Med Oncol (2011) 28:S214–S218
DOI 10.1007/s12032-010-9701-2

ORIGINAL PAPER

Clinical significance of microsatellite instability for stage II or III colorectal cancer following adjuvant therapy with doxifluridine

Byung Woog Kang · Jong Gwang Kim · Soo Jung Lee · Yee Soo Chae · Joon Ho Moon · Sang Kyun Sohn · Seong Woo Jeon · Min Kyu Jung · Kyoung-Hoon Lim · You Seok Jang · Jun Seok Park · Soo Han Jun · Gyu-Seog Choi

Received: 22 August 2010 / Accepted: 21 September 2010 / Published online: 16 October 2010

Abstract Microsatellite instability (MSI) is a molecular marker that can provide valuable prognostic information for colorectal cancer (CRC). However, the predictive role of the MSI status remains less clear than its role in prognostication due to mixed results from previous studies. Therefore, this study investigated the usefulness of the MSI status as a predictive factor for stage II or III CRC patients who received adjuvant doxifluridine therapy. Among 3030 patients with CRC who underwent surgical resection between 1997 and 2006, 564 patients were diagnosed with stage II or III, and adjuvant doxifluridine therapy was administered to 394 patients (70.0%). The MSI status was assessed using the markers BAT25 and BAT26, and samples with instability at both markers were scored as exhibiting high-frequency MSI (MSI-H). Among the 564 patients, 290 patients (51.4%) had stage II, and MSI-H was found in 41 patients (7.3%). With a median follow-up duration of 35.1 months (range, 0.5–135.2), the 5-year overall survival (OS) rate and relapse-free survival (RFS) rate were 87.5 and 76.2%, respectively. MSI-H showed a favorable survival trend for OS ($P = 0.098$) and significant survival benefit for RFS ($P = 0.037$) in all patients. In a univariate analysis, the doxifluridine-treated patients with MSI-H showed improved RFS compared to those with low or stable MSI (MSI-L/S) ($P = 0.036$), while the MSI status was not significantly associated with OS ($P = 0.107$). In a multivariate analysis, MSI-H was not significantly associated with RFS (Hazard ratio = 2.467, $P = 0.125$). In conclusion, this study confirmed the positive prognostic role of MSI-H. However, MSI-H patients with stage II or III CRC did not seem to benefit from doxifluridine adjuvant therapy.

Keywords Colorectal cancer · Microsatellite instability · Doxifluridine · Adjuvant therapy

Jong Gwang Kim and Gyu-Seog Choi are contributed equally to this work.

B. W. Kang · J. G. Kim (✉) · S. J. Lee · Y. S. Chae · J. H. Moon · S. K. Sohn
Department of Hematology/Oncology, Kyungpook National University Hospital, Kyungpook National University School of Medicine, 200 Dongduk-Ro, Daegu, Jung-Gu 700-712, South Korea
e-mail: jkk21c@mail.knu.ac.kr

S. W. Jeon · M. K. Jung
Department of Gastroenterology, Kyungpook National University Hospital, Kyungpook National University School of Medicine, 200 Dongduk-Ro, Daegu, Jung-Gu 700-712, South Korea

K.-H. Lim · Y. S. Jang · J. S. Park · S. H. Jun · G.-S. Choi (✉)
Department of Surgery, Kyungpook National University Hospital, Kyungpook National University School of Medicine, 200 Dongduk-Ro, Daegu, Jung-Gu 700-712, South Korea
e-mail: kyuschoi@knu.ac.kr

Introduction

The clinical impact of molecular markers in colorectal cancer (CRC) has been the focus of extensive investigation, with microsatellite instability (MSI) attracting a lot of attention. Approximately 15–20% of sporadic CRC develops via an alternative pathway of tumorigenesis characterized by MSI, a molecular marker of a defective function of the DNA mismatch repair system [1]. The instability is termed MSI-high (MSI-H) or MSI-low/stable (MSI-L/S), based on the percentage of loci showing instability [2]. The positive prognostic role of MSI-H has also been demonstrated in several studies and a systemic review, representing improved overall survival (OS) independent of the tumor stage [3–6].

