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Review

Microsatellite Instability as a Prognostic Factor in Stage II Colon Cancer Patients, a Meta-Analysis of Published Literature

IOANNIS GKEKAS[1,2], JAN NOVOTNY[2], LADISLAV PECEN[3], KARIN STRIGÅRD[1], RICHARD PALMQVIST[4] and ULF GUNNARSSON[1]

[1]*Department of Surgical and Perioperative Sciences, Umea University, Umea, Sweden;*
[2]*Department of Surgery, Sunderby hospital Lulea, Lulea, Sweden;*
[3]*Faculty Hospital Pilsen, Charles University, Prague, Czech Republic;*
[4]*Department of Medical Biosciences/Pathology, Umea University, Umea, Sweden*

Abstract. *Background/Aim: The prognostic role of microsatellite instability (MSI) in stage II colon cancer patients remains controversial despite the fact that it has been investigated in a number of studies. Hazard ratios differ considerably among these studies. We performed a meta-analysis to define the significance of MSI in this group of patients. Materials and Methods: Studies indexed in PubMed presenting separate data on MSI status and survival outcomes for stage II colon cancer patients have been analyzed using fixed-effect meta-analysis of hazard ratio (HR) according to the method of Peto. Results: Analysis was performed on 19 studies including 5,998 patients. A 47.3% of patients received postoperative chemotherapy and included 52.8% males and 47.2% females. Eight studies included some rectal cancer patients although this cohort was not clearly defined in 3 of these. MSI observed in 20.8% (mean) of patients (median 19.9%). HR for overall survival (OS) of MSI vs. microsatellite stable (MSS) tumors for the entire population: 0.73 (95% confidence interval (CI)=0.33-1.65); HR for disease-free survival (DFS):0.60 (95%CI=0.27-1.32). No statistical significant difference was found when studies analyzing MSI with genotyping (MG) and immunohistochemistry (IHC) were compared separately (MG vs. IHC: HR OS 0.45, 95%CI=0.10-2.05 vs. 0.95, 95%CI=0.57-1.58; HR DFS 0.51, 95%CI=0.14-1.85 vs. 0.67, 95%CI=0.26-1.70). However, numerically MSI determination with genotyping shows significantly lower hazard ratios for both DFS and OS. Separate analysis of studies describing colon cancer patients only showed HR OS 0.72 (95%CI=0.31-1.71); HR DFS 0.60 (95%CI=0.27-1.31). Conclusion: No significant relation was found between MSI status and OS or DFS. Routine determination of MSI status to guide postoperative management of stage II colon cancer patients cannot be recommended. New large scale high quality studies are needed to answer this question definitively, since currently analyzed studies vary considerably.*

Correspondence to: Dr. Ioannis Gkekas, Dept. Surgery, Sunderby sjukhus, Sjukhusvägen 10, Luleå, 971 80, Sweden. Tel: +46 703660249, e-mail: ioannis.gkekas@nll.se

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Colorectal (CRC) cancer is the third most common cancer worldwide (1). Approximately one third of the CRC patients is diagnosed with stage II disease according to current UICC/AJCC systems. Most patients are cured by surgery alone. Only a small proportion of stage II patients with more aggressive tumors benefits from additional postoperative treatment with fluorouracil-based chemotherapy (2).

There is still no generally accepted definition of the term "more aggressive tumor" in stage II but generally T4 tumors, tumors causing bowel obstruction or perforation, tumors with lymphovascular or perineural invasion and microsatellite (MSS) tumors with GIII or mucinous histology are currently considered to have a poorer prognosis that encourage the administration of chemotherapy (3, 4).

Microsatellite instability (MSI) is deemed to be an additional positive prognostic and concurrently a negative predictive factor, both speaking against the use of postoperative adjuvant treatment (5-8). However, there are several contradictory issues that have never been addressed. Most authors have not presented separate data for CRC patients with stage II and III tumors and for colon and rectal cancers respectively. Many retrospective single-center studies have been neglected in former meta-analyses or have been published later.

Therefore, a meta-analysis with an appropriately selected cohort of stage II colon cancer patients was performed to avoid potential bias and to specifically target the prognostic role of microsatellite instability.

Table I. *Scrutinized terms and their combination, inclusion criteria, simplifications.*

Terms/combination	Colon cancer OR rectal cancer OR colorectal cancer AND outcome OR prognosis OR survival OR recurrence OR relapse OR prognostic significance AND MMR OR mismatched repair OR replication error OR microsatellite instability AND hazard ratio OR relative risk OR log-rank test AND localized disease OR stage II OR stage III OR Dukes B OR Dukes C.
Inclusions criteria	A study was included if at least one hazard ratio for overall survival or/and disease free survival for stage II disease was presented or could be easily derived from published results.
Simplificatiosn	MSI-low, MSS and RER-negative tumors are considered as MSS. Only MSI-high and RER-positive tumors are classified as MSI.

MMR: Missmatch repair system; MSI: microsatellite instability; MSS: microsatellite stable tumors; RER: replication error.

Materials and Methods

Articles indexed in the PubMed electronic database was manually and independently scrutinized by two authors for the terms listed in Table I.

There were no restrictions for the selection of studies regarding language, size, method for determination of MSI (genotyping *vs.* immunohistochemistry) or design (prospective *vs.* retrospective, randomized *vs.* non-randomized). In case of duplicate reports, the most recent publication, often including longer follow-up, was selected for further analysis. A mandatory inclusion criterium was assessment of the relationships between MSI status and OS, disease specific survival (DSS), DFS, metastasis free survival (MFS) or any combination of these endpoints regardless of whether postoperative chemotherapy has been administered or not. Studies were included only if separate statistical analysis for stage II disease was included. No additional studies were found when assessing the Cochrane database.

Deadline for inclusion was set to 22nd November 2016. Disagreements regarding selection of publications were resolved by consensus between three assessors.

