who submitted archival tumor tissue specimens yielded sufficient high quality RNA for testing, respectively. TS/ β -actin $\times 10^{-3}$ ranged from 2.16 to 31.46, DPD/ β -actin $\times 10^{-3}$ ranged from 0.72 to 44.80, and TP/ β -actin $\times 10^{-3}$ ranged from 1.07 to 28.53.

Exploratory analyses suggested that individuals who were homozygous for the triple repeat variant (3R/3R) in the 5' end of TS did not express higher levels of TS mRNA than subjects who were heterozygous for the repeat variant (2R/3R) or homozygous for the double repeat variant (2R/2R), and that a germline polymorphism in the 3' end of TS did not appear to be related to the relative expression of TS in tumor cells. The relative tumor expression of TS did not appear to be correlated with DPD or TP and there was no indication of a correlation between

DPD and TP in this set of patients with a diagnosis of persistent or recurrent squamous cell carcinoma of the cervix.

Genotype either in the 5' or 3' end of TS was not associated with tumor response or the occurrence of grade 3 or 4 gastrointestinal toxicity, dermatologic toxicity or anemia. There was no indication that the relative tumor expression of TS, DPD or TP predicted tumor response or the occurrence of a severe (grade 3 or 4) gastrointestinal, dermatologic. Relative tumor expression of TP, but not TS or DPD, appeared to predict an increase in the estimated odds of experiencing grade 3 or 4 anemia (odds ratio = 1.137; exact 95% confidence interval = 1.01 to 1.308; exact midpoint p-value = 0.016). Given the small size of this trial, the limited number of events observed and the number of exploratory tests performed, the latter observation should be interpreted cautiously.

DISCUSSION

This is the first study to evaluate the activity of capecitabine in patients with recurrent squamous cell carcinoma of the cervix who had not received prior systemic chemotherapy for treatment of metastatic disease. In this homogenous population, capecitabine was associated with modest activity. All of the patients received prior radiotherapy. Of the 22 that received a radiation sensitizer, 18 were treated with concurrent cisplatin and radiation, one with concurrent carboplatin and radiation and three with concurrent 5-FU, cisplatin and radiation. Overall response was 15.4%. There were no responders among the the 3 patients who had received prior 5-FU (2 patients with increasing disease, 1 with stable disease). The median time to treatment failure of the first therapy was 16.0 months and median survival from diagnosis was 21.8 months.

At the beginning of the study, capecitabine was administered at the FDA approved dose of 2500 mg/m². However, this dose was associated with unacceptable toxicity which prompted the GOG to reduce the dose to 1800 mg/m². In our study, the frequency of serious dermatologic toxicities, pain and anemia appeared to decrease once the starting dose was reduced from 2500 mg/m² to 1800 mg/m²: 30% and 12% experienced grade 3 dermatologic toxicity, 17% and 0% experienced grade 3 pain and 22% and 0% experienced grade 3 anemia. On the other hand, grade 3 gastrointestinal toxicities were common with both doses and were seen in 27% and 50% of patients, respectively. Reports in other tumors suggest also that the FDA approved dose is associated with significant toxicity [27]. As capecitabine and its metabolites are excreted predominantly in the urine, increased exposure is observed in patients with impaired renal function. Patients with cervical cancer may exhibit abnormal renal function secondary to obstructive uropathy or prior therapy with cisplatin. Therefore, abnormal renal function could explain, at least in part, the toxicity observed in this study. However, we believe this is unlikely as patients required to have an estimated creatinine clearance > 50 ml/min at baseline.