The benefits of adjuvant treatment have already been clearly established for CRC. Almost all adjuvant chemotherapy for CRC involves the agent 5-fluorouracil (5-FU), typically in combination with leucovorin [7]. With the recent availability of new drugs, such as oxaliplatin and irinotecan, the potential use of MSI for identifying patients that may respond better to particular drugs could be important. However, while a positive prognosis is well established for patients with MSI-H CRC, the issue of whether the MSI status of a patient can predict their response to adjuvant chemotherapy remains more controversial. Several studies have suggested that patients with MSI-H CRC did not derive benefit from 5-FU-based chemotherapy when compared to patients with a non-MSI-H tumor [4, 5, 8, 9]. In contrast, Bertagnolli et al. reported equal outcomes for MSI-H and non-MSI-H tumors when treated with irinotecan and 5-FU as the adjuvant chemotherapy regimen [10]. Furthermore, the gene expression of the DNA mismatch repair gene MSH2 has also been evaluated as a predictive marker for advanced CRC treated with capecitabine [11].

In current clinical practice, orally administered 5-FU drugs are widely used as an adjuvant chemotherapy regimen. Doxifluridine is an oral fluoropyrimidine that was designed to generate 5-FU, preferentially at the tumor site, via an enzymatic process that exploits the significantly higher activity of thymidine phosphorylase in tumors [12]. Doxifluridine, which is an intermediate of capecitabine, has also been shown to be effective in patients with CRC [13]. However, the clinical effect of the MSI status on the treatment response to doxifluridine as an adjuvant regimen has not yet been evaluated. Accordingly, the current authors conducted a retrospective study to determine the usefulness of the MSI status as a predictive factor for stage II or III CRC patients who received adjuvant doxifluridine therapy.

Patients and Methods

Patients

Between July 1997 and August 2006, 3030 patients with histologically confirmed CRC underwent complete surgical resection at Kyungpook National University Hospital (KNUH). Among these 3030 patients, 564 patients were diagnosed with stage II or III, according to the 6th Edition of the Guidelines of the American Joint Committee on Cancer (AJCC) [7]. These 564 patients were then retrospectively analyzed, including an assessment of their MSI status. The following clinical data were collected from medical records for each patient: surgical and pathologic reports, imaging, treatment modalities, and MSI status. This study was reviewed by the Institutional Review Board.

Treatment

After complete surgical resection, adjuvant doxifluridine therapy was administered to 394 patients (70.0%), where doxifluridine was administered orally three times a day after every meal. The daily dosages of doxifluridine were based on the body surface area (BSA): 600 mg (3 cap); <1.48 m^2, 800 mg (4 cap); 1.48–1.91 m^2, 1000 mg (5 cap); >1.91 m^2. The chemotherapy was given daily and continued for 1 year until disease relapse, patient refusal, or intolerable toxicity.

MSI analysis

Tumor tissue samples were obtained from each patient during surgery. The laboratory analysis was then conducted at KNUH, where the DNA extracted from each tumor was amplified by polymerase chain reaction-denaturing high performance liquid chromatography (PCR-DHPLC), and the MSI testing was performed based on the two most sensitive markers (BAT25 and BAT26) among the 5-marker panel proposed by the National Cancer Institute [2]. Using BAT25 and BAT26, the samples with instability at both markers were classified as exhibiting MSI-H [5]. Plus, a tumor was classified as MSI-L if one locus showed instability and MSI-S if all the loci were stable.

