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The role of microsatellite instability to predict clinical benefit from irinotecan-based regimens in metastatic colorectal cancer

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ABSTRACT

The aim of our study was to assess the relationship between microsatellite instability (MSI) and the clinical outcome in metastatic colorectal cancer (CRC) patients treated with irinotecan-based regimens. We assessed best objective response, progression free survival (PFS) and overall survival (OAS) in relation to MSI analysis that was performed using BAT-25, BAT-26, D5S346, D2S123, D17S250 markers in normal and tumor DNA. The best objective response was significantly and negatively related with the D17S250 (an adjacent locus to p53) microsatellite marker (p=0.047). However, MSI score was not related with the best objective response (p=0.88). There was again no relationship between PFS, OAS and MSI score. In conclusion, this study allowed us to establish in a prospective study design that MSI status did not predict survival in metastatic colorectal cancer patients treated with irinotecan-based regimens. [Turk J Cancer 2008;38(2):49-56]

KEY WORDS:

Colon cancer, microsatellite instability, irinotecan, PCR

INTRODUCTION

For a long time, the therapeutic options for patients with advanced colorectal cancer (CRC) have been almost exclusively based on fluorouracil. However, the overall treatment results remained unsatisfactory (1). The results of systemic therapy for advanced colorectal cancer have improved significantly with the availability of cytotoxic drugs such as irinotecan and oxaliplatin, and monoclonal antibodies against growth factors and their receptors (1,2). Irinotecan is a potent inhibitor of topoisomerase I, a nuclear enzyme involved in the unwinding of DNA during replication (3). This results in an increase in the number of single-strand breaks, as well as an inhibition of both replication and transcription. Microsatellite instability (MSI) is characterized by the inactivation of the mismatch repair (MMR) machinery thereby increasing the rate of mutations in DNA molecules containing microsatellite repeats (4). Several studies have used MSI as a marker for prognosis and response to therapy of colon cancer (5-8).

Germ-line alterations of MMR genes, usually hMSH2 or hMLH1, cause susceptibility to hereditary non-polipo-

sis colorectal cancer (HNPCC), a genetic disorder that accounts for nearly 5% of all cases of CRC (9). In HNPCC tumors, inactivation of the wild-type allele of the inactive MMR gene most often results from loss of heterozygosity or somatic mutation (10). These tumors which display biallelic inactivation of one of the MMR genes are characterized by high levels of MSI, defined by the accumulation of mutations, mostly insertions or deletions in short tandem repeats throughout the genome. MSI phenotype is not confined to HNPCC tumors but also occurs in ≤15% of sporadic CRC (11).

CRC with high levels of MSI are more likely to be of high histological grade, located in the proximal colon and associated with improved overall survival (5,12). The possible involvement of the DNA mismatch repair (MMR) system in the cytotoxicity of topoisomerase inhibitors has been investigated in the CRC cell lines (13). In a retrospective study in metastatic CRC patients with progressive disease after treatment with fluorouracil-based regimen it has been shown that MSI phenotype is a criteria for selecting patients who could benefit from chemotherapy with irinotecan (14). However, in the literature, no study that prospectively evaluated the prognostic role of MSI in metastatic CRC patients who were treated with irinotecan-based regimens could be found.

Therefore the aim of our study was to prospectively assess the relationship between microsatellite instability (MSI) and the clinical outcome in metastatic colorectal cancer patients treated with irinotecan-based regimens.

PATIENTS AND METHODS

Patients

During a period of 1 year between December 2003 and December 2004 metastatic CRC patients who were admitted to the Akdeniz University Medical Faculty Department of Medical Oncology were recruited to the study. The eligibility criteria were: histologically proven metastatic adenocarcinoma of the colon or rectum with measurable disease, no prior chemotherapy for metastatic disease, age ≥18 and ≤76 years, Eastern Cooperative Oncology Group (ECOG) performance status ≤2, life expectancy >3 months, adequate hematological and biochemical parameters (hemoglobin ≥10 g/dl, neutrophils ≥2x10^9/l, platelets ≥100x10^9/l, bilirubin ≤1.25x institutional upper limit of normal range (ULN), aspartate aminotransferase and alanine aminotransferase ≤5 times ULN, serum creatinine ≤1.25 ULN or creatinine clearance >50 ml/min). All patients gave written consent before the treatment they received. Main exclusion criteria were: prior exposure to irinotecan, severe concomitant conditions, inflammatory bowel disease, malabsorption (15). Prior 5-FU based adjuvant chemotherapy was not accepted as an exclusion criteria. Hypertension, diabetes mellitus and coronary heart disease were scored as positive for comorbidities.