A major mechanism of action of capecitabine is the inhibition of TS, its detoxification by DPD and its activation by TP. Variations in intratumoral TS gene expression levels among patients' tumors have been shown to be a statistically significant predictor for response in colorectal cancer patients receiving 5-FU chemotherapy. Those tumors with high TS gene expression are generally non-responsive to protocols that include the TS-directed combination of 5-FU and leucovorin [17,18,20]. However, while high TS gene expression levels effectively predict non-response, low TS gene expression did not necessarily predict response: all responding patients had low TS expression, but a subset of patients with low TS gene expression levels did not respond to treatment [17]. These non-responders therefore may have other mechanisms of resistance that the favorable condition of low TS gene expression is insufficient to overcome. These other mechanisms are increased DPD gene expression levels or decreased TP level. There is a significant variation between patients in the clearance of 5-FU. These variations may

be due to genetic differences in the activity of the DPD enzyme [28–30]. Previous data has suggested that DPD gene expression predicts for response and survival in gastrointestinal tumors as was seen in patients with colorectal cancer patients and gastric cancer treated with 5-FU based therapy [20,31]. Patients with low expression levels of DPD are more likely to respond to 5-FU therapy. DPD also seems to be an independent predictor beyond TP and TS gene expression [32].

The small number of patients and responders in this phase II trial presented a significant limitation for the translational research that was embedded into this phase II protocol. Archival formalin-fixed and paraffin-embedded tumor tissue as well as a pre-treatment blood specimen were collected and used to study biomarkers with potential to predict response and toxicity to capecitabine. In this series, patients were either homozygous short, homozygous long or heterozygous for the polymorphic site in the 5' end of TS, and either homozygous long or heterozygous, but not homozygous short, for the polymorphic site in the 3' end of the TS gene. In addition, all of the tumors expressed detectable levels of TS, DPD and TP transcripts. There was no evidence to suggest a relationship between germline polymorphisms in the 5' end of TS and the relative expression of TS. This finding differs from a metastatic colon cancer study that demonstrated that individuals who were homozygous for the triple repeat variant (3R/3R) in the 5' end of TS exhibited higher TS mRNA levels compared to subjects who were heterozygous for the repeat variant (2R/3R) [21], but is consistent with a study in platinum-sensitive recurrent ovarian and primary peritoneal cancers [33]. Additional larger studies will be needed to study the factors that control whether or not TS genotype influences TS gene expression including loss of heterozygosity (LOH) at the TS locus which was shown by Kawakami and colleagues to be important for TS protein expression in colorectal cancer [34].

The study reported herein did not provide any evidence to suggest a relationship between the expression of these enzymes involved in capecitabine action, detoxification and activation. This is consistent with results obtained in a set of 33 colorectal cancers [20] but not with data generated from a series of 18 platinum-sensitive recurrent ovarian and primary peritoneal cancers [33]. The latter study provided suggestive evidence that DPD and TP were coordinately regulated, but a larger study is needed to validate this finding. If this result is confirmed then organ-specific differences in gene expression and prior exposure to a platinum compound may contribute at least in part to the apparent disparity between these studies.

In addition, there was no evidence that TS genotype or the relative expression of TS, DPD or TP predicted a tumor response or severe (grade 3 or 4) gastrointestinal toxicity or dermatologic toxicity following treatment with capecitabine. There was also no evidence that the relative expression of TS or DPD predicted severe anemia. In contrast, relative expression of TP predicted a significant increase in the estimated odds of experiencing grade 3 or 4 anemia. These exploratory findings may simply reflect the small sample size and the limited number of responders observed in this trial. Additional studies are, therefore, required to make a definitive determination regarding the predictive value of these biomarkers and to validate the exploratory finding that tumor expression of TP predicts severe anemia.

In this study, capecitabine was shown to have modest activity in recurrent or metastatic squamous cell carcinoma of the cervix. Recently, Jenkins and collaborators reported the results of clinical trial of capecitabine in patients with previously treated cervical cancer [35]. This study differed from ours in that patients were heavily pretreated and received a lower dose of capecitabine. Treatment was well tolerated, but no partial or complete responses were observed. Our study evaluated the activity of capecitabine in patients with recurrent or metastatic squamous cell carcinoma of the cervix who have not received prior systemic chemotherapy for treatment of metastatic disease. All of the patients received prior

radiotherapy and 22 received a radiation sensitizer. In this setting, few chemotherapy drugs, including cisplatin, paclitaxel and ifosfamide, have consistently shown response rates of approximately 20%. Other agents, such as topotecan [36], irinotecan [37,38] and vinorelbine [39] have response rates similar to those observed with capecitabine in this study. The low response rate observed in this study may be explained in part because they had received prior chemotherapy as a radiation sensitizing agent.