Statistical analysis

The continuous variables were compared using a two-sample *t test*, while the categorical data were analyzed using a Chi-square test. OS was defined as the time from surgery until death from any cause. Relapse-free survival (RFS) was defined as the time from surgery until relapse or death from any cause. The OS and RFS were analyzed using the Kaplan–Meyer test, and both groups were compared using a log-rank test or Breslow test. The Cox regression model was used to determine the clinical predictors for RFS. The parameters with a P value of less than 0.1 in a univariate analysis were then used in a multivariate analysis. The variables were analyzed using forward and backward methods. The statistical analyses used Statistical Package for the Social Sciences (SPSS) software version 14 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

The patient characteristics at diagnosis according to their MSI status are shown in Table 1. The total cohort included 564 patients, where the median age was 63 years (range,

Table 1 Clinicopathological characteristics according to MSI status

	Total patients ($n = 564$)	MSI-L/S ($n = 523$)	MSI-H ($n = 41$)	P value
Age (year)	63 (21–89)	64 (21–89)	60 (27–84)	0.143
Sex (male)	327 (60.0%)	300 (57.4%)	27 (65.9%)	0.327
Site				0.088
Colon	318 (56.4%)	286 (54.7%)	32 (78.0%)	
Rectum	236 (41.8%)	231 (44.2%)	5 (12.2%)	
Multiple	10 (1.8%)	6 (1.1%)	4 (9.8%)	
Histology				0.216
Adenocarcinoma	529 (93.8%)	235 (44.9%)	31 (75.6%)	
Mucinous carcinoma	34 (6.0%)	24 (4.6%)	10 (24.4%)	
Others	1 (0.2%)	1 (0.2%)	0	
Stage				0.019
IIA	259 (45.9%)	235 (44.9%)	24 (58.5%)	
IIB	31 (5.5%)	25 (4.8%)	6 (14.6%)	
IIIA	24 (4.3%)	24 (4.6%)	0	
IIIB	160 (28.4%)	152 (29.1%)	8 (19.5%)	
IIIC	90 (16.0%)	87 (16.6%)	3 (7.3%)	
CEA (ng/ml)	2.8 (0–807)	2.8 (0–807)	2.0 (0–137.6)	0.954
Adjuvant doxifluridine therapy				0.113
Yes	394 (70.0%)	270 (70.7%)	24 (58.5%)	
No	170 (30.0%)	153 (29.3%)	17 (41.5%)	

21–89) and 60.0% were male. Two hundred and ninety patients (51.4%) had a stage II disease, and adjuvant doxifluridine therapy was administered to 394 patients (70.0%). Forty-one tumors (7.3%) were MSI-H, and 523 (92.7%) were MSI-L/S. The MSI-H and MSI-L/S groups were not significantly different in terms of age, gender, the primary site of the tumor, tumor histology, level of CEA, and use of adjuvant doxifluridine therapy. However, the patients with MSI-H tumors did exhibit a significant difference as regards being in an earlier stage of the disease ($P = 0.019$).

Survival and MSI status

At a median follow-up of 35.1 months (range, 0.5–135.2), 109 patients (19.3%) had relapsed and 56 patients (9.9%) had died. The 5-year overall survival (OS) rate and relapse-free survival (RFS) rate were 87.5 and 76.2%, respectively. The patients with MSI-H showed a favorable survival trend for OS ($P = 0.098$) and a significant survival benefit for RFS ($P = 0.037$) (Fig. 1). For the patients who received doxifluridine adjuvant therapy, the MSI status was not significantly associated with OS ($P = 0.107$); however, the doxifluridine-treated patients with MSI-H showed an improved RFS when compared to those with low or stable MSI (MSI-L/S) ($P = 0.036$) (Fig. 2). Nonetheless, in a multivariate analysis adjusted for age, sex, stage, and the primary site of the tumor, MSI-H was not significantly associated with RFS (Hazard ratio = 2.467, 95% CI 0.778–7.819, $P = 0.125$), and the tumor stage was the only independent prognostic factor (Hazard ratio = 3.821, 95% CI 2.312–6.315, $P < 0.001$) (Table 2).