All studies matching the inclusion criteria were individually analyzed. Data Extraction from selected publications was performed by two authors. Review articles, case reports, abstracts, letters and former meta-analyses were not included but were screened for additional publications in the reference lists. Finally, one author independently evaluated the information collected (Figure 1).

Due to the non-standardized terminology of microsatellite (in)stability, simplification has been made (see Table I). Primary authors of selected publications have not been contacted.

Inclusion criteria are listed in Table I. A fixed-effect meta-analysis of hazard ratio according to the method of Peto was applied (9) in the analysis of the papers included.

Firstly, the natural logarithm (ln) of the hazard ratio (HR) from each study was calculated. Standard errors for the ln(HR) were calculated from CIs if available. Weighted mean of ln(HR) together with weighted standard error of mean HR estimations between different studies were calculated as well as the mean of the standard errors from each study. Weights were equaled to the number of patients where MSI was accessed. Because standard errors were not available from all studies included, the formula weight = 1/(standard error)^2 could be used. Standard errors between-studies and mean standard errors from involved studies were appropriately combined according to Peto's method.

Key message. Microsatellite instability was detected in approximately 20% of stage II CRC patients. This subgroup of tumors differs from stage III cases in behavior, sensitivity to chemotherapy and is deemed to have better prognosis. However, data regarding prognosis are contradictory. Our recent meta-analysis made on 5990 patients including 19 studies does not support a statistically significant relationship between MSI/MSS status and prognosis.

Results

Study characteristics. Our search using PubMed online database retrieved a total of 153 references. A PRISMA diagram (Figure 1) shows the different reasons for exclusion from the final meta-analysis. Characteristics of the selected studies are given in Tables II and III. From these 19 studies totally, 16 were eligible for analysis of overall survival and 14 for analysis of DFS (10-28). In all studies, determination of MSI status has been performed retrospectively. Analysis of the prognostic significance of MSI has been done in 13 retrospectively selected cohorts of patients (single or multicenter studies), in several cohorts of patients participating in randomized controlled trials [PETACC-3, QUASAR, CALGB 9581 and former protocols 784852, 794604, 844652, 874651, 894651, 794751, 864751 that served as a source of clinical data and tissue for analysis published by Halling *et al*. (28)] whereas one included trial was designed as a retrospective case-control study. However, MSI status has never been used as prospective stratification marker in any study. In one study, a population of young adults was selected (12) while another study included only patients who did not receive adjuvant chemotherapy (11).

Specific results for patients with stage II colon cancer have been published in 11 studies. In five studies, a proportion of stage II rectal cancer patients have been analyzed (7.5%-35%). Results were given only for these mixed cohorts without any possibility to evaluate data separately for colon and rectal cancer patients. In 3 studies only, information lacked whether rectal cancer patients were included or not.

Patients with stage pT4 are insufficiently represented across all studies (range=0-40.2% per study, mean 8.8%

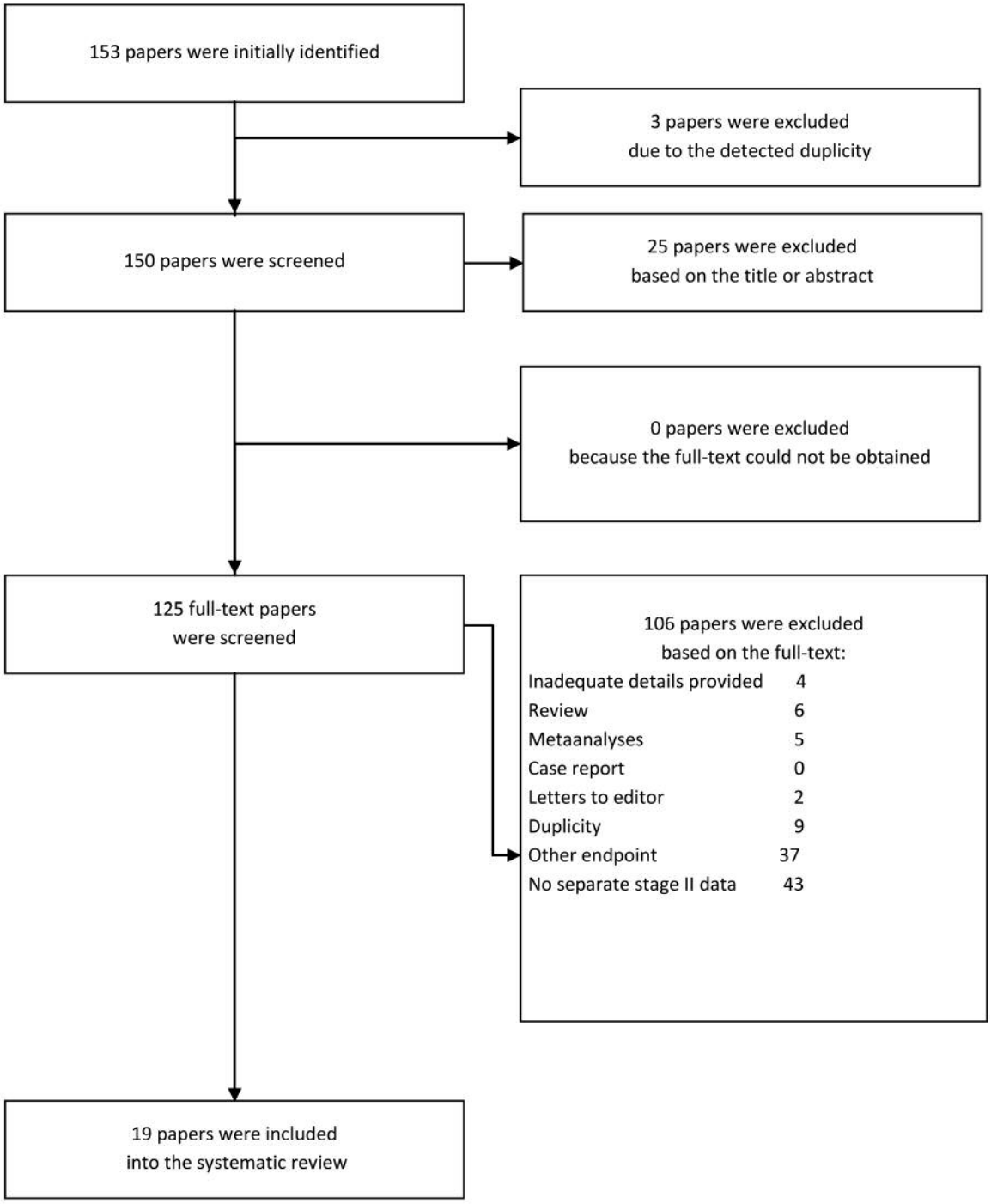

Figure 1. *Details of the included and excluded studies.*

(305/3468 patients with available information)). Distribution of T stage was not given in 7 out of 19 included studies.