Although capecitabine has modest activity when used as single agent in this patient population, rationale combination with synergistic agents (i.e. vinorelbine or docetaxel) may be beneficial. Nonetheless, the introduction of novel biologic therapies may be the focus of the new generation of clinical trials in cervical cancer. The GOG plans to evaluate the combination of capecitabine and vinorelbine in previously treated cervical cancer and is currently conducting clinical trials with inhibitors of angiogenesis and antagonists of the epidermal growth factor receptor. The results of these studies are eagerly awaited.

In summary, our trial suggests that single agent capecitabine is associated with modest activity in persistent or recurrent squamous cell carcinoma of the cervix.

Acknowledgements

The authors thank Anne Reardon for her assistance in preparing this manuscript for publication, and Barbara Saczynski for coordinating the clinical data for this protocol. A special acknowledgement is also extended to the clinic staff at the various GOG institutions for their efforts in obtaining tissue and blood specimens for this study. Drs. Alan Hutson, Dan Gaile and Dong Yang are recognized for their assistance with the statistical analysis of the laboratory data for this study. Ms. Eleanor Cashmore is thanked for her careful review of the manuscript and suggestions. Finally, we also thank the GOG Publications Subcommittee for their critical review of the manuscript and for their helpful recommendations.

References

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, et al. Cancer Statistics, 2005. *CA Cancer J Clin* 2005;55:10–30. [PubMed: 15661684]
2. Thigpen JT, Vance R, Punecky L, Khansur T. Chemotherapy as a palliative treatment in carcinoma of the uterine cervix. *Semin Oncol* 1995;22:16–24. [PubMed: 7537898]
3. Thigpen JT, Shingleton H, Homesley H, Lagasse L, Blessing JA. Cis-platinum in treatment of advanced or recurrent squamous cell carcinoma of the cervix. *Cancer* 1981;48:899–903. [PubMed: 7196794]
4. Sutton GP, Blessing JA, Adcock L, Webster KD, DeEulis T. Phase II Study of ifosfamide and mesna in patients with previously treated carcinoma of the cervix: a Gynecologic Oncology Group study. *Invest New Drugs* 1989;7:341–3. [PubMed: 2513286]
5. McGuire WP, Blessing JA, Moore D, Lentz SS, Photopulos G. Paclitaxel has moderate activity in squamous cervix cancer: a Gynecologic Oncology Group study. *J Clin Oncol* 1997;14:792–5. [PubMed: 8622025]
6. Omura GA. Chemotherapy for stage IV B or recurrent cancer of the uterine cervix. *Monogr Natl Cancer Inst* 1996;21:123–6. [PubMed: 9023841]
7. Look KY, Blessing JA, Valea FA, McGehee R, Manetta A, Webster KD, et al. Phase II trial of 5-fluorouracil and high-dose leucovorin in recurrent adenocarcinoma of the cervix: a Gynecologic Oncology Group study. *Gynecol Oncol* 1997;67:255–8. [PubMed: 9441772]
8. Look KY, Blessing JA, Gallup DG, Lentz SS. A phase II trial of 5-fluorouracil and high-dose leucovorin in patients with recurrent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. *Am J Clin Oncol* 1996;19:439–41. [PubMed: 8823469]
9. Budman DR, Meropol NJ, Reigner B, Creaven PJ, Lichtman SM, Berghorn E, et al. Preliminary studies of a novel oral fluoropyrimidine carbamate: capecitabine. *J Clin Oncol* 1998;16:1795–1802. [PubMed: 9586893]
10. O'Shaughnessy JA, Blum J, Moiseyenko V, Jones SE, Miles D, Bell D, et al. Randomized, open-label, phase II trial of oral capecitabine (Xeloda) vs. a reference arm of intravenous CMF

(cyclophosphamide, methotrexate and 5-fluorouracil) as first-line therapy for advanced/metastatic breast cancer. *Ann Oncol* 2001;12:1247–54. [PubMed: 11697835]