Treatment

We conducted a non-randomised prospective, observational study recruiting consecutive patients. Five patients were treated with the IFL (irinotecan 125 mg/m^2 IV, 5-FU 500 mg/m^2 IV bolus, leucovorin 20 mg/m^2 IV bolus weekly for four weeks every six weeks) regimen and the other twenty-five patients were treated with XELIRI (irinotecan 250 mg/m^2 IV day 1 and capecitabine 2000 mg/m^2/day days 1-14, every 3 weeks) regimen as the first-line treatment by the decision of their own treating physicians (15,16). Treatment was continued till disease progression and unacceptable toxicity. Patients were followed up every 3 months after completion of treatment to evaluate overall survival. Response was evaluated by RECIST criteria at every 2-3 cycles during treatment. Toxicity was evaluated during treatment according to the National Cancer Institute Common Toxicity Criteria (version 3, 2003). Protocol specified dose reductions and delays were based on previous cycle toxicity utilizing both hematological nadirs and hematological/biochemical parameters on the day of next treatment.

Tumor specimen collection

Detailed descriptions of the specific characteristics of the specimen collection have been published previously (17). Tissue samples from 30 patients with metastatic CRC were studied. One section from the tumor together with a section from the adjacent normal tissue, if available, were blocked. These sections were cut at 5 µm thickness and stained with hematoxylin-eosin (H&E). The sections stained with H&E were reviewed and re-examined by the same pathologist. DNA was isolated from the tis-

sue specimens of the tumor, adjacent normal tissue and also from the blood samples. Serial 5 µm thick sections of selected tissue blocks were obtained on glass slides, and the areas of interest were microdissected after matching with an adjacent section stained with H&E. To eliminate cross-contamination, disposable microtome blades were used (18).

PCR and MSI analysis

The method utilized for the characterization of microsatellite alterations was based on PCR amplification (5). Samples of genomic DNA were used to amplify sequences from 5 of the following mononucleotide and dinucleotide microsatellite loci: BAT-25, BAT-26, D5S346, D2S123, D17S250. These specific microsatellite loci were derived from the National Cancer Institute (NCI) reference (19). Using this reference panel, microsatellite instability-high (MSI-H) tumors were defined as having instability in two or more markers, whereas microsatellite instability-low (MSI-L) tumors were defined as having instability in one marker. Lack of instability in any marker described the microsatellite stable (MSS) group (20). Genomic DNA was amplified by PCR in a total of 25 µl of reaction mixture, which included 100 nm primers, 100 µM each of dNTP, 1xPCR buffer, 1.5 mM magnesium chloride and 2 units of Tag DNA polymerase (Fermentase, USA) (21). Aliquots (5 µl) of the PCR products were added to 5 µl of loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol) and the entire was denatured at 94°C for 2 minutes and placed on ice. Aliquots (8 µl) were electrophoresed on 8% polyacrylamide gels (PAGE) and silver nitrate was used for the detection of bands (22). MSI was identified as either a deletion or a band shift. A band shift was defined as an abnormal and reproducible pattern which revealed expansion, contraction or rearranged bands (23). All altered cases were analyzed at least twice by an additional PCR and an electrophoretic run to confirm the results.