Details about chemotherapy treatment have been reported in 12 out of 19 selected studies (4339 patients). Approximately 47.3% of the patients have been treated with postoperative adjuvant chemotherapy whereas for 1659 patients this information was not available. In one of the clinical trials (CALGB 9581) 50% of the patients were

Table II. *Study characteristics I.*

Author	Ref	Country	Year of publication	Year of patients CRC diagnosis	Follow up: Median, range (months)	Number of patients	Method of MSI determination (genotyping *vs.* IHC)	MSI (%)	% of rectal cancer patients	Male/ female (%)	Patients with/ without adjuvant (%)	Proportion T3/T4 tumors (%)	Age median, range (years)	Proximal/ distal colon (%)
Hansen TF	10	Denmark	2014	2003-NA	NA	560	unknown	28.40	0	46/57	NA	87/13	74, NA	51/49
Vogelaar FJ	11	Netherlands	2015	2002-2008	NA	186	G	23.10	0	53/47	0/100	99/1	NA[1]	54/46
Gryfe R	12	Canada	2000	1989-1993	mean 7.2±0.1 y.[2]	173	G	26.60	35[2]	50/50[2]	NA	79/4[2]	43.1[2]	34/32[2]
Guidoboni M	13	Italy	2001	1988-1992	74, 50-120	55	G	49.10	0	41.3/58.7[2]	12.7/87.3	NA	NA[3]	100/0
Shin US	14	South Korea	2014	2006-2009	NA	115	G	13.00	NA	58/42[2]	87.8/12.1	NA	NA	24.9/75.1[2,4]
Klingbiel D	15	Multicentric RCT	2015	NA	NA	395	G	21.80	0	NA	100/0	NA	NA	11.7/88.3
Kim JE	16	South Korea	2015	2003-2008	60.3, NA	860	IHC	14.70	0	61.3/40.5	85.8/14.2	95/5	61, NA	51.5/48.5
Sargent DJ	17	The U.S.A./Canada	2011	1999-2006	60, NA	229	IHC	33.20	0	42.3/57.7	NA	95.2/4.8	74, 34-96	76.8/23.2
Malesci A	18	Italy	2007	1997-2005	NA	246	IHC	17.10	27[2]	58.1/41.9	45.5/53.5[1]	NA	NA	33.4/39.2[2]
Bertagnolli M	19	Multicentric RCT	2011	NA	NA	935	IHC+G	21.30	0	52.4/47.6	0/100[5]	NA	NA, mean 65, 30-90	60.1/39.2
Kopetz S	20	Spain, Germany, Austria, Italy, USA	2015	1987-2009	81, 56-178	416	IHC+G	19.20	7.5	58.4/41.6	29.8/70.2[6]	88.5/11.5[6]	67[7]	42.5/48.3
Donada M	21	Italy	2013	NA	113, NA	120	IHC	15.00	0	47.5/52.5	50/50	88.3/11.7	Approx. mean 67.5	38.3/61.7
Merok MA	22	Norway	2013	1993-2003	NA	291	G	19.90	25[2]	46/54[2]	NA	93/7	NA[8]	41/34[2]
Kevans D	23	Ireland	2011	1990-2004	NA	258	IHC	11.60	28[9]	55/45	NA	95.7/4.3	70.6, 32.9-88.8	30/42
Wang W	24	China	2010	1996-2001	NA	102	G	17.60	0	61.8/38.2	83.3/16.7	59.8/40.2	NA, NA[10]	45.1/54.9
Liang JT	25	Taiwan	1999	1991-1992	NA, 60-84	78	G	32.10	NA	34.6/65.4	100/0	100/0	NA	56.4/43.6
Hutchins G	26	Multicentric RCT	2011	1994-2003	NA	636	IHC	17.45	0	55.8/44.2	48.9/51.1	NA	NA[11]	100/0
Touchefeu	27	France	2016	2001-2009	NA, but at least 3 years	195	G	26.73	0	54.88/45.12	19.7/80.3	82/18	NA, mean 73.4, 23-97	43.3/56.7
Halling	28	USA	1999	NA	8.4, 4.9-17[2]	148	G	25.67	NA	51/49[12]	NA	NA	NA	50/50[12]

[1]≤65 y. 28%; 66-75 y. 33%; ≥76 39%. [2]Data apply to the whole group of patients (all examined stages), separate analysis of stage II patients is not given. [3]<60 y. 22.9%; ≥60 y. 77.1%. [4]unknown if „distal" also includes rectal cancer patients. [5]50% of patients have been randomized to receive edrecolomab. [6]Cumulative data for stage II colon and rectal cancer. [7]<65 y. 41.3 %; > 65 y. 58.7. [8]16%<60 y., 18% 60-70 y., 41% 70-80 y., 25% >80 y. [9]Not specified for MSI and MSS cohorts separately. [10]56.9% ≤60 y., 43.1% >60 y. [11]10.4%<50 y., 26.7% 50-59 y., 40.4% 60-70 y., 22.5% >70 y. [12]Data apply to the whole cohort of stage II patients, not to the subgroup of these patients selected for MSI/MSS determination. MSI: Microsatellite instability; IHC: immunohistochemistry.

