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ORIGINAL REPORT

The TP53 Colorectal Cancer International Collaborative Study on the Prognostic and Predictive Significance of *p53* Mutation: Influence of Tumor Site, Type of Mutation, and Adjuvant Treatment

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Members of the TP53-CRC Collaborative Group are found in the Appendix.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

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ABSTRACT

Purpose
The aims of the TP53 Colorectal Cancer (CRC) International Collaborative Study were to evaluate the possible associations between specific *TP53* mutations and tumor site, and to evaluate the prognostic and predictive significance of these mutations in different site, stage, and treatment subgroups.

Patients and Methods
A total of 3,583 CRC patients from 25 different research groups in 17 countries were recruited to the study. Patients were divided into three groups according to site of the primary tumor. *TP53* mutational analyses spanned exons 4 to 8.

Results
TP53 mutations were found in 34% of the proximal colon tumors and in 45% of the distal colon and rectal tumors. They were associated with lymphatic invasion in proximal tumors. In distal colon tumors, deletions causing loss of amino acids were associated with worse survival. In proximal colon tumors, mutations in exon 5 showed a trend toward statistical significance ($P < .05$) when overall survival was considered. Dukes' C tumors with wild-type *TP53* and those with mutated *TP53* (proximal tumors) showed significantly better prognosis when treated with adjuvant chemotherapy.

Conclusion
Analysis of *TP53* mutations from a large cohort of CRC patients has identified tumor site, type of mutation, and adjuvant treatment as important factors in determining the prognostic significance of this genetic alteration.

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INTRODUCTION

In Western countries, cancers of the colon and rectum are second only to lung cancer both in terms of incidence and mortality.[1] Although the mortality rate has declined in recent years, particularly for rectal cancer, the incidence continues to increase. Mutation of the *TP53* tumor suppressor gene is thought to play an important role in the progression of colorectal cancer (CRC) and might therefore represent a clinically useful marker of prognosis. The frequency of *TP53* mutations in CRC is approximately 40% to 50%.[2] The majority (approximately 80%) are missense mutations comprising GC to AT transitions at cytosine phosphate guanine dinucleotides and occur principally in five hotspot codons (175, 245, 248, 273, and 282).[3] Most *TP53* mutations occur in exons 5 to 8, in highly conserved areas, and in three principal structural domains of the TP53 protein (L2, L3, and loop-sheet-helix [LSH]).[4,5]

Several groups have reported that different types of *TP53* mutation are differentially

associated with CRC prognosis. These include mutations in exon 7,[6] codon 245,[7] conserved areas,[8] and the L3 structural domain.[5,9] Results between groups have not always been consistent, however, and this is likely to reflect the insufficient statistical power of individual studies. Several important issues should be considered when evaluating the prognostic significance of *TP53* mutations in CRC. First, loss of *TP53* function is a late event in adenoma-carcinoma progression.[10,11] Second, *TP53* mutations have a different incidence and perhaps also prognostic impact depending on the site of origin of the tumor in the large bowel. The frequency of *TP53* mutations is higher in distal colon and rectal tumors than in proximal colon tumors.[12,13] Third, different frequencies of individual *TP53* mutations between populations may also account for previous discordant results on the prognostic significance of this genetic alteration.

Furthermore, several clinical studies have reported that CRC patients with wild-type *TP53* derive a survival benefit from fluorouracil (FU) -based chemotherapy but those with mutant *TP53* do not.[2] Hence, the prognostic impact of *TP53* mutation should be evaluated separately for patients treated with or without adjuvant chemotherapy to avoid this interaction. The aim of the TP53-CRC International Collaborative Study was to pool data from a large number of individual studies to evaluate the prognostic and predictive significance of *TP53* mutations in CRC according to site of origin in the large bowel, tumor stage, type of mutation, and use of adjuvant treatment.

PATIENTS AND METHODS

Recruitment

Beginning in September 2001, different research groups around the world were invited to participate in this International Collaborative Study. The selection of groups for contact was by means of a Medline search for publications on *TP53* mutations and CRC. A Web page created within the site http://www.p53.free.fr and entitled "*TP53* Mutation Analysis in Colorectal Cancer: Call for an International Collaborative Study" was also used to alert research groups to the study. Appropriate groups identified by the Medline search received a formal invitation to participate in the study. If an affirmative answer was received, a questionnaire was sent for the collection of information required for the study. In all, 25 groups agreed to participate in the study and returned the completed questionnaires. Individual groups have published much of the data in this study previously.[9,13-33] The names of the communicating authors from each group, their country of origin, and the number of patients contributed to the study are shown in Table 1.