11. Blum JL, Jones SE, Buzdar AU, LoRusso PM, Kuter I, Vogel C, et al. Multicenter phase II study of capecitabine in paclitaxel-refractory metastatic breast cancer. *J Clin Oncol* 1999;17:485–93. [PubMed: 10080589]
12. Van Cutsem E, Twelves C, Cassidy J, Allman D, Bajetta E, Boyer M, et al. Xeloda Colorectal Cancer Study Group. Oral capecitabine colorectal cancer: results of a large phase III study. *J Clin Oncol* 2001;19:4097–106. [PubMed: 11689577]
13. Ishikawa T, Utoh M, Sawada N, Nishida M, Fukase Y, Sekiguchi F, et al. Tumor selective delivery of 5-fluorouracil by capecitabine, a new oral fluoropyrimidine carbamate, in human cancer xenografts. *Biochem Pharmacol* 1998;55:1091–7. [PubMed: 9605432]
14. Hindenburg AA, Matthews L. Complete and sustained remission of refractory cervical cancer following a single cycle of capecitabine. A case report. *Int J Gynecol Cancer* 2003;13:898–900. [PubMed: 14675331]
15. Miwa M, Ura M, Nishida M, Sawada N, Ishikawa T, Mori K, et al. Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumors by enzymes concentrated in human liver and cancer tissue. *Eur J Cancer* 1998;34:1274–81. [PubMed: 9849491]
16. Takebayashi Y, Yamada K, Miyadera K, Sumizawa T, Furukawa T, Kinoshita T, et al. The activity and expression of thymidine phosphorylase in human solid tumors. *Eur J Cancer* 1996;32A:1227–32. [PubMed: 8758258]
17. Leichman CG, Lenz HJ, Leichman L, Danenberg K, Baranda J, Groshen S, et al. Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infusion 5-fluorouracil and weekly leucovorin. *J Clin Oncol* 1997;15:3223–9. [PubMed: 9336359]
18. Leichman L, Lenz HJ, Leichman CG, Groshen S, Danenberg K, Baranda J, et al. Quantitation of intratumoral thymidylate synthase expression predicts for resistance to protracted infusion of 5-fluorouracil and weekly leucovorin in disseminated colorectal cancers: preliminary report from an ongoing trial. *Eur J Cancer* 1995;31:1306–10. [PubMed: 7577041]
19. Metzger R, Danenberg K, Leichman CG, Salonga D, Schwartz EL, Wadler S, et al. High basal level gene expression of thymidine phosphorylase (platelet derived endothelial cell growth factor) in colorectal tumors is associated with non-response to 5-fluorouracil. *Clin Cancer Res* 1998;4:2371–6. [PubMed: 9796967]
20. Salonga D, Danenberg KD, Johnson M, Metzger R, Groshen S, Tsao-Wei DD, et al. Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin Cancer Res* 2000;6:1322–7. [PubMed: 10778957]
21. Pullarkat ST, Stoehlmacher J, Ghaderi V, Xiong YP, Ingles SA, Sherrod A, et al. Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. *Pharmacogenomics* 2001;1:65–70.
22. Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei D, Groshen S, Lenz HJ. Thymidylate synthase gene polymorphism predicts response to capecitabine in advanced colorectal cancer. *Int J Colorectal Dis* 2002;17:46–9. [PubMed: 12018454]
23. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16. [PubMed: 10655437]
24. Shirota Y, Stoehlmacher J, Brabender J, Xiong Y, Danenberg KD, Groshen S, et al. ERCC1 mRNA Levels and Thymidylate Synthase (TS) mRNA Levels Predict Survival Colorectal Cancer Patients Receiving Combination Oxaliplatin and Fluorouracil Chemotherapy. *J Clin Oncol* 2001;19:4298–4304. [PubMed: 11731512]
25. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. *Genome Res* 1996;6:986–994. [PubMed: 8908518]

