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Gender-specific genomic profiling in metastatic colorectal cancer patients treated with 5-fluorouracil and oxaliplatin

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Abstract

Aims—Survival and response rates in metastatic colorectal cancer remain poor, despite advances in drug development. There is increasing evidence to suggest that gender-specific differences may contribute to poor clinical outcome. We tested the hypothesis that genomic profiling of metastatic colorectal cancer is dependent on gender.

Materials & methods—A total of 152 patients with metastatic colorectal cancer who were treated with oxaliplatin and continuous infusion 5-fluorouracil were genotyped for 21 polymorphisms in 13 cancer-related genes by PCR. Classification and regression tree analysis tested for gender-related association of polymorphisms with overall survival, progression-free survival and tumor response.

Results—Classification and regression tree analysis of all polymorphisms, age and race resulted in gender-specific predictors of overall survival, progression-free survival and tumor response. Polymorphisms in the following genes were associated with gender-specific clinical outcome: estrogen receptor , EGF receptor, xeroderma pigmentosum group D, voltage-gated sodium channel and phospholipase A2.

Conclusion—Genetic profiling to predict the clinical outcome of patients with metastatic colorectal cancer may depend on gender.

Keywords

colorectal cancer; estrogen receptor ; gender; oxaliplatin; polymorphism

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Colorectal cancer is the second leading cause of cancer-related death in the USA, with an estimated 147,000 new cases and 50,000 deaths in 2009 as a result of this disease [1]. The current standard of care for metastatic colorectal cancer involves 5-fluorouracil (5-FU) in combination with other agents such as oxaliplatin and irinotecan. However, survival and response rates remain poor. Germline polymorphisms contribute to interindividual variation in response to therapy and clinical outcome in numerous malignancies, including colorectal cancer [2–6]. Significant polymorphisms have been identified in genes involved in multiple molecular pathways relevant to cancer. By analyzing germline polymorphisms from interconnected molecular pathways in combination, rather than individually, it may be possible to develop a more accurate molecular signature with which to predict clinical outcome. We have taken this approach and have selected candidate genes in molecular pathways relevant to colorectal cancer to test for association with clinical end points. These pathways include drug metabolism, DNA repair, oxidative stress response, angiogenesis, tyrosine kinase signaling and others. A total of 13 genes were analyzed in this study and their functions are described below; genes were selected from the aforementioned pathways based on their biological function and/or their harboring of functional SNPs.

TS is an enzyme responsible for the synthesis of thymidine monophosphate; it is the main target of the chemotherapeutic agent 5-FU [7]. TS expression levels and germline polymorphisms have been associated with clinical outcome in colorectal cancer patients [4,8]. MTHFR is an enzyme that regulates intracellular folate levels. The MTHFR substrate 5,10-MTHF is required to convert deoxyuridine monophosphate to deoxythymidine monophosphate by TS, which affects DNA synthesis [9]. *MTHFR* polymorphisms are predictive of clinical outcome in colorectal cancer patients [10].

The DNA repair enzymes ERCC1 and XPD function in the nucleotide excision repair pathway, which repairs lesions induced by platinum-based chemotherapies such as oxaliplatin [11]. Upregulation of ERCC1 induces the resistance of colon cancer cells to oxaliplatin [12]. ERCC1 and XPD have been shown to affect drug sensitivity in colorectal cancer patients [13,14].

Genes encoding voltage-gated sodium channels such as *SCN1A* have a role in cancer progression, as well as in oxaliplatin-induced neurotoxicity. Increased expression of *SCN1A* has been associated with proliferation, progression and metastasis of breast and prostate cancers [15–17]. In addition, oxaliplatin induces changes in sodium channel function [18,19].

The detoxifying enzyme glutathione *S*-transferase P1 catalyzes the conjugation of reduced glutathione to electrophiles. It is overexpressed in colorectal cancer, promotes colon cancer cell growth and has been associated with clinical outcome in colorectal cancer patients treated with 5-FU/oxaliplatin [20–22]. Cyclooxygenase 2 catalyzes prostaglandin synthesis, and prostaglandins in turn affect multiple processes such as angiogenesis and carcinogenesis. Cyclooxygenase 2 is overexpressed in colon cancer and promotes tumor growth [23].

The proinflammatory cytokine IL-8 and its receptors, CXCR1 and CXCR2, are involved in angiogenesis, a process that is critical for tumor growth [24–26]. IL-8 also causes an increase in cellular proliferation, migration and invasion in colon cancer cell-line models and is a negative prognostic marker in colorectal cancer [27,28].