Table III. *Study characteristics according to microsatellite status.*

Author	MSI MSS	Ref.	GIII (+/-GIV)	Mucinous (%)	Lymphovasc. invasion present	Perineural invasion present	Median age	B-RAFmut	K-RASmut	Male/ Female	Left/ right[2]	pT3/ pT4[3]	Obstruction present	Perforation present
Hansen TF	MSI	10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSS													
Vogelaar FJ	MSI	11	**44.2**	NA	2.3	NA	ns	**60.5**	**14.0**	**39.5/57.3**	**9.3/90.7**	100/0	9.3	7.0
	MSS		**14.0**		3.5			**6.3**	**41.3**	**60.5/42.7**	**56.6/43.4**	99.3/0.7	12.6	2.8
Gryfe R[1]	MSI	12	**42**	7	NA	NA	**41.3±0.7**	NA	NA	52/48	**9/71**	74/11	NA	NA
	MSS		**18**	21			**43.5±0.3**			50/50	**36/26**	80/3		
Guidoboni M[1]	MSI	13	**59.6**	38.3	NA	NA	**ss[4]**	NA	NA	42.6/57.4	0/100	NA	NA	NA
	MSS		**27.4**	16.1						40.3/59.7	0/100			
Shin US[1]	MSI	14		**35[5]**	50	5	59.1±16.0	NA	NA	65/35	**10/90**	NA	35	NA
	MSS			**7.1**	52.5	34.2	62.5±10.5			57/43	**80.9/19.1**		19.1	
Klingbiel D	MSI	15	**15.1**	**60.8[1]**	NA	4.6	**54[1]**	**23.5[1]**	**31.5[1]**	NA	**24.2/75.8[1]**	76.7/23.3	18.6	8.1
	MSS		**2.3**	**85.0**		3.9	**61**	**5.0**	**40.2**		**66.4/33.6**	81.6/18.4	27.8	6.1
Kim JE	MSI	16	**18.3**	**11.1**	13.5	7.1	**56**	NA	NA	59.5/40.5	**21.4/78.6**	94.4/5.6	**19[6]**	
	MSS		**3.8**	**3.7**	15.9	8.4	**62**			61.6/38.4	**53.1/46.9**	95.1/4.9	**10.5**	
Sargent DJ	MSI	17	**30.7**	NA	15.1	1.4	**78**	NA	NA	**26.3/73.7**	**5.2/94.8**	94.7/5.3	6.8	0
	MSS		**11.2**		5.8	3.4	**71**			**51/49**	**33.1/66.9**	95.4/4.6	7.3	100
Malesci A[1]	MSI	18	**39.3**	**13.5**	NA	NA	65.9±14.7	NA	NA	60.7/39.3	14.3/85.7	67.4/17.8	NA	NA
	MSS		**16.7**	**5**			65.1±11.1			57.8/42.2	60/40	66.8/10.7		
Bertagnolli M	MSI	19	**32.3**	NA	NA	NA	65	NA	NA	**42.7/57.3**	**13.6/86.4**	NA	NA	NA
	MSS		**10.8**				64			**55/45**	**46.2/53.8**			
Kopetz S	MSI	20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSS													
Donada M	MSI	21	**16.7**	NA	NA	NA	ns[7]	**27.8**	NA	39/61	**33/67**	NA	NA	NA
	MSS		**1**					**10.8**		59/41	**67/33**			
Merok MA	MSI	22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSS													
Kevans D	MSI	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSS													
Wang W	MSI	24	**16.7**	**72.2**	NA	NA	**ss[8]**	NA	NA	50/50	**16.7/83.3**	61.1/38.9	NA	NA
	MSS		**25**	**14.3**						64./35.7	**63.1/36.9**	59.5/40.5		
Liang JT	MSI	25	16	**44**	48	NA	**ss[9]**	NA	48	32/68	**24/76**	100/0	NA	NA
	MSS		11.3	**3.8**	45.3				41.5	35.8/64.2	**52.8/47.2**	100/0		
Hutchins G	MSI	26	**24**	**24**	NA	NA	NA	**41.7**	**19.2**	46.7/53.3	0/100	84.4/15.0		
	MSS		**7**	**12**				**6.9**	**50**	59.1/40.1	0/100	83.8/15.8	NA	NA
Touchefeu	MSI	27	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSS													
Halling	MSI	28	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	MSS													

Statistically significant differences in bold. [1]Data apply to the whole group of patients (all examined stages and including rectal cancer patients, if present), separate analysis of stage II patients is not given. [2]Rectal cancer patients account for the rest of cases to 100%. [3]pT1 and pT2 cases account for the rest of cases to 100%. [4]MSI *vs*. MSS: <60 y. 12.8% *vs*. 30.7%; ≥60 y. 87.2% *vs*. 69.4%. [5]Combined analysis mucinous + GIII. [6]Combined analysis bowel obstruction + bowel perforation. [7]Patients matched according to age and sex in this case-control study. [8]MSI *vs*. MSS: ≤60 y. 61.1% *vs*. 38.9%; >60 y. 56% *vs*. 44%. [9]MSI *vs*. MSS: <50 y. 60% *vs*. 11.3%; ≥50 y. 40% *vs*. 88.7%. MSI: Microsatellite instability; MSS: microsatellite stable tumors.