26. Gibson UE, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. *Genome Res* 1996;6:995–1001. [PubMed: 8908519]
27. Hennessy BT, Gauthier AM, Michaud LB, Hortobagyi G, Valero V. Lower dose capecitabine has a more favorable therapeutic index in metastatic breast cancer: retrospective analysis of patients treated at M. D. Anderson Cancer Center and a review of capecitabine toxicity in the literature. *Ann Oncol* 2005;16:1289–96. [PubMed: 15890665]
28. Diaso RB, Lu Z. Dihydropyrimidine dehydrogenase activity and fluorouracil chemotherapy. *J Clin Oncol* 1994;12:2239–42. [PubMed: 7964937]
29. Diaso RB, Beavers TL, Carpenter JT. Familial deficiency of DPD: biochemical basis for familial pyrimidinemia severe 5-FU induced toxicity. *J Clin Invest* 1988;81:47–51. [PubMed: 3335642]
30. Lu Z, Zhang R, Diasio RB. DPD activity in human peripheral blood mononuclear cells and liver: population characteristics, newly identified deficient patients and clinical implications in 5-FU chemotherapy. *Cancer Res* 1993;53:5433–8. [PubMed: 8221682]
31. Danenberg K, Salonga D, Park CG, Leichman CG, Leichman L, Johnson M, et al. DPD and TS gene expressions identify a high percentage of colorectal tumors responding to 5-FU (abstract). *Proc Amer Soc Clin Oncol* 1998;17:992.
32. Uetake H, Ichikawa W, Takechi T, Fukushima M, Nihei Z, Sugihara K. Relationship between intratumoral dihydropyrimidine dehydrogenase activity and gene expression in human colorectal cancer. *Clin Cancer Res* 1999;5:2836–9. [PubMed: 10537350]
33. Garcia AA, Blessing JA, Lenz HJ, Darcy KM, Mannel RS, Miller DS, et al. Phase II clinical trial of capecitabine in ovarian carcinoma recurrent 6–12 months after completion of primary chemotherapy, with exploratory TS, DPD and TP correlates: A Gynecologic Oncology Group study. *Gynecol Oncol* 2005;96:810–7. [PubMed: 15721430]
34. Kawakami K, Ishida Y, Danenberg KD, Omura K, Watanabe G, Danenberg PV. Functional polymorphism of the thymidylate synthase gene in colorectal cancer accompanied by frequent loss of heterozygosity. *Jpn J Cancer Res* 2002;93:1221–9. [PubMed: 12460463]
35. Jenkins AD, Ramondetta LM, Sun C, Johnston T, Wolf JK, Bodurka DC, et al. Phase II trial of capecitabine in recurrent squamous cell carcinoma of the cervix. *Gynecol Oncol* 2005;97:840–4. [PubMed: 15882895]
36. Muderspach LI, Blessing JA, Levenback C, Moore JL Jr. A Phase II study of topotecan in patients with squamous cell carcinoma of the cervix: a Gynecologic Oncology Group Study. *Gynecol Oncol* 2001;81:213–5. [PubMed: 11354055]
37. Lhomme C, Fumoleau P, Fargeot P, Krakowski Y, Dieras V, Chauvergne J, et al. Results of a European Organization for Research and Treatment of Cancer/Early Clinical Studies Group phase II trial of first-line irinotecan in patients with advanced or recurrent squamous cell carcinoma of the cervix. *J Clin Oncol* 1999;17:3136–42. [PubMed: 10506610]
38. Look KY, Blessing JA, Levenback C, Kohler M, Chafe W, Roman LD. A phase II trial of CPT-11 in recurrent squamous carcinoma of the cervix: A Gynecologic Oncology Group study. *Gynecol Oncol* 1998;70:334–38. [PubMed: 9790784]
39. Muggia FM, Blessing JA, Method M, Miller DS, Johnson GA, Lee RB, et al. Evaluation of vinorelbine in persistent or recurrent squamous cell carcinoma of the cervix: a Gynecologic Oncology Group Study. *Gynecol Oncol* 2004;92:639–43. [PubMed: 14766259]

TABLE 1**PATIENT CHARACTERISTICS**

Age	
30–39	4
40–49	13
50–59	7
60–69	2
Performance Status	
0	9
1	10
2	7
Tumor Grade	
1	0
2	15
3	11
Disease Site	
Pelvic	2
Extra-pelvic	22
Both	2
Prior Chemotherapy for recurrent or metastatic disease	0
Prior Radiotherapy	26
Without radiation sensitizing agent(s)	4
With radiation sensitizing agent(s)	
5-FU and Cisplatin	3
Cisplatin	18
Carboplatin	1
Cycles of capecitabine	
1	7
2–4	15
>4	4