The EGF receptor is a receptor tyrosine kinase that is activated by EGF ligand and activates multiple downstream pathways resulting in cellular growth and proliferation [29]. Targeted

therapeutics such as cetuximab and panitumumab have been developed to inactivate EGFR and inhibit its effect on a number of cancer types, including colon cancer [30,31].

PLA2 belongs to an enzyme family that release fatty acids from glycerol. Cytosolic PLA2 contributes to the production of eicosanoids, which are active lipids implicated in inflammation and cancer progression [32,33]. Cytosolic PLA2 is overexpressed in colorectal cancers, and may have a role in tumor progression [34].

Estrogen receptor (ER-), which is expressed in normal colonic epithelium, may have a protective role against colorectal cancer development and progression [35–37]. The loss of ER- is a common event in malignant transformation of the colon and in tumor progression, and the binding of estrogen to ER- has antiproliferative effects in the colon [36,38,39].

There is increasing evidence that gender-specific differences play a role in the development and progression of colorectal cancer. Women have a lower incidence of colorectal cancer than men, and recent studies have found that polymorphisms in genes such as *EGFR*, *MTHFR* and *ER-* are associated with risk and clinical outcome in colorectal cancer in a sex-specific manner [10,40–42]. Therefore, we designed the current study in order to determine if men and women with metastatic colorectal cancer have different genomic profiles that are predictive of clinical outcome. The candidate genes we selected for this study have known roles in colorectal cancer progression, and their corresponding polymorphisms may lead to functional alterations. We have analyzed these polymorphisms in combination and have generated gender-specific genomic profiles to predict clinical outcome in metastatic colorectal cancer patients treated with 5-FU/oxaliplatin.

Materials & methods

Eligible subjects

Subjects included in this study were enrolled in the 3C-01-7 clinical trial. The study was based on a Phase II study of oxaliplatin in combination with continuous infusion of 5-FU in patients with colorectal cancer, refractory to 5-FU-/irinotecan-based chemotherapy. This study was conducted at the Norris Comprehensive Cancer Center (University of Southern California, CA, USA) and was approved by the institutional review board of the University of Southern California for Medical Sciences. Patients were accrued from September 2001 to August 2004. A total of 173 patients were included in this trial. All patients signed informed consent prior to entering this study. The study was an observational study that was not designed to compare antitumor activity of the regimen to historical controls because oxaliplatin was already established as a standard of care in patients with metastatic colorectal cancer when the study was conducted. The primary objectives of the study were to evaluate the associations between germline polymorphisms of genes in relevant molecular pathways and clinical outcome.

Tumor biopsy from time of diagnosis and peripheral blood samples were collected for each patient. All patients were aged at least 18 years, had a Southwest Oncology Group (SWOG) performance status of 2 or less and had stage IV metastatic colorectal cancer. The dose of oxaliplatin administered was 103 mg/m² every 3 weeks and 5-FU was 200 mg/m²/day CI for 10 weeks followed by 2 weeks rest. Treatment was administered until one of the following occurred: disease progression; intercurrent illness that prevented further administration of treatment; unacceptable toxicity; patient's decision to withdraw from the study; or general or specific changes in the patient's condition which rendered the patient unacceptable for further treatment in the judgment of the investigator. Baseline evaluations were conducted within 1 week prior to the administration of the study drug. Scans and x-rays were conducted at 4 weeks or earlier prior to the start date of therapy. The Response Evaluation

Criteria In Solid Tumors (RECIST) and Common Toxicity Criteria (CTC) 2.0 were used. Tumor response was evaluated every 6 weeks and toxicity was evaluated every 3 weeks or as needed. Response parameters were defined as follows: complete response, disappearance of all target lesions; partial response, at least a 30% decrease in the sum of longest diameter (LD) of target lesions taking as reference the baseline sum LD; stable disease, neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progression of disease (PD) taking as a references the smallest sum LD since the start of treatment; PD, at least a 20% increase in the sum of LD of target lesions taking as references the smallest sum LD recorded from the start of treatment or the appearance of one or more new lesions.