Statistical analysis

The association of clinical and MSI related factors with the best objective response was analyzed with the univariate logistic regression analysis. Survival curves for the progression-free survival (PFS) and the overall survival (OAS) were drawn according to the method of Kaplan and Meier (23); Cox regression analysis was used to test associations with survival. A multivariate regression analysis was planned if more than two clinic factors had p values <0.10. P values of less than 0.05 were considered to indicate statistical significance. All statistical calculations were performed by using SPSS for Windows version 11.0 (SPSS Inc. Chicago, IL).

RESULTS

Patient characteristics

Thirty patients with metastatic CRC were included in the study, 14 females and 16 males with a mean age of 55.1±14.4 year (Range: 22-76). Clinical characteristics of the patients are given in table 1. All the patients except one had a good performance score (performance score ≤2). Median of one metastatic site was found in our patients.

Table 1
Demografic and clinic parameters of metastatic CRC patients

Characteristics	All patients (n=30)
Age (mean±SD)	55.1±14.4
Gender, n (%)	
Male	16 (53.3%)
Female	14 (46.7%)
Comorbidities, n (%)	
Present	13 (43.3%)
Absent	15 (50%)
Adjuvant chemotherapy, n (%)	
Yes	12 (40%)
No	18 (60%)
Adjuvant radiotherapy, n (%)	
Yes	7 (23.3%)
No	22 (73.3%)
Performance status, n (%)	
1	21 (70%)
2	8 (26.7%)
3	1 (3.3%)
Liver metastasis, n (%)	
Present	21 (70%)
Absent	9 (30%)

Table 2
Analysis of microsatellite markers and microsatellite instability status of the patients

No	Gender	Age	BAT25	BAT26	D5S346	D2S123	D17S250	MSI
1	F	70	(-)	(-)	(-)	(-)	(-)	MSS
2	F	39	(-)	(-)	(-)	(+)	(-)	MSI-L
3	F	58	(+)	(-)	(-)	(-)	(+)	MSI-H
4	M	52	(+)	(-)	(-)	(-)	(-)	MSI-L
5	M	58	(-)	(-)	(+)	(-)	(+)	MSI-H
6	F	26	(-)	(-)	(-)	(-)	(+)	MSI-L
7	M	44	(-)	(+)	(-)	(+)	(-)	MSI-H
8	M	69	(-)	(-)	(-)	(+)	(-)	MSI-L
9	M	22	(-)	(-)	(-)	(+)	(-)	MSI-L
10	M	65	(-)	(-)	(+)	(-)	(-)	MSI-L
11	M	41	(-)	(-)	(-)	(-)	(-)	MSS
12	F	52	(-)	(+)	(-)	(-)	(-)	MSI-L
13	F	64	(+)	(+)	(-)	(-)	(-)	MSI-H
14	F	50	(-)	(-)	(+)	(-)	(-)	MSI-L
15	M	67	(-)	(-)	(-)	(-)	(-)	MSS
16	M	74	(-)	(-)	(-)	(-)	(-)	MSS
17	M	63	(-)	(+)	(-)	(+)	(-)	MSI-H
18	M	61	(-)	(-)	(+)	(-)	(+)	MSI-H
19	M	76	(+)	(-)	(-)	(+)	(+)	MSI-H
20	M	64	(-)	(-)	(-)	(-)	(-)	MSS
21	F	48	(-)	(-)	(-)	(-)	(+)	MSI-L
22	F	51	(-)	(-)	(+)	(-)	(-)	MSI-L
23	F	34	(-)	(-)	(-)	(-)	(-)	MSS
24	M	70	(-)	(-)	(-)	(+)	(-)	MSI-L
25	F	44	(+)	(+)	(-)	(-)	(-)	MSI-H
26	F	41	(+)	(+)	(+)	(-)	(+)	MSI-H
27	F	53	(-)	(-)	(-)	(-)	(-)	MSS
28	M	72	(+)	(-)	(-)	(+)	(+)	MSI-H
29	M	73	(-)	(+)	(-)	(-)	(+)	MSI-H
30	F	67	(-)	(-)	(+)	(-)	(-)	MSI-L

MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability - low; MSI-H: Microsatellite instability - high

MSI status

Tumor MSI analysis was performed with mononucleotide and dinucleotide markers. The results of the MSI analysis can be viewed in tables 2 and 3. MSI-L group (12 patients, 40%) was the most common group in our study according to the NCI criteria. D2S123 (Figures 1, 2) and D17S250 were the most common markers (26.7%, each) (Table 3).