TABLE 2
ADVERSE EVENTS BY INITIAL DOSE N = 26

Adverse Event	2500mg (n=18)					1800mg (n=8)*				
	0	1	2	3	4	0	1	2	3	4
Leukopenia	14	1	3	0	1	6	1	0	0	0
Neutropenia	16	1	1	0	1	6	0	0	0	1
Thrombocytopenia	16	2	0	0	1	6	1	0	0	0
Anemia	4	6	4	4	1	3	1	3	0	0
Hemorrhage	16	3	0	0	0	6	0	1	0	0
Coagulation	16	1	0	1	1	7	0	0	0	0
Transfusion	16	0	1	2	0	7	0	0	0	0
Gastrointestinal	3	4	7	5	0	0	1	2	4	0
Stomatitis	15	1	2	0	1	6	0	1	0	0
Neurologic	17	0	1	1	0	3	1	2	1	0
Pain	8	2	5	3	1	2	1	4	0	0
Dermatologic	10	3	0	6	0	4	1	1	1	0
Constitutional	4	6	7	2	0	7	1	1	0	0
Metabolic	11	5	1	2	0	5	1	0	1	0
Hepatic	12	3	2	2	0	5	1	0	1	0
Cardiovascular	10	4	2	3	0	4	0	1	1	1
Pulmonary	17	1	1	0	0	4	0	1	1	1
Infection	16	0	2	1	0	7	0	0	0	0
Ocular	17	0	2	0	0	7	0	0	0	0
Lymphatics	17	0	2	0	0	7	0	0	0	0
Lymphodema	18	0	0	1	0	7	0	0	0	0
Renal	18	1	0	0	0	6	0	1	0	0
Genitourinary	18	0	1	0	0	7	0	0	0	0
Urinary Tract Infection	19	0	0	0	0	6	0	1	0	0
Fatigue	18	1	0	0	0	5	1	0	0	1
Fever	19	0	0	0	0	6	0	0	0	1
Auditory	19	0	0	0	0	6	0	1	0	0
Sexual	19	0	0	0	0	6	0	1	0	0
Endocrine	19	0	0	0	0	6	1	0	0	0
Musculoskeletal	18	1	0	0	0	7	0	0	0	0

* One patient enrolled to receive a starting dose of 1800 mg/m²/day received instead 3000 mg/m² day during cycle 1 and 1350 mg/m²/day during cycle 2. The appropriate dose of 1800 mg/m²/day was administered on subsequent cycles.

TABLE 3

CASE SUMMARY OF SPECIMEN SUBMISSION STATUS, LABORATORY TESTING RESULTS, TUMOR RESPONSE AND MOST COMMON SEVERE ADVERSE EVENTS BY STARTING DOSE