Genotyping

Of the 173 patients enrolled in the trial, 152 patients were evaluable for genotyping and statistical analysis. The remaining 21 patients were inevaluable owing to a lack of peripheral blood samples. Genomic DNA was extracted from peripheral blood using the QIAamp® kit (Qiagen, Hilden, Germany). A total of 21 polymorphisms in 13 cancer-related genes were tested by PCR; primers and restriction enzymes are summarized in Supplementary Table 1 see www.futuremedicine.com/doi/suppl/10.2217/pgs.10.163. Polymorphisms were tested using three available methods: PCR-RFLP, commercially available TaqMan®-based allelic discrimination assay (Applied Biosystems, CA, USA), or PCR-based radiolabeling technique. *ER-CA* repeat and *EGFR* CA repeat were determined using a PCR-based radiolabeling technique as previously described [43]. *SCN1A* polymorphisms were determined using the TaqMan assay, as previously described [44,45]. Remaining polymorphisms were determined using the PCR-RFLP technique. Briefly, PCR was followed by restriction enzyme digestion, and resulting DNA fragments were visualized on 3–4% ethidium bromide-stained agarose gel.

Statistical analysis

The primary end points in the study were progression-free survival (PFS), overall survival (OS), tumor response and toxicity. Tumor response was categorized as three groups: complete response and partial response, stable disease and PD. Toxicity was defined as the maximum grade of toxicity that patients experienced and categorized as grade 1–2 and grade 3–4. The OS was calculated as the period from the first day of starting the treatment until death from any cause, or until the date of the last follow-up, at which point OS was censored. The PFS was calculated from the time of the first day of the treatment until the first observation of disease progression or death from any cause. If a patient had not progressed or died, PFS was censored at the time of the last follow-up.

The distribution of alleles for each SNP was tested for Hardy–Weinberg equilibrium using a χ^2 test with one degree of freedom. Univariate analysis was performed for each polymorphism and clinical outcome was determined using the exact conditional test for tumor response. Fisher's exact test tested for toxicity, and the log-rank test was performed to determine OS and PFS assuming a codominant, dominant or recessive genetic model when appropriate. Cut-off values of the number of (CA)_n repeats of *EGFR* and *ER-* were chosen from the most frequent ones. All analyses were performed in male and female patients separately.

A classification and regression tree (CART) method based on recursive partitioning (RP) was used to explore gene polymorphisms for identifying homogeneous subgroups for various clinical end points, including OS, PFS and tumor response [46,101]. All polymorphisms, age (continuous variable), and race were considered in the CART analysis. RP analysis is a nonparametric statistical method for modeling a response variable and

multiple predictors. RP analysis includes two essential processes: tree growing and pruning. The tree-growing process started with all patients in one group (the root node) and binary recursive splits were generated based on a cut-off point of a covariate that yielded the greatest dissimilarity in survival functions at each node. For continuous covariates, such as age, all potential values were searched at each step of tree growing and the split with the maximal discrimination function was selected. The crossvalidation approach was applied to prune the initial tree to avoid overfitting [28]. Patients with missing values on a particular variable that was used to define a split were grouped into a daughter node using surrogate splits. The end branches of a survival tree were terminal nodes representing combinations of covariates associated with contrasting risks. Finally, the terminal nodes with similar PFS or OS patterns were merged. Patients were separated by gender and regression trees were developed for males and females separately.

Finally, formal tests for interactions between gender and gender-specific terminal nodes developed from RP were conducted to determine tumor response, PFS and OS using the likelihood ratio test within logistic regression or Cox proportional hazards regression model whenever appropriate.

All analyses were performed using the SAS statistical package version 9.2 (SAS Institute Inc. NC, USA), and Recursive Partitioning (RPART) function in the S-Plus library written by [101].

Results

Clinical & demographic variables

Baseline information and clinical outcome for this Phase II clinical trial are summarized in Table 1. There were no statistically significant differences in baseline characteristics between the 173 patients enrolled in the trial and the 152 patients evaluable for genotyping (data not shown). Baseline patient characteristics and clinical outcome differences between males and females are summarized in Tables 2 & 3. There were no statistically significant differences in age, race or site of tumor based on gender. PFS, OS and tumor response were not statistically significantly different between males and females. Toxicity was the only variable that was statistically significantly different between males and females, with females having increased rates of grade 3–4 toxicity ($p = 0.02$).