Discussion

To our knowledge, this is the first study to evaluate the predictive role of the MSI status in CRC patients who received adjuvant doxifluridine therapy, and the results confirmed the prognostic value of MSI-H, as found in previous studies [4, 5, 8, 9]. However, for the patients who received adjuvant doxifluridine therapy, the predictive value of MSI-H was not demonstrated in a multivariate analysis adjusted for the tumor stage.

MSI represents a promising molecular marker for CRC, due to the favorable prognosis associated with MSI-H. Plus, recent data suggest that it may also serve as a reliable marker for the response to chemotherapy. An intact DNA mismatch repair system would appear to be necessary to mediate the cytotoxicity of several chemotherapeutic agents, including 5-FU [14]. Both cell cycle arrest and cell death following exposure to 5-FU have been shown to be dependent on mismatch repair (MMR) proteins, and the recognition of 5-FU incorporation into the DNA by the MMR proteins would appear to be a critical step in this process [15].

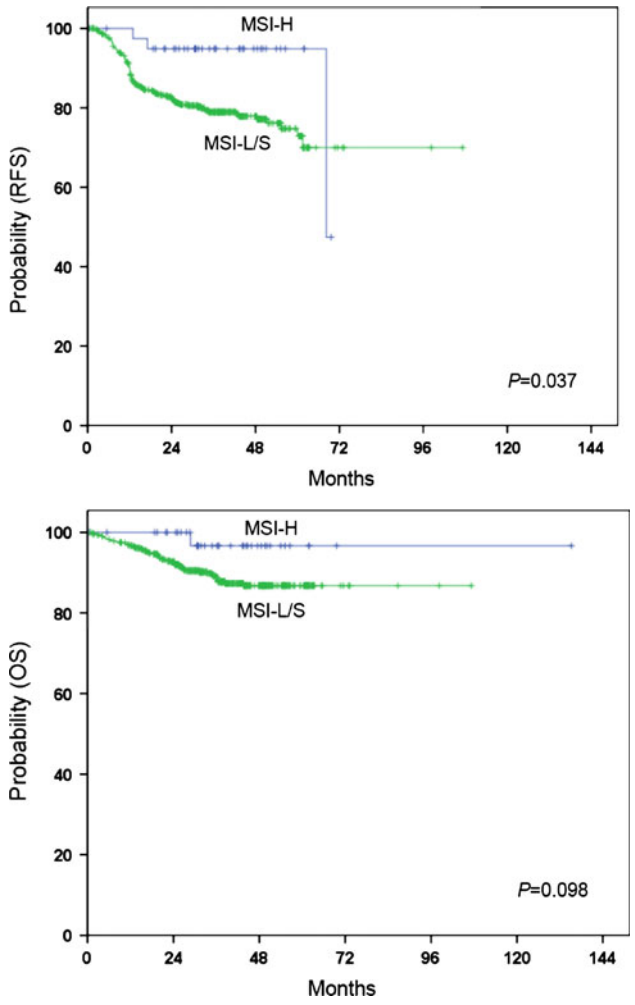

Fig. 1 Relapse-free survival and overall survival of total patients with MSI-H and MSI-L/S tumors