Tumor response

The response rate was found to be 46.7% (14/30 patients) in the study group. Although the response evaluation could not be done in 3 patients, 14 patients (46.7%) gave response to irinotecan-based chemotherapy. 27 patients were included in the tumor response analysis. One of these 3 patients who could not be evaluated for the response died suddenly at home after the first cycle of IFL

Table 3
The sum of microsatellite instability results

MSI	N	%
Total	30	100
Bat25^{+}	7	23.3
Bat26^{+}	7	23.3
D5S346^{+}	7	23.3
D2S123^{+}	8	26.7
D17S250^{+}	8	26.7
MSS	7	23.3
MSI-L	12	40.0
MSI-H	11	36.7

MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability - low; MSI-H: Microsatellite instability - high

with no known reason. The other two were lost to follow up after two cycles of chemotherapy. The best objective response was only negatively related with D17S250 marker

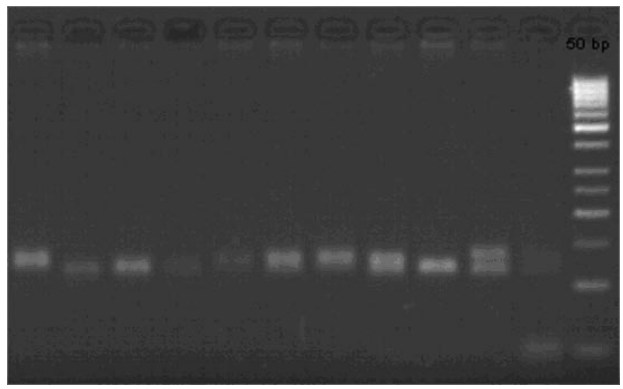

Fig 1. An example for the amplification results of D2S123 marker (1-1T/2-1N/3-1L, 4-2T/5-2N/6-2L, 7-3T/8-3N/9-3L, 10 and 11/positive amplification control)

(exp(B)=0.16, p=0.047). The MSI scores were not found to be related with the best objective response (p=0.88). The relation of D17S250 marker and the response can be seen in table 4 and figure 3.

Survival analysis

A total of 30 patients were included in the survival analysis. Median PFS was 152 days (95% CI: 91-213

Table 4
The relation of progression free survival, overall survival and tumor response with the other factors

Characteristics	OAS Exp(B)	P	PFS Exp(B)	P	Response Exp(B)	P
Age	0.98	0.42	0.98	0.24	1.04	0.17
Gender	0.78	0.65	0.72	0.44	2.88	0.19
Grade	2.82	0.13	1.92	0.25	0.21	0.20
Performance status	1.61	0.30	1.35	0.38	1.18	0.81
Metastatic site	0.77	0.63	1.98	0.13	1.60	0.55
MSI						
MSI-L vs. MSS	0.47	0.27	1.23	0.72	0.25	0.17
MSI-H vs. MSI-L	0.64	0.51	1.29	0.67	0.93	0.95
MSI score (0-5)	0.74	0.33	1.12	0.70	1.08	0.88
Bat25	0.39	0.24	0.61	0.34	6.54	0.11
Bat26	1.43	0.56	1.58	0.35	2.29	0.38
D5S346	0.43	0.27	1.14	0.77	0.48	0.41
D2S123	2.23	0.16	0.79	0.62	3.0	0.23
D17S250	0.31	0.14	1.21	0.80	0.16	0.05*

OAS: Overall survival, PFS: Progression free survival, MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability - low; MSI-H: Microsatellite instability - high
*P=0.047