Polymorphisms & clinical outcome

Univariate analyses of polymorphisms with clinical end points are summarized in Supplementary Tables 2–5; see www.futuremedicine.com/doi/suppl/10.2217/pgs.10.163. Polymorphisms in *SCN1A*, *ER- α* , *XPD*, *TS*, *EGFR*, *CXCR2*, and *IL-8* were individually statistically significantly associated with gender-specific clinical outcome. The allelic frequencies for all polymorphisms were within the probability limits of Hardy–Weinberg equilibrium ($p > 0.05$, exact test for Hardy–Weinberg equilibrium), with the exception of *XPD* 156 (data not shown).

Sex-specific genomic profiling by CART analysis

Classification and regression tree analysis was performed to differentiate male and female genomic profiles to predict clinical outcome. CART analyses by gender resulted in different regression trees for men versus women for all clinical end points. CART and RP resulted in patients being grouped into three categories for each clinical end point: low risk (group I), medium risk (group II) and high risk (group III). The survival curves in Figures 1 & 2 indicate that patients in group I had the longest median OS and PFS, patients in group II had

an intermediate median OS and PFS, and patients in group III had the shortest median OS and PFS.

Progression-free survival in male patients was predicted by two *ER*- polymorphisms: *ER*-(CA)_n repeat and *ER*- A730G SNP (Figure 1A). Males carrying two long alleles of the *ER*-(CA)_n repeat polymorphism had a shorter PFS (group III). Males carrying any short allele (<22 repeats) of the *ER*-(CA)_n repeat polymorphism and who were homozygous G/G at the *ER*- A730G locus had intermediate PFS (group II). Males carrying any short allele (<22 repeats) of the *ER*-(CA)_n repeat polymorphism and any A allele at the *ER*- A730G locus had increased PFS (group I). PFS in female patients was predicted by polymorphisms in *XPD* and *EGFR* (Figure 1B). Females carrying homozygous C/C genotype at *XPD* 156 had shortened PFS (group III). Females carrying any A allele at *XPD* 156 and two short repeats of the *EGFR* (CA)_n repeat polymorphism had intermediate PFS (group II). Females carrying any A allele at *XPD* 156 and any long repeat of the *EGFR* (CA)_n repeat polymorphism had the longest PFS (group I).

Overall survival for male patients was predicted by *SCN1A* IVS591 and *ER*- A730G (Figure 2A). Males homozygous G/G at the *SCN1A* IVS591 locus had shorter OS (group III). Males carrying any A allele at the *SCN1A* IVS591 locus and who were homozygous G/G genotype at the *ER*- A730G locus, had intermediate OS (group II). Males carrying any A allele at the *SCN1A* IVS591 locus and any A allele at the *ER*- A730G locus, had the longest OS (group I). For female patients, OS was predicted by *SCN1A* T1067A and *PLA2* polymorphisms (Figure 2B). Females carrying the heterozygous T/A genotype at the *SCN1A* T1067A locus had decreased OS (group III). Females carrying *SCN1A* T/T genotype and *PLA2* C/C genotype had intermediate OS (group II). Patients carrying *SCN1A* T/T genotype and any *PLA2* T allele had the longest OS (group I).

Tumor response in male patients was predicted by two *ER*- polymorphisms, *ER*- A730G and *ER*-(CA)_n repeat (Figure 3). Males carrying any A allele at *ER*- A730G and two long repeats (>22 repeats) at *ER*-(CA)_n repeat locus had poor tumor response (group 3). Males carrying any A allele at the *ER*- A730G locus and any short repeat (<22 repeats) at the *ER*-(CA)_n repeat locus had intermediate response (group II). Males who were homozygous G/G at the *ER*- A730G locus had better tumor response (group I). Tumor response in female patients was predicted by age and *XPD* 156 polymorphism (Figure 3). Females who were aged 65 years or older had poor tumor response (group III). Females who were aged less than 65 years and who carried homozygous C/C genotype at the *XPD* 156 locus had intermediate tumor response (group II). Females who were aged less than 65 years and who carried any A allele at the *XPD* 156 locus had improved tumor response (group I).