Table IV. *Molecular markers used for the microsatellite instability determination.*

Author	MSI (%)	Markers used
Vogelaar *et al.* (11)	23.10	BAT26
Gryfe *et al.* (12)	26.60	BAT25, BAT26, D5S346, D2S123, D17S250, BAT40, TGF-β RII, D18S58, D18S69, D17S787
Guidoboni *et al.* (13)	49.10	BAT25, BAT26, D17S250, D2S123, D5S346; additional set: BAT40, D10S197, D18S58, D18S69, L-myc
Shin *et al.* (14)	13.00	BAT25, BAT26, D5S346, D2S123, D17S250
Klingbiel *et al.* (15)	21.80	BAT25, BAT26, D5S346, D2S123, D17S250, BAT40, TGF-β RII, D18S58, D18S69, D17S787
Bertagnolli *et al.* (19)	21.30	BAT25, BAT26, D5S346, D17S250, BAT40, ACTC, D18S55, D10S197, BAT34c4, L-myc
Kopetz *et al.* (20)	19.20	Set 1: BAT25, BAT26, D2S123, D5S346, D17S250; Set 2: D21S415, D21S1235, D12S95, D4S2948, SIT2, BAT26; Set 3: BAT25, BAT26, NR21, NR24, Mono27
Merok *et al.* (22)	19.90	BAT25, BAT26, D5S346, D2S123, D17S250
Wang *et al.* (24)	17.60	D18S474, D18S55, D18S58, D18S61, D18S64
Liang *et al.* (25)	32.10	1P34.3, D2S123, 2P15-16, D3S1029, 3P21.2-21.3, D11S988, 11P15, 178261, 17P11-12, D17S588, 17q12-21, BAT26, P11-22, L-myc
Touchefeu *et al.* (27)	26.73	BAT25, BAT26, NR21, NR22, and NR24.
Halling *et al.* (28)	25.67	D5S346, D5S107, D8S254, ACTC, D17S261, TP53.D18S34, D18S49, D18S35, D18S58.

MSI: Microsatellite instability.

randomized to the adjuvant treatment with the monoclonal antibody edrecolomab. Due to the proven lack of the anticancer efficacy of this drug, these patients were considered not receiving adjuvant chemotherapy (29). The number of patients included in the selected studies varied between 55 and 935, allowing for analysis of a total of 5998 patients of whom 4647 provided data for OS and 5087 for DFS.

MSI status has been determined by genotyping in 12 studies and by immunohistochemistry in 6 studies. The methodology was not defined in one study. In two studies both genotyping and immunohistochemistry was used. Genotyping has been performed using different numbers of markers (range=1-14, Table IV). Only some studies adhered to the Bethesda's criteria for the selection of markers and criteria for interpretation.

No significant difference in the proportion of MSI-positive cases was revealed with respect to the methodology used for determination of MSI status (MSI positivity IHC/genotyping: mean=17.2±2.5%, range=11.6-33.2%; resp. 23.5±2.1%, 13.0-49.1%; p=0.0670).

One study reported disease specific survival (DSS) (18) whereas another (24) defined DFS (according to a description in their original article) as DSS. For the purpose of this meta-analysis, this result has been calculated together with other studies reporting overall survival as endpoint. DFS is defined as the time from randomization or diagnosis to the first recurrence or relapse, second cancer, or death. OS is defined as the time from randomization or diagnosis to the date of death from any cause.

Only two studies provided some information on family history in the cohorts studied. Malesci (18) analyzed two separate cohorts of MSI tumors – one on a hereditary basis and one with sporadic cases. Wang (24) describes that no relevant family history had been noticed, leading to the assumption that all MSI tumors analyzed in that article were MSI sporadic cases.

Overall survival data. Six out of 16 studies available for analysis of OS revealed HR for OS >1.0 (1 unknown methodology of MSI determination, 3 using genotyping and 2 using immunohistochemistry). Among 10 studies with HR for OS <1.0, 6 used genotyping, 3 immunohistochemistry and 1 both methods. p-Values are given in 13 studies, of which 3 show statistical significance. These three studies with statistical significance reported HR for OS <1.0, but evaluated outcomes from only 575 patients in total. Overall survival in the entire group of patients did not differ significantly between MSI and MSS patients (weighted mean for HR for OS 0.73 (95%CI=0.33-1.65, Figure 2). Almost identical results were observed for the subgroup of studies including exclusively colon cancer patients (OS for HR showing a weighted mean of 0.72 (95%CI=0.31-1.71). No significant difference was noticed when studies using immunohistochemistry or genotyping were analyzed separately (immunohistochemistry: OS for HR showing a weighted mean of 0.95 (95%CI=0.57-1.58); genotyping 0.45 (95%CI=0.10-2.05), but there was an apparent non-significant trend to lower HR for OS in studies using genotyping.

Disease-free survival data. Disease-free survival data resemble overall survival data. Four out of 14 studies available for analysis of DFS revealed HR for DFS >1.0 (2 using genotyping and 2 using immunohistochemistry). Among 10

Table V. *Survival results by individual study.*

Study	Overall survival			Disease free survival		
	HR	95%CI	*p*-Value	HR	95%CI	*p*-Value
Hansen *et al*. (10)	1.09	0.81-1.45	0.59	0.72	0.47-1.11	0.14
Vogelaar *et al*. (11)	1.80	0.60-4.90	NS(>0.05)	1.60	0.70-3.90	NS(>0.05)
Gryfe *et al*. (12)	0.38	NA	NA	NA	NA	NA
Guidoboni *et al*. (13)	0.33	0.08-1.31	0.20	0.23	0.05-1.15	0.08
Shin *et al*. (14)	4.06	NA	NA/0.24*	3.34	NA	**NA/0.03***
Klingbiel *et al*. (15)	**0.16**	**0.04-0.64**	**0.01**	0.26	0.10-0.65	**0.004**
Kim *et al*. (16)	1.10	0.72-1.68	0.65	1.09	0.63-1.90	0.76
Sargent *et al*. (17)	1.18	0.71-1.96	0.53	0.50	0.19-1.32	0.13
Malesci *et al*. (18)	0.81	NA	NA/0.59*	NA	NA	NA
Bertagnolli *et al*. (19)	0.76	0.54-1.07	0.12	0.65	0.47-0.89	**0.008**
Kopetz *et al*. (20)	NA	NA	NA	0.39	0.16-0.99	**0.046**
Donada *et al*. (21)	0.97	NA	NA	1.54	NA	NA
Merok *et al*. (22)	NA	NA	NA	0.52	NA	**NA/0.010***
Kevans *et al*. (23)	0.55	NA	NA/0.45*	NA	NA	NA
Wang *et al*. (24)	**0.23**	**NA**	**0.045**	0.46	NA	0.155
Liang *et al*. (25)	**0.46**	**NA**	**NA/<0.05***	NA	NA	NA
Hutchins *et al*. (26)	NA	NA	NA	0.33	NA	**NA/<0.0001***
Touchefeu *et al*. (27)	0.25	0.06-1.09	NS(0.07)	0.26	0.06-1.12	NS(0.072)
Halling *et al*. (28)	**0.51**	**0.31-0.82**	**0.006**	**0.42**	**0.24-0.74**	**0.003**