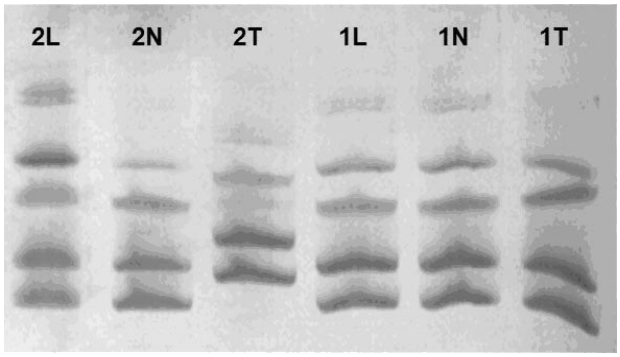

Fig 2. An example for PAGE results of D2S123 marker (T: Tumor tissue DNA; N: Normal tissue DNA; L: Lymphocyte DNA)

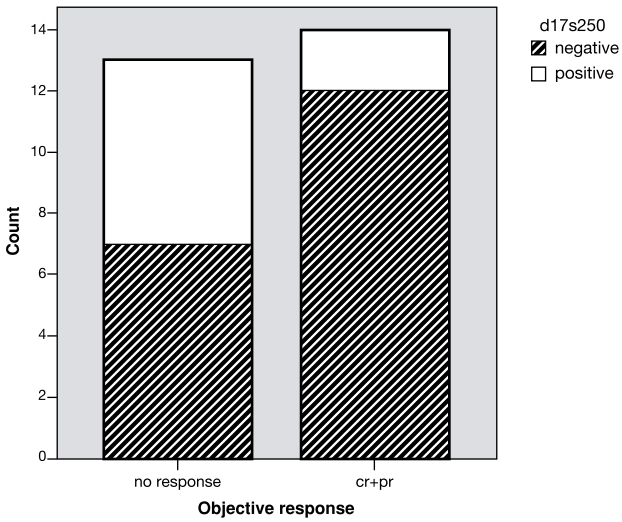

Fig 3. The relation of D17S250 microsatellite instability marker and the best objective response (cr: complete response; pr: partial response)

days) in our study group. None of the investigated factors were found to be significantly associated with the PFS in the univariate analysis (Table 4). Also, MSI score was not found to be related with the PFS (p=0.70).

Median OAS was 486 days (95% CI: 246-726). Similar to PFS, none of the factors in this study was found to be related for the OAS in the univariate analysis (Table 4).

DISCUSSION

In the present study, we have failed to demonstrate an improved clinical outcome by irinotecan-based regimens in MSI-H metastatic CRC patients. In detail, we could not find a positive correlation with MSI status and DFS, OAS or tumor response. We suppose that the issue in therapeutic response prediction to irinotecan is the same as with the 5-FU response which was studied much more in detail than with irinotecan. There may be other pathways to test any association between the DNA repair system activity and irinotecan response like the case with oxaliplatin. ERCC1 (excision repair cross-complementing) gene and thymidilate syntase gene were demonstrated as predictive factors of survival for CRC patients receiving combination oxaliplatin and fluorouracil chemotherapy (25,26). Although small and retrospective, similar to the oxaliplatin/5FU story, a study suggests that gene expression levels of ERCC1 may be useful in predicting the clinical outcome of patients with metastatic CRC patients treated with first line irinotecan-based chemotherapy (27).

There was no prospective or a well designed retrospective study for establishing the relation of MSI and irinotecan response in metastatic CRC patients up to the initiation of this study. Therefore, our aim was to assess the relationship prospectively between MSI and tumor response in metastatic CRC patients treated with irinotecan-based regimens. It was impossible to test irinotecan monotherapy prospectively, because of the accumulation of the evidence that supported the benefit of the combination chemotherapy options in metastatic CRC patients (28). We had recruited patients consecutively into this study. Although, our study population was very small, they received standard regimens. Also, the objective response rate (46.7%) in our study is similar with the previous studies (15,16,29). We have not found a relation with the best objective response and the MSI score. The best objective response was only negatively related with D17S250 marker (an adjacent locus to p53). This was the first report demonstrating the negative relation of response to irinotecan-based regimens in D17S250 marker positive metastatic CRC patients. In a study, loss of heterozygosity of D17S250 was described as a criterion for suppressor/p53-type tumors of mucinous adenocarcinoma of the colorectum which had a significant association with distal colon location, venous invasion, extent of lymph node metastasis and higher tumor stage (30). Mutator-type tumors which had a dysfunction of the DNA MMR system have been related with a high frequency of MSI. Tumors with high frequency MSI are more likely to be right-sided with mucinous histology, poor differentiation, tumor-infiltrating lymphocytes and associated with improved survival (5,12). But, we are not sure whether and how these factors might have affected our results.