Formal test for interactions between gender-specific genomic profiles & gender

Statistical analyses were performed to determine if markers that associated with PFS, OS or tumor response for females predicted outcomes in males or *vice versa*. Markers that were predictive of PFS in females were tested to determine if there was a statistically significant difference in their predictive ability in males versus females. The formal test for interaction demonstrated that female markers only predicted PFS in females, and that the difference in predictability between males and females was statistically significant ($p = 0.021$, Supplementary Figures 1A & 1B; see www.futuremedicine.com/doi/suppl/10.2217/pgs.10.163). A similar analysis was performed to test for markers of tumor response in females. Female markers predicted tumor response only in females, and the differences in predictability between females and males was statistically significant ($p < 0.001$). A similar analysis was performed to determine if markers of OS in females were predictive of male OS, and if there was a difference in predictability between females and males. Markers predictive of OS in females were not predictive in males, but the interaction only showed a

trend for association ($p = 0.073$, Supplementary Figure 2). Similar analyses were performed for markers predictive of male PFS, OS and tumor response; these interactions were not found to be statistically significant ($p = 0.951$, $p = 0.483$, $p = 0.468$, respectively; Supplementary Figures 3 & 4; see www.futuremedicine.com/doi/suppl/10.2217/pgs.10.163).

Discussion

Our results suggest that genetic profiling to predict clinical outcome of patients with metastatic colorectal cancer treated with 5-FU/oxaliplatin may depend on gender. In each of the three clinical end points we examined in this study; OS, PFS and tumor response, there was a difference in the genotypes that predicted outcome in men versus women (Figures 1–3). Given that several of the polymorphisms associated with outcome have known functional significance in relation to colorectal cancer, our current findings support the hypothesis that gender has an effect on the molecular etiology and clinical outcome of metastatic colorectal cancer. In the present study, polymorphisms in *XPD*, *SCN1A*, *EGFR*, *PLA2*, and *ER* were associated with clinical end points in a gender-related manner.

The *XPD* 156 polymorphism was associated with PFS and tumor response in females. This finding lends further clinical support to the role of *XPD* in oxaliplatin response, but in a gender-specific manner. The function of the *XPD* 156 polymorphism has not yet been determined, but the variant A allele has been associated with increased risk of lung cancer [47], as well as increased toxicity in platinum-treated non-small-cell lung cancer patients [48], suggesting that the A allele is associated with decreased DNA repair capacity. Our results support this hypothesis, as female patients carrying the A allele had improved tumor response and PFS. *XPD* is involved in nucleotide excision repair, a pathway that impacts oxaliplatin efficacy; we speculate that this polymorphism will lose clinical significance in patients not treated with oxaliplatin. The gender-related role of this polymorphism remains unclear; however, several studies have found *XPD* polymorphisms to be associated with cancer risk in a gender specific manner [49,50].

The *SCN1A* IVS591 polymorphism was associated with OS in males, and *SCN1A* T1067A polymorphism was associated with OS in females. *SCN1A* IVS591 alters the consensus sequence of the 5' splice donor site of an alternative exon [51]. *SCN1A* T1067A is a coding variant with unknown function that was originally identified in epileptic patients [52,53]. Voltage-gated sodium channel function is altered in response to oxaliplatin [54], and it has been demonstrated that sodium channel expression enhances metastatic potential [15,55]. Although the function of these SNPs in relation to oxaliplatin is unknown, it is possible that they alter the function of sodium channels thereby inhibiting proliferation and enhancing the efficacy of oxaliplatin. The functional basis of the gender-specific associations of these two polymorphisms is unknown and should be determined in future studies.

The *EGFR* CA repeat polymorphism was associated with PFS in females. Females carrying fewer than 20 repeats had shorter PFS. Short repeats in this polymorphism have been previously associated with increased *EGFR* expression [56,57], which is a negative prognostic marker in colorectal cancer [58]. Our results confirm that this polymorphism is a negative prognostic marker, but in female patients only. A previous study has shown that *EGFR* may have a gender-specific role in the outcome of colorectal cancer [40].

The *PLA2* polymorphism was associated with OS only among female patients. The SNP occurs in intron 1 of cytosolic PLA2, and its effect on PLA2 function is not known, but it may have an effect on PLA2 activity [59,60]. Cytosolic PLA2 is activated by estradiol [61], indicating that this gene may have a gender-specific role in cancer progression.