NA: Not available; NS: not statistically significant. *Log rank test for KM curves comparison, no testing of HR from Cox model. Statistically significant differences are highlighted in bold.

studies with HR for DFS <1.0, 1 used unknown methodology of MSI determination, 5 used genotyping, 2 immunohistochemistry and 2 both methods. *p*-Values were given in 13 studies, 6 of which showed statistical significance (five of them with HR <1, one with HR >1). Analysis of all included patients together did not show any significant difference between MSI and MSS groups (DFS HR weighted mean 0.60 (95% CI=0.27-1.32, Figure 3). Neither separate analysis of cohorts including colon cancer patients only, reached the threshold for statistical significance – DFS HR showing a weighted mean of 0.60 (95% CI=0.27-1.31). No difference in outcome of statistical analysis was revealed between studies using immunohistochemistry (DFS HR showing a weighted mean of 0.67, 95%CI=0.26-1.70); and genotyping (DFS HR weighted mean 0.51, 95%CI=0.14-1.85).

Discussion

In this meta-analysis, the first that has analyzed patients with colon cancer stage II, we have not been able to demonstrate a statistically significant association between MSI status and OS or DFS.

While almost all stage I colon cancer patients are cured with surgery alone and stage III patients almost uniformly receive adjuvant postoperative chemotherapy to achieve optimal treatment results, the treatment algorithm is less clear for stage II patients. Approximately 20% of the patients experience distant relapse and finally die due to metastatic disease (30). Postoperative treatments with the same schedules as used for stage III patients has been investigated in several randomized clinical trials and have been summarized in former systematic reviews. The use of this treatment modality is reported in two meta-analyses. The first is a joint analysis of the NSABP C01-C04 trials describing a 30% reduction of the overall mortality (31). The second study is a robust analysis of 12 RCTs including 8,276 patients showing 2-4% improvement in 5-year mortality (2). The large multicenter clinical trial QUASAR with 3,239 randomized patients is commonly cited as a positive trial confirming significant benefits of adjuvant chemotherapy in terms of relative risk reduction for death from any cause and recurrence. However, the subgroup analysis of patients with stage II colon cancer shows no significant influence of chemotherapy on survival – RR 0.82 (95%CI=0.63-1.08) (32). In contrast, neither the large randomized controlled trial IMPACT 2 (33), the other two meta-analyses (30, 34) nor a retrospective analysis of SEER data support routine use of adjuvant chemotherapy (35).

In this situation with contradictory results from clinical studies there is an urgent need to identify positive or negative prognostic factors as a basis for evidence based decision-making.