In their retrospective study, Fallik et al. (14), found that among the 7 tumors that displayed a MSI-H phenotype, 4 responded to irinotecan-based regimens, whereas only 7 of the 65 MSI-L/MSS tumors did so (p=0.009). Interestingly, a complete response to irinotecan was observed in 1 (1.4%) patient and a partial response in 10 (13.9%) patients, whereas 61 (84.7%) patients did not respond in the same study. However, different irinotecan and fluorouracil combinations in second-line with minor modifications in the dose and schedule have been tested in metastatic CRC patients and the median overall response rate of these trials was around 22% (31). It is possible that, a group of metastatic CRC patients who had resistance to irinotecan-based therapy were included in their retrospective study. In addition, retrospective nature and study size could have affected their results. Therefore, their study does not justify a standard role for MSI for the purpose of patient selection in metastatic CRC for treatment with irinotecan-based regimens. Our study, in contrast, excludes this role for MSI in this patient population treated with irinotecan-based regimens.

It is still controversial whether MSI testing could predict the 5-FU response in CRC patients (32). Ribic et al. (6) used specimens from resected stage II or stage III colon cancer who were previously enrolled in prospective randomized trials of 5-FU-based chemotherapy. Of the 570 tumor samples tested, 16.7% were categorized as MSI-H which appears to be lower than our cohort of Turkish patients (36.7%). Among the patients who had not received adjuvant chemotherapy, those with MSI-H tumors had longer overall survival and higher rates of 5 year disease-free survival than patients with MSI-L or MSS tumors. However, fluorouracil-based adjuvant chemotherapy benefited patients with stage II or stage III colon cancer with MSS tumors or exhibiting MSI-L tumors but not those with tumors exhibiting MSI-H. Also, Kim et al. (33) and Lamberti et al. (34) do not support the use of MSI-H as a predictive marker of chemotherapy benefit in 5FU treated patients. These results suggest that the improved prognosis for MSI-H patients, as previously reported, may be more dependent on the specific biology of these cancers as opposed to their response to adjuvant 5-FU based chemotherapy.

In conclusion, we report for the first time that there is a relation between the best objective response and the presence of D17S250 marker in metastatic CRC patients that were treated with first-line irinotecan-based chemotherapy. However, we found no correlation with the MSI score and clinical outcome in our cohort. Further evaluation is warranted to further define the role of MSI and D17S250 as potential predictive markers in metastatic CRC.

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References

1. Vanhoefer U, Harstrick A, Achterrath W, et al. Irinotecan in the treatment of colorectal cancer: clinical overview. J Clin Oncol 2001;19:1501-18.
2. Punt CJA. New options and old dilemmas in the treatment of patients with advanced colorectal cancer. Ann Oncol 2004;15:1453-9.
3. Kawato Y, Aonuma M, Hirota Y, et al. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. Cancer Res 19912;51:4187-91.
4. Diaz LA Jr. The current clinical value of genomic instability. Semin Cancer Biol 2005;15:67-71.
5. Gryfe R, Kim H, Hsieh ET, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med 2000;342:69-77.
6. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite instability status as a predictor of benefit from fluorouracil based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349:247-57.
7. Carethers JM, Smith EJ, Behling CA, et al. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. Gastroenterology 2004;126:394-401.
8. Brueckl WM, Moesch C, Brabletz T, et al. Relationship between microsatellite instability, response and survival in palliative patients with colorectal cancer undergoing first-line chemotherapy. Anticancer Res 2003;23:1773-7.
9. Wheeler JM, Bodmer WF, Mortensen NJ. DNA mismatch repair genes and colorectal cancer. Gut 2000;47:148-53.