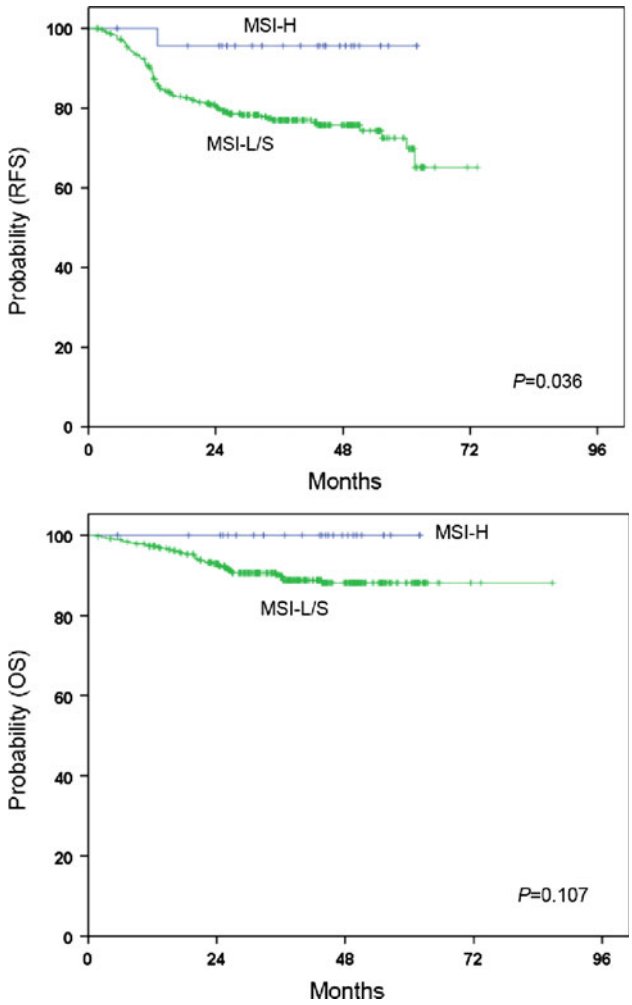

Fig. 2 Relapse-free survival and overall survival of patients with MSI-H and MSI-L/S tumors following adjuvant doxifluridine chemotherapy

To date, several studies have revealed a lack of benefit from 5-FU-based chemotherapy for patients with MSI-H tumors [14], which is consistent with the present study in terms of survival. Ribic et al. studied tissue specimens from 570 patients enrolled in five large clinical trials investigating the role of adjuvant chemotherapy compared with surgery alone. More than 50% of these specimens were from patients with stage II colon cancer. MSI predicted a lack of response to adjuvant chemotherapy even after adjustment for stage and grade, with a trend toward worse overall survival for the MSI-H group treated with adjuvant chemotherapy. Plus, recent prospective trials reported that the survival benefit of 5-FU treatment was confined to non-MSI tumors or those with intact mismatch repair proteins [9, 16]. In contrast, a retrospective analysis of patients with stage III colon cancer revealed a highly improved survival for MSI-H patients treated with 5-FU-based chemotherapy [17]. Meanwhile, various other retrospective studies have failed to demonstrate a predictive impact of MSI [18, 19].

Table 2 Multivariate analysis of factors affecting relapse-free survival

Factor	Hazard ratio	95% confidence interval	*P* value
Female sex	0.989	0.676–1.448	0.955
Tumor site (rectum)	1.037	0.730–1.473	0.839
Stage III	3.821	2.312–6.315	<0.001
Adjuvant doxifluridine therapy	0.705	0.410–1.215	0.208
MSI-L/S	2.467	0.778–7.819	0.125

However, in two recent studies, the addition of oxaliplatin or irinotecan was found to overcome the negative impact of 5-FU on MSI-H tumors in an adjuvant setting [10, 20]. Thus, when taken together, studies on the association of MSI and adjuvant chemotherapy would seem indicate that

MSI can play a predictive role depending on the chemotherapeutic agent.

Doxifluridine, an oral fluoropyrimidine that is converted to 5-FU predominantly in tumors, is an intermediate form of capecitabine that was developed due to the clinical need for efficient, tolerable, and convenient agents that do not require continuous infusion. Currently, doxifluridine is used as an adjuvant chemotherapy regimen for CRC in Asia [12, 13, 21]. Interestingly, Jensen et al. previously evaluated the gene expression of the DNA mismatch repair gene MSH2 as a predictive marker in advanced CRC treated with capecitabine [11] and found that a higher gene expression of MSH2 was associated with overall survival.

In the present study, the incidence of MSI-H (7.3%) was relatively low, probably as only 2 mononucelotide repeats (BAT25 and BAT26) were checked to diagnose the MSI status. Plus, the current study included more stage III patients, yet there is a higher incidence of MSI-H tumors in stage II patients [22].