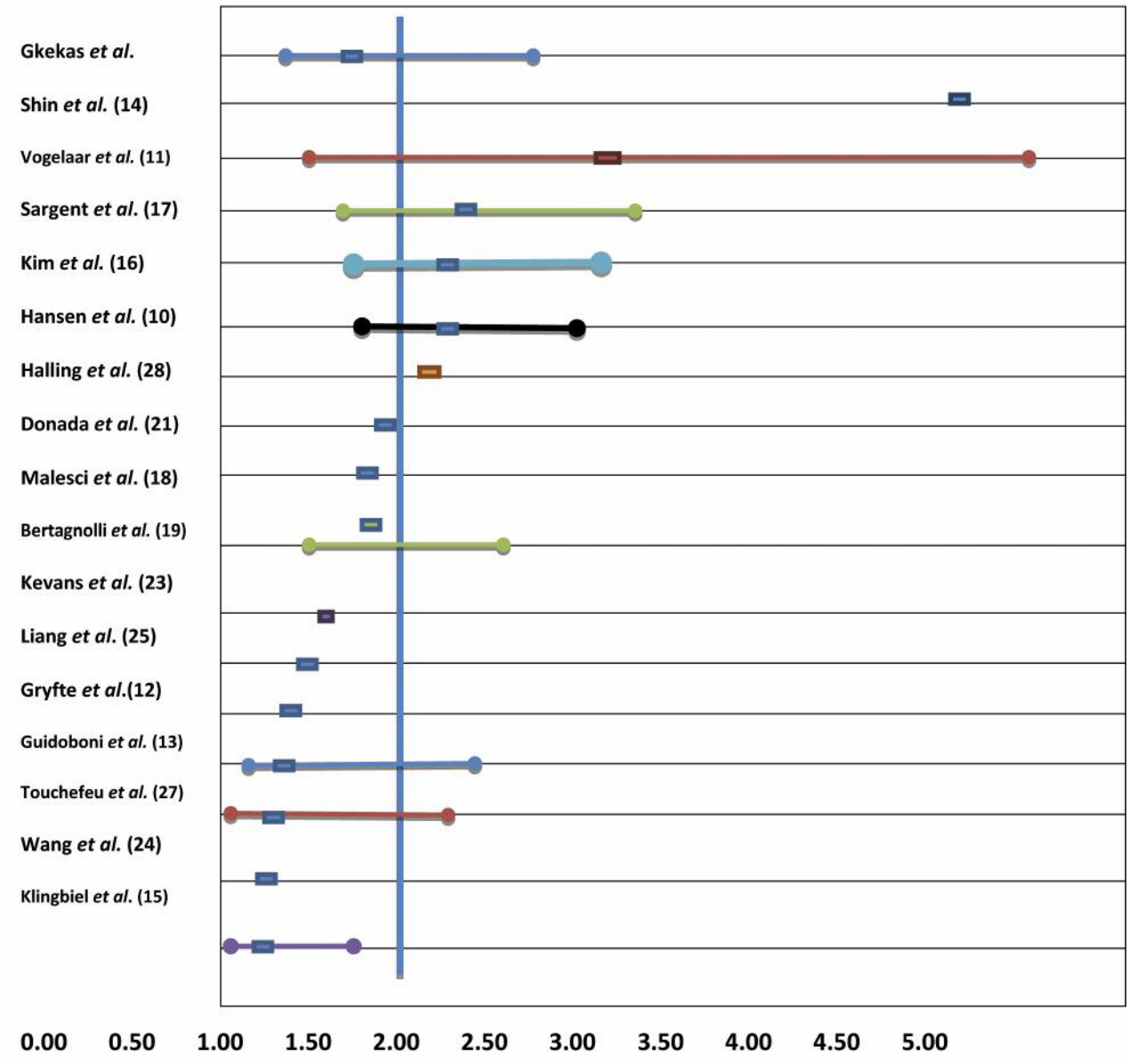

Figure 2. *Overall survival – Hazard ratio (with 95%CI if available) MSI versus MSS. MSI: Microsatellite instability; MSS: microsatellite stable tumors.*

Currently, several factors have been identified that are associated to increased risk of recurrence leading to the recommendation of postoperative chemotherapy while present. Such factors include T4-tumors, perforated tumors, high grade tumors, and mucinous histological pattern without concomitant MSI status, bowel obstruction, inadequate resection with respect to surgical margins or the number of removed/examined lymph nodes. Despite this, the discrimination capability is relatively low and none of these markers has been specifically evaluated within prospectively designed clinical trials. Given the advances of techniques for molecular genetics, several other genetic markers have been tested as prognostic factors (36). Microsatellite instability has been one of the most promising candidates. In 2005 (7), the first meta-analysis summarized the results of 32 eligible studies including 7,642 patients of all stages of whom 1,277 were MSI cases. The combined HR estimate for overall survival associated with MSI was 0.65 (95%CI=0.59-0.71). Unfortunately, the isolated impact of MSI status in stage II patients has not been evaluated.

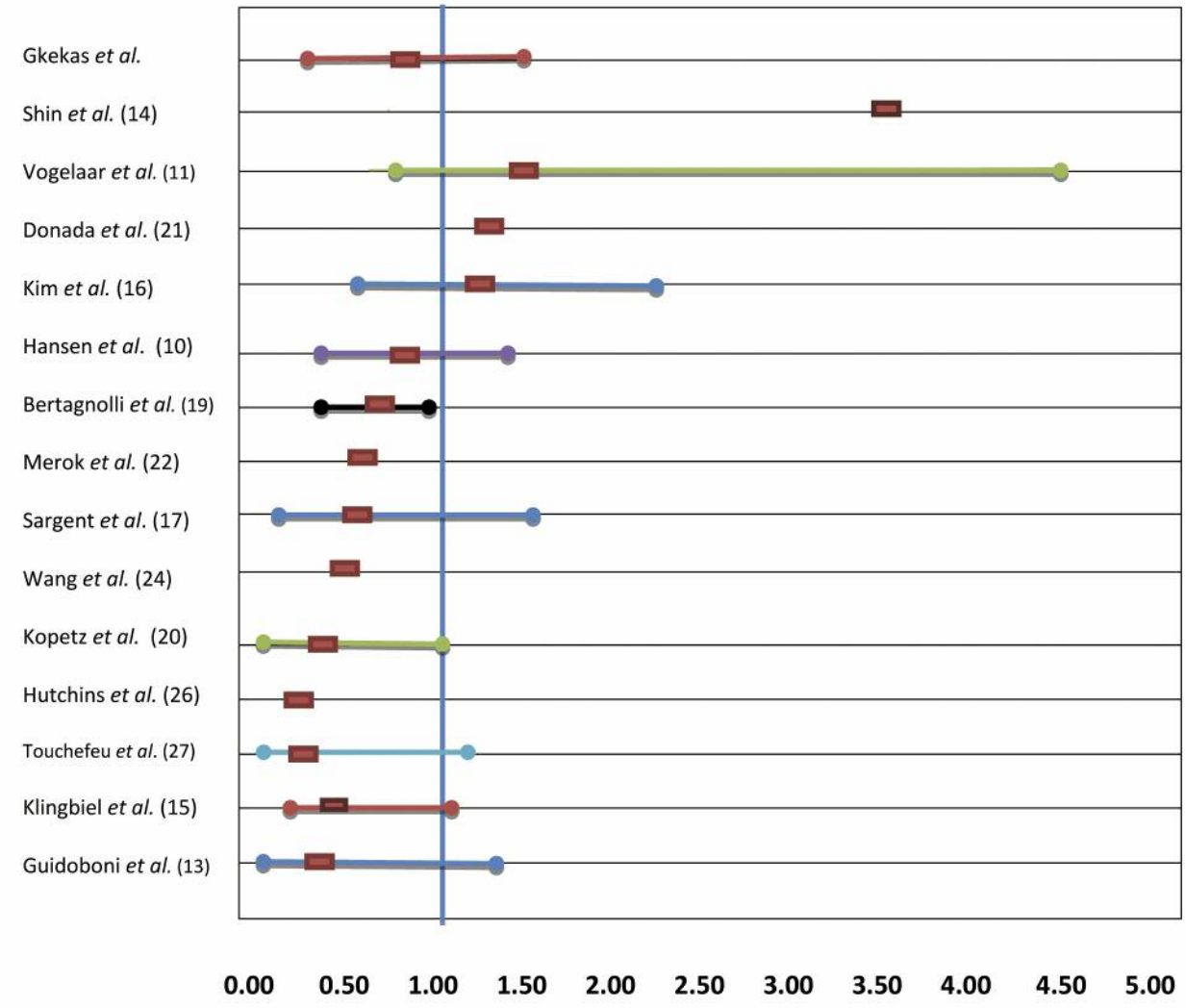

Figure 3. *Disease free survival – Hazard ratio (with 95%CI if available) MSI versus MSS. MSI: Microsatellite instability; MSS: microsatellite stable tumors.*

The second meta-analysis was performed on 3,690 patients, 810 of them with stage II and the rest with stage III colorectal cancer (6). The authors studied interaction between MSI status and postoperative treatment. Unfortunately, the prognostic significance of MSI was not analyzed. Authors found, in concordance with the former meta-analysis, no benefit from chemotherapy in patients with MSI tumors (global HR for OS 0.70, 95%CI=0.44-1.09; global HR for RFS 0.96, 95%CI=0.62-1.49). The third meta-analysis (5) confirmed the association between MSI and favorable prognosis in terms of OS and RFS and found a significant benefit from 5-FU-based treatment in MSS tumors. Unfortunately, also this study evaluates patients with stage II and III cancer as one cohort.