In the current study, polymorphisms in the *ER*- gene were predictive of clinical outcome among male patients, but not among female patients. *ER*- intron 5 CA repeat polymorphism was predictive of tumor response and PFS in males only. This polymorphism has been demonstrated to be associated with a risk of colorectal cancer among women, but not among men [62]. Higher numbers of CA repeats were found to be associated with an increased risk in that study, which correlates with our current findings. However, females were associated with risk in the study by Slattery *et al.*, but males were associated with clinical outcome in the present study [61]. These opposite findings require further analysis. Two studies have examined the functional impact of the *ER*- CA repeat polymorphism, with discrepant results. The first study examined the gender-specific effects of the *ER*- repeat polymorphism in a large cohort of metastatic colon cancer patients, as well as the effects of the repeat polymorphism on *ER*- gene expression [63]. There was an association between *ER*- 1 gene expression and the *ER*- (CA)_n repeat polymorphism, where tumors with any short repeat alleles had increased intratumoral *ER*- 1 mRNA expression. These findings support our current results, where male patients with short repeats, and hence potentially increased *ER*- 1 gene expression, had improved clinical outcome. The second analysis of the *ER*- CA repeat polymorphism showed a trend for association between short repeats and increased expression using an *in vitro* luciferase reporter assay [64]. This trend would support our results, that male patients with shorter repeats had improved clinical outcome owing to increased expression of *ER*- . The *ER*- CA repeat polymorphism requires further study in colorectal cancer. In addition to the CA repeat, a SNP at nucleotide 730 in the *ER*- gene was predictive of tumor response among male patients, but not among female patients. This SNP has been associated with a risk of rectal cancer, and is also associated with decreased risk of colon cancer among women taking hormone replacement therapy [62]. The function of this SNP has not been determined and requires further study.

It is notable that the formal test for interaction to check whether the markers that associated with PFS, OS and tumor response for males predicted outcomes in females or *vice versa* were not all statistically significant. The markers predicting PFS and tumor response among females were statistically significant for females only, and the markers predicting OS among females showed a trend for association in females only; however, the markers predicting OS, PFS and tumor response among males were not statistically significant. Reasons for the observed lack of associations could be owing to the relatively small size of the study. Nevertheless, the associations observed for female predictors of clinical outcome warrant validation in larger cohorts.

Conclusion

The results of this hypothesis-generating study indicate the need to account for gender in the genetic analysis of colorectal cancer progression. Functional polymorphisms that have been previously characterized in colorectal cancer should be re-evaluated with respect to gender. Polymorphisms in five of the genes that were tested in this study should be better characterized in colorectal cancer patients, especially polymorphisms with little known function such as the *ER*- , *PLA2*, *XPD* and *SCN1A* polymorphisms that were described here. Given the relatively small sample size of this exploratory study, results should be validated in a larger cohort of metastatic colorectal cancer patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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of interest

of considerable interest

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Executive summary

Genetic profiling to predict clinical outcome of patients with metastatic colorectal cancer treated with 5-fluorouracil/oxliplatin may depend on gender.

The examination of a panel of 21 polymorphisms in 13 cancer-related genes revealed that males and females have different predictors of overall survival (OS), progression-free survival (PFS) and tumor response.

Classification and regression tree (CART) analysis generated a subset of polymorphisms that grouped patients based on gender into high-risk, medium-risk and low-risk groups for each of the three clinical end points: OS, PFS and tumor response.

The following polymorphisms were predictive of clinical outcome among males: *ER- (CA)n repeat*, *ER- A730G*, *SCN1A IVS591*. These polymorphisms were not associated with clinical outcome in male patients.

The following polymorphisms were predictive of clinical outcome among females: *XPD 156*, *EGFR (CA)n repeat*, *SCN1A T1067A*, *PLA2 C/T*. These polymorphisms were not associated with clinical outcome in male patients.

Notably, polymorphisms in the estrogen receptor (*ER-*) gene were associated with OS, PFS and tumor response among male patients, but not among female patients.

The panel of polymorphisms described here may be clinically useful to predict patients who have a more aggressive disease and require aggressive treatment, and should be validated in larger prospective studies.

Figure 1. CART analysis of progression-free survival by gender

Ovals represent intermediate subgroups; squares represent terminal nodes. Rectangles indicate predictive polymorphism. Fractions within nodes indicate patients who progressed/total patients with that genotype. Group I represents low-risk patients or increased PFS; group II represents intermediate risk patients; group III represents high-risk patients or decreased PFS. Groups defined in regression trees correspond to groups in gender-specific survival curves. (A) Denotes PFS in males. (B) Denotes PFS in females. 5-FU: 5-fluorouracil; CART: Classification and regression tree analysis; PFS: Progression-free survival.