In conclusion, MSI-H patients with stage II or III CRC did not seem to benefit from adjuvant doxifluridine therapy, suggesting that adjuvant oral 5-FU-based chemotherapy may not be useful for patients with MSI-H. The present study also serves to emphasize the importance of a large-scale prospective study to explore the association between the MSI status and oral 5-FU drugs, such as doxiflurine or capecitabine, in an adjuvant setting.

References

1. Sinicrope FA, Sargent DJ. Clinical implications of microsatellite instability in sporadic colon cancers. Curr Opin Oncol. 2009;21: 369–73.
2. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, 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.
3. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med. 2000; 342:69–77.
4. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol. 2005;23:609–18.
5. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, 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.
6. Samowitz WS, Curtin K, Ma KN, Schaffer D, Coleman LW, Leppert M, et al. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. Cancer Epidemiol Biomarkers Prev. 2001;10:917–23.
7. Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, et al. Colorectal cancer. Lancet. 2010;375: 1030–47.
8. Des Guetz G, Uzzan B, Nicolas P, Schischmanoff O, Perret GY, Morere JF. Microsatellite instability does not predict the efficacy of chemotherapy in metastatic colorectal cancer. A systematic review and meta-analysis. Anticancer Res. 2009;29:1615–20.
9. Jover R, Zapater P, Castells A, Llor X, Andreu M, Cubiella J, et al. The efficacy of adjuvant chemotherapy with 5-fluorouracil in colorectal cancer depends on the mismatch repair status. Eur J Cancer. 2009;45:365–73.
10. Bertagnolli MM, Niedzwiecki D, Compton CC, Hahn HP, Hall M, Damas B, et al. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. J Clin Oncol. 2009;27:1814–21.
11. Jensen LH, Danenberg KD, Danenberg PV, Jakobsen A. Predictive value of MSH2 gene expression in colorectal cancer treated with capecitabine. Clin Colorectal Cancer. 2007;6:433–5.
12. Niitani H, Kimura K, Saito T, Nakao I, Abe O, Urushizaki I, et al. Phase II study of 5′-deoxy-5-fluorouridine (5′-DFUR) in patients with malignant cancer–a multi-institutional cooperative study. Gan To Kagaku Ryoho. 1985;12:2044–51.
13. 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 tumours by enzymes concentrated in human liver and cancer tissue. Eur J Cancer. 1998;34:1274–81.
14. Jo WS, Carethers JM. Chemotherapeutic implications in microsatellite unstable colorectal cancer. Cancer Biomark. 2006;2: 51–60.
15. Bellizzi AM, Frankel WL. Colorectal cancer due to deficiency in DNA mismatch repair function: a review. Adv Anat Pathol. 2009; 16:405–17.
16. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol. 2010;28:3219–26.
17. Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. Lancet. 2000;355: 1745–50.
18. Kim GP, Colangelo LH, Wieand HS, Paik S, Kirsch IR, Wolmark N, et al. 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.
19. Lamberti C, Lundin S, Bogdanow M, Pagenstecher C, Friedrichs N, Buttner R, et al. Microsatellite instability did not predict individual survival of unselected patients with colorectal cancer. Int J Colorectal Dis. 2007;22:145–52.
20. Kim ST, Lee J, Park SH, Park JO, Lim HY, Kang WK, et al. Clinical impact of microsatellite instability in colon cancer following adjuvant FOLFOX therapy. Cancer Chemother Pharmacol. 2010;66(4):659–67.
21. Yamada Y. Current status of chemotherapy for advanced colorectal cancer in Japan. Clin Colorectal Cancer. 2008;7:15–24.
22. Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer. 2009;9:489–99.

Note: This paper has been processed and peer-reviewed according to the journal standard procedure, identical to all of the regular issues of Medical Oncology. This issue (28_Supp1_2011) has been published in an effort to reduce the publication cycle and is not a sponsored supplement.