10. Liu B, Nicolaides NC, Markowitz S, et al. Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. Nat Genet 1995;9:48-55.

11. Thibodeau SN, French AJ, Cunningham JM, et al. Microsatellite instability in colorectal cancer: different mutator phenotypes and the principal involvement of hMLH1. Cancer Res 1998;58:1713-8.

12. Ward R, Meagher A, Tomlinson I, et al. Microsatellite instability and the clinicopathological features of sporadic colorectal cancer. Gut 2001;48:821-9.

13. Jacob S, Aguado M, Fallik D, et al. The role of the DNA mismatch repair system in the cytotoxicity of the topoisomerase inhibitors camptothecin and etoposide to human colorectal cancer cells. Cancer Res 2001;61:6555-62.

14. Fallik D, Borrini F, Boige V, et al. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. Cancer Res 2003;63:5738-44.

15. Rea DW, Nortier JW, Ten Bokkel Huinink WW, et al. A phase I/II and pharmacokinetic study of irinotecan in combination with capecitabine as first-line therapy for advanced colorectal cancer. Ann Oncol 2005;16:1123-32.

16. Saltz LB, Cox JV, Blanke C, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med 2000;343:905-14.

17. Pehlivan S, Koyuncuoglu M, Pehlivan M, et al. Premalignant lesions of the kidney share the same genetics changes as conventional renal cell carcinoma. World J Urol 2004;22:120-3.

18. Goelz SE. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. Biochem Biophys Res Commun 1985;130:118.

19. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248-57.

20. Pandey V, Prabhu JS, K P, Rajan V, et al. Assessment of microsatellite instability in colorectal carcinoma at an Indian center. Int J Colorectal Dis 2007;22:777-82.

21. Liengswangwong U, Nitta T, Kashiwagi H, et al. Infrequent microsatellite instability in liver fluke infection-associated intrahepatic cholangiocarcinomas from Thailand. Int J Cancer, 107:375-80, 2003.

22. Erdem H, Pehlivan S, Topaloglu H, et al. Allele distribution of D5S125, MAP1B5' and D5S679 microsatellite markers in Turkish spinal muscular atrophy families. Turk J Pediatr 1997;39:447-52.

23. Leung WK, Kim JJ, Kim JG, et al. Microsatellite instability in gastric intestinal metaplasia in patients with and without gastric cancer. Am J Pathol 2000;156:537-43.

24. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.

25. Stoehlmacher J, Park DJ, Zhang W, et al. A multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. Br J Cancer 2004;91:344-54.

26. Shirota Y, Stoehlmacher J, Brabender J, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. J Clin Oncol 2001;19:4298-304.

27. Vallbohmer D, Iqbal S, Yang DY, et al. Molecular determinants of irinotecan efficacy. Int J Cancer 2006;119:2435-42.

28. Venook A. Critical evaluation of current treatments in metastatic colorectal cancer. Oncologist 2005; 10: 250-61.

29. Douillard JY, Cunningham D, Roth AD, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000;355:1041-7.

30. Li D, Semba S, Wu M, et al. Molecular pathological subclassification of mucinous adenocarcinoma of the colorectum. Pathol Int 2005;55:766-74.

31. Atalay G, Cardoso F, Paesmans M, et al. Second-line treatment in advanced colon cancer: are multiple phase II trials informative enough to guide clinical practice? Anticancer Drugs 2003;14:703-13.

32. Niv Y. Biologic behavior of microsatellite-unstable colorectal cancer and treatment with 5-fluorouracil. Isr Med Assoc J 2005;7:520-4.

33. Kim GP, Colangelo LH, Wieand HS, et al. National Cancer Institute. Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. J Clin Oncol 2007;25:767-72.

34. Lamberti C, Lundin S, Bogdanow M, et al. Microsatellite instability did not predict individual survival of unselected patients with colorectal cancer. Int J Colorectal Dis 2007;22:145-52.