Figure 2. CART analysis of overall survival by gender

Ovals represent intermediate subgroups; squares represent terminal nodes. Rectangles indicate predictive polymorphism. Fractions indicate patients who died versus total patients with that genotype. Group I represents low-risk patients or increased OS; group II represents intermediate risk patients; group III represents high-risk patients or decreased OS. Groups defined in regression trees correspond to groups in gender-specific survival curves. **(A)** Denotes OS in males. **(B)** Denotes OS in females. 5-FU: 5-fluorouracil; CART: Classification and regression tree analysis; OS: Overall survival.

Figure 3. CART analysis of tumor response by gender

Ovals represent intermediate subgroups; squares represent terminal nodes. Rectangles indicate predictive polymorphism. Fractions indicate number of complete response + partial response/stable disease/progression of disease with that genotype. Group I represents low-risk patients or best tumor response; group II represents intermediate risk patients; group III represents high-risk patients or worst tumor response.

CART: Classification and regression tree analysis.

Table 1

Baseline information and clinical outcome among patients in the protocol 3C-01-7 (n = 173).

Characteristics	Frequency	%
Median age, years (range)	60 (25–87)	
Age (years)		
50	36	21
51–60	55	32
61	82	47
Sex		
Female	84	49
Male	89	51
Race		
Asian	27	16
Black	7	4
Caucasian	115	66
Hispanic	24	14
Anatomical site		
Appendix	1	1
Colon	102	59
Rectosigmoid	43	25
Rectum	27	16
Best response		
Complete response	1	1
Partial response	27	17
Stable disease	75	46
Progressive disease/symptomatic deterioration	59	36
Inevaluable	11	
Hematologic toxicity		
Grade 0–2	156	97
Grade 3–4	5	3
Not evaluable	12	
Nonhematologic toxicity		
Grade 0–2	68	42
Grade 3–4	93	58
Not evaluable	12	
Dose reduction		
No	48	30
Yes	113	70
Not evaluable	12	
Other information		

Characteristics	Frequency	%
No. of cycles of treatment received: median (range) [†]	5 (1–28)	
Median follow-up time, months (range)	16.1 (1.1–39.5)	
Median overall survival, months (158 patients dead) (95% CI) [‡]	9.4 (8.2–11.0)	
Median progression-free survival, months (138 patients progressed or dead) [§]	4.1 (3.2–4.7)	

[†]One cycle of treatment is 3 weeks in the toxicity data.

[‡]Median follow-up for patients who were still alive at the latest contact: 18.4 months (range: 1.1–47.5 months).

[§]Defined as the time from first day of treatment to the date of disease progression, death due to any cause, or off-study, whichever comes first. If a patient has not progressed or died, progression-free survival is censored at the time of off-study.

Table 2

Baseline characteristics by sex (n = 152).

Characteristics	Male (n = 78)	Female (n = 74)	p-value [‡]
Median age, years (range)	59 (29–87)	61 (25–82)	0.74
Age (years)			
50	16 (21%)	15 (20%)	
51–60	29 (37%)	22 (30%)	0.59
61	33 (42%)	37 (50%)	
Race			
Asian	10 (13%)	14 (19%)	
Black	3 (4%)	2 (3%)	0.67
Caucasian	54 (69%)	51 (69%)	
Hispanic	11 (14%)	7 (9%)	
Site of primary tumor			
Appendix	1 (1%)	0 (0%)	
Colon	46 (59%)	45 (61%)	0.97
Rectosigmoid	19 (24%)	19 (26%)	
Rectum	12 (15%)	10 (14%)	

[‡]Based on Fisher's exact test except comparing median age based on Mann–Whitney U-test.

Table 3

Clinical outcome by sex.

	Male (n = 78)	Female (n = 74)	p-value [†]
Response			
CR + PR	16 (21%)	12 (17%)	0.33
SD	37 (48%)	29 (40%)	
PD	24 (31%)	31 (43%)	
Toxicity			
Grade 1–2	37 (50%)	21 (30%)	0.017
Grade 3–4	37 (50%)	50 (70%)	
PFS			
Median, months (95% CI)	4.2 (3.4–5.3)	4.2 (3.1–5.3)	0.52
OS			
Median, months (95% CI)	9.0 (7.4–12.0)	11.3 (9.7–13.5)	0.69

[†]Based on the exact conditional test for response, Fisher's exact test for toxicity, and the log-rank test for PFS and OS.

CR: Complete reponse; OS: Overall survival; PD: Progression of disease; PFS: Progression-free survival; PR: Partial response.