Starting Dose Level 2500 mg/m ² /day	Case	Blood	TS Polymorphism ^a		Tumor	Relative Gene Expression Level ^b			Tumor Response ^c	Toxicity Grade ^d		
			5' end 2R/3R	3' end -6bp/ +6bp		TS	DPD	TP [*]		GI	Derm	Anemia [*]
	2	Yes	3R/3R	+6bp	Yes	-	-	-	PR	3	3	1
	16	Yes	3R/3R	+6bp	Yes	8.67	2.90	11.37	PR	1	1	0
	19	Yes	3R/3R	+6bp	Yes	2.16	13.08	-	PR	3	3	2
	3	Yes	2R/3R	+6bp	Yes	6.00	2.96	25.49	SD	1	3	3
	20	Yes	2R/3R	+6bp	Yes	19.33	5.99	1.31	SD	0	0	0
	8	Yes	2R/3R	+6bp	Yes	-	-	-	SD	0	3	2
	1	Yes	3R/3R	+6bp	Yes	24.40	44.80	6.28	SD	2	0	1
	13	Yes	3R/3R	+6bp	Yes	3.99	4.28	6.05	SD	3	3	1
	9	No	-	-	No	-	-	-	SD	3	0	2
	4	Yes	2R/3R	+6bp	Yes	4.14	4.91	26.16	ID	2	1	3
	6	Yes	2R/2R	+6bp	Yes	18.59	0.72	7.01	ID	1	0	1
	11	Yes	2R/3R	+6bp	Yes	4.77	3.91	28.53	ID	2	0	3
	15	Yes	2R/3R	+6bp	Yes	-	-	-	ID	1	0	2
	18	Yes	2R/2R	+6bp	Yes	-	-	-	ID	2	0	0
	7	Yes	3R/3R	+6bp	Yes	-	-	-	ID	0	0	1
	10	Yes	3R/3R	+6bp	Yes	31.46	1.56	11.68	ID	2	0	3
	5	Yes	3R/3R	+6bp	Yes	-	-	-	ID	2	1	1
	12	Yes	2R/3R	+6bp	Yes	7.79	1.60	24.64	-	3	3	0
1800 mg/m ² / day	23	Yes	3R/3R	+6bp	Yes	5.32	10.67	2.76	PR	3	3	2
	21	Yes	2R/3R	+6bp	Yes	6.83	5.81	1.07	SD	2	0	4
	26	Yes	2R/3R	+6bp	Yes	14.33	8.00	3.29	SD	2	0	2
	25	Yes	3R/3R	+6bp	Yes	14.72	21.99	2.88	SD	3	2	0
	17	Yes	2R/3R	+6bp	Yes	3.81	1.85	14.19	ID	3	0	1
	22	Yes	2R/3R	+6bp	Yes	7.33	23.01	7.96	ID	1	1	0
	24	Yes	3R/3R	+6bp	Yes	3.42	3.34	2.63	ID	2	0	2
	14	Yes	2R/3R	+6bp	Yes	7.99	25.38	2.80	-	3	0	0

- ^a PCR coupled with gel electrophoresis was used to evaluate the presence of a triple repeat (3R) or a double repeat (2R) of a 28 base pair region in the 5' end of the TS gene or a insertion (+6bp) or deletion (-6bp) of a 6 base pair region of the 3' end of the TS gene. Polymorphisms in the 5' end of TS were expressed as 3R/3R for alleles with the triple/triple 28 base pair repeat, 2R/3R for alleles with the double/triple 28 base pair repeat and 2R/2R for alleles with the double/double 28 base pair repeat. Polymorphisms in the 3' end of TS were expressed as +6bp/+6bp for alleles with the insertion/insertion 6 base pair variant, -6bp/-6bp for alleles with the deletion/deletion 6 base pair variant and -6bp/-6bp for alleles with the deletion/deletion 6 base pair variant. Twenty-three cases were evaluable for TS genotype and tumor response while 25 were evaluable for TS genotype and toxicity.
- ^b Quantitative RT-PCR was used to measure the expression of TS, DPD or TP relative to β -actin expression $\times 10^{-3}$. The association between relative expression of TS, DPD or TP and tumor response was evaluated in 17, 17 or 16 cases, respectively while the association between relative expression of TS, DPD or TP and toxicity was evaluated in 19, 19 or 18 cases, respectively.
- ^c Tumor response was evaluated in 24 patients. ID indicates increasing disease; SD denotes stable disease; PR signifies partial response. None of the patients experienced a complete response (CR) to capecitabine treatment.
- ^d Capecitabine adverse events were evaluated in 26 patients according NCI Common Toxicity Criteria v2.0. Gastrointestinal toxicity (GI), dermatological toxicity (Derm) and anemia were the most frequent grade 3 or 4 adverse events reported in this study.
- * Exact logistic regression and descriptive univariate ROC analysis for experiencing grade 3 or 4 anemia provided an exact odds ratio of 1.137, an exact 95% confidence interval for the odds ratio of 1.01 to 1.308, an exact midpoint p-value of 0.016 and an estimate of the area under the ROC curve (c-statistic) of 0.769.
- ** One patient enrolled to receive a starting dose of 1800 mg/m²/day received instead 3000 mg/m²/day during cycle 1 and 1350 mg/m²/day during cycle 2. The appropriate dose of 1800 mg/m²/day was administered on subsequent cycles.