Our meta-analysis focus strictly on stage II colon cancer patients making it possible to provide a deep insight on the true significance of MSI/MSS status in this patient population. We did not find any prognostic significance of MSI in stage II colon or colon and rectal cancer patients for any of the clinically relevant outcomes studied. Several reasons could explain this negative result. We assume that the published source articles could contain selection bias. In our meta-analysis, almost 47.3% of the patients received adjuvant chemotherapy. This is more than twice as much as in the population based SEER database (33). This factor could mitigate the prognostic effect of MSI, since MSS patients could have experience increased survival rate due to the administered chemotherapy while the same

chemotherapy did not improve outcome for MSI cases. Negative effect on the possibility to estimate MSI significance could also have an opposite type of patient`s selection. One study exclusively included patients who had never received chemotherapy (11). According to local guidelines for stage II disease are candidates for adjuvant treatment only high risk MSS patients. All MSI patients, despite the presence of high risk features, do not receive adjuvant chemotherapy. Thus, the possible bias in this study could be inappropriate comparison of any risk MSI patients *vs*. low risk MSS patients.

It should also be emphasized that the proportion of high risk T4 tumors in all studies included was very low with T4 cases constituting only 10% of all patients analyzed. Moreover, we were able to analyze colon and rectal cancer patients separately. It is known that colon cancer has a better prognosis when adjusted for the stage. This logically leads to a relatively low number of events despite the large number of cases collected. We suppose that the low risk of events is also caused by improving adequacy of surgical procedures and pathological examination of the specimens. This mainly affects recent studies published after the previous meta-analyses.

Methodology for determination of MSI status had only a non-significant impact on outcome, but in the studies using genotyping lower hazard ratios has been observed, favoring better outcomes of patients with MSI tumors. In general, there is a lack of standardization of the technique used for determination of MSI among all studies analyzed.

From a statistical point of view, the non-significant results and broad confidence intervals seem primarily to be caused by the fact that several studies included have contradictory results, emphasizing the need for further well-designed studies with enough power (11, 14, 16). Ten out of 14 studies (totally 3,806 patients) provided data for analysis of DFS with a hazard ratio < 1.0 whereas also 4 studies (totally 1,281 patients) showed a HR >1.0, one of them including 860 patients. Three studies showed divergent results regarding HR for DFS and OS respectively. Hansen's study included 560 patients (10) and Sargent's 229 patients (17) with a HR for DFS <1 and concurrently a HR for OS >1. A study published by Donada (21) also observed diverging results with a HR for DFI >1 whereas HR for OS was <1, (Table V, Figures 2 and 3).

Beyond above mentioned outcomes, our meta-analysis confirms additionally former observations (Tables II and III). MSI tumors are consistently associated with higher grade, mucinous pattern, high incidence of *B-RAF* V600E mutation, low incidence of *K-Ras* mutations and a more proximal location, predominantly in the right colon.

In summary, the factors discussed here including a low risk profile of many CRC tumors included in these meta-analyses together with some degree of "overtreatment" may explain the absence of prognostic significance of a relatively weak factor as MSI.

Due to the lack of adequate discrimination potential of current conventional as well as molecular prognostic factors, several groups of investigators have tried to test new approaches aimed to improve the prognostic precision in an individual level. This is especially important in patients undergoing radical surgery for stage II colon cancer. In recent years, intensive efforts have focused on understanding of the interactions between the immune system, colon cancer development and treatment. Some immunomodulatory agents are currently tested in large phase III clinical trials. Pretreatment measurement of neutrophil to leukocyte ratio (NLR) or platelet to lymphocyte ratio (PLR) can be used as a surrogate marker of immune response. Recently, Li *et al*. (37) found a negative prognostic value of high pretreatment NLR. Similarly, Szkandera *et al*. (38) described the same observation in a cohort of stage II+III colon cancer patients for PLR. The other approach involves the design of multigene essays. ColoPrint is a developer based 18-gene expression signature on genes with the highest correlation to relapse of tumor. In a prospectively monitored cohort of 416 stage II CRC cases, ColoPrint identified 63% of the patients as low risk cases with a 5-year relapse risk of 10% whereas high-risk patients (37%) had a 5-year relapse risk of 21% (HR 2.16, p=0.004). This relationship remained significant also in a multivariate model including the number of lymph nodes retrieved and MSI. A validation of ColoPrint has recently been published. The ability of ColoPrint to distinguish between groups with poor and good prognosis was much higher than the combination of commonly used clinical high-risk factors. Recently, CDX2 positivity has been linked to good prognosis in a large set of stage II and III colon cancer patients (20). The hazard ratio for stage II disease reached a HR of 2.71 (95%CI=1.57-4.67). This represents a 5-years DFS of 80% and 51%, respectively (p-value=0.004) (39). These results are promising and other confirmatory studies are desirable.

It is likely that an improved understanding of carcinogenesis in colorectal cancer allows for identification of appropriate prognostic factors with high discrimination ability leading to the possibility to make personalized therapeutic decisions for each individual patient. In conclusion, MSI status determined in stage II colon cancer patients does not, as judged from the presently available studies, predict patient outcome.

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