Information Requested by Questionnaire

The information requested for each patient included patient age and sex, presence of predisposing factors, use of adjuvant therapies, and site of relapse, if any. Other information included the date of surgery, Dukes' stage, surgical outcome, and site of the primary tumor. Tumor site was classified as follows: proximal colon included cecum through to and including transverse colon (Original Operations Details [OOD]-2 codes CP1, CP3), distal colon included splenic flexure through to and including the descending colon (OOD-2 code CP2), and rectal cancer group comprising the sigmoid colon and rectum (OOD-2 code CP4). Other information included the date of last follow-up or death (perioperative, cancer or unrelated to cancer), tumor type (flat or polypoid), histologic grade (well-differentiated [G1], moderately differentiated [G2], poorly differentiated [G3]), lymphocyte infiltration (prominent, not prominent), vascular invasion, mucinous status (0% to 50%, $>$ 50%), lymphatic invasion, and regional lymph node involvement.

Information on the type of tissue was also requested (frozen or paraffin embedded) as well as the control tissue used (normal mucosa, blood, or other). Information on the methods used for mutational analysis of the *TP53* gene were also requested, including polymerase chain reaction–single-stranded conformation polymorphism (PCR-SSCP), PCR–denaturing gradient gel electrophoresis (DGGE), PCR sequencing, or other.

Information on the type of *TP53* gene mutation (point or frameshift) and site of mutation (codon, exon, functional domain, or conserved area) was also requested. For tumors with more than one mutation, the data for each is included as a separate entry. Where specific information was not available, this was entered in the database as not available.

Patient Characteristics

This collaborative study included data from a total of 3,583 CRC patients (from 17 different countries) with information on *TP53* gene mutation status. Patients were divided into three groups according to site of the primary tumor: 1,017 (28%) sites were proximal colon, 426 (12%) were distal colon, and 2,031 (57%) were sigmoid colon and rectum. For another 109 (3%) patients, it was not possible to establish the site of the original tumor and hence these were not included in the analyses relating to tumor site. Table 2 shows the clinicopathologic features of the three patient groups classified according to site of tumor origin and includes patient age and sex; tumor size, stage, and grade; lymphatic and vascular invasion; and treatment with chemotherapy for the Dukes' C subgroup. Median follow-up times for patients were 58 months (range, 1 to 194 months), 61 months (range, 1 to 173 months), and 61 months (range, 1 to 235 months) for the proximal colon, distal colon, and rectal tumor groups, respectively. Additional information on patient and tumor characteristics from each of the contributing centers is shown in the Appendix, Supplementary Table.

TP53 Mutation Screening Techniques

TP53 mutational analyses spanned exons 4 to 8. Exon 4 was screened in 1,880 patients (53%). For mutational analysis, 14 groups used frozen material for a total of 1,191 specimens (34%), and nine groups used paraffin-embedded specimens for 1,878 specimens (52%). Fresh tissue was used for 63 specimens (1.7%), whereas the storage method was not specified for 514 specimens (14%). Normal mucosa was used as the non-neoplastic control in 90% (3,243 of 3,583) of patients. A total of 2,397 patients were screened by PCR-SSCP followed by sequencing, 158 patients were screened by PCR-DGGE followed by sequencing, 281 patients were screened directly by DNA sequencing, 114 patients were screened by SSCP alone, and 454 patients were screened by temperature-gradient gel electrophoresis or DGGE alone. No information on the *TP53* mutation screening technique was provided for 179 patients.

Table 1. Groups Included in the TP53-CRC International Collaborative Study

Author	Country	Total No. of Patients	*TP53* Mutation Frequency		Patients for Survival Analyses		Median Follow-Up (months)	Patients for Clinical and Pathologic Associations	Patients With Unknown Tumor Site
			No.	%	No.	%			
Iacopetta et al[14]	Australia	1,135	406	36	1,090	97	82	1,121	14
Kandioler et al[15]	Austria	74	53	71	71	97	35	73	1
Lung et al[16]	China	99	35	35	16	16	9	97	2
Yuen et al[17]	China	67	29	43	66	98	89	67	0
Crapez et al†	France	91	33	36	87	96	48	91	0
Leahy et al[18]	Ireland	66	27	41	66	100	121	66	0
Chieco-Bianchi et al[19]	Italy	335	139	41	0		NA	329	6
Giaretti et al[20]	Italy	60	35	58	13	23	31	56	4
Ricevuto et al†	Italy	44	18	41	43	100	89	43	1
Russo et al[9]	Italy	160	68	43	160	100	71	160	0
Miyaki[21]	Japan	58	34	59	0	45	NA	57	1
Onda[22]	Japan	45	27	60		100	98	45	0
Kampman[23]	Netherlands	184	57	31	140	81	55	172	12
Lothe et al[24]	Norway	221	100	45	218	99	63	221	0
Guzinska et al†	Poland	47	21	45	43	91	34	47	0
Ostrowski et al[25]	Poland	50	23	46	48	96	58	50	0
Capella et al[26]	Spain	163	81	50	161	99	18	163	0
Lonnroth/Lundholm K et al[27]	Sweden	98	37	38	93	99	103	94	4
Sun et al[28]	Sweden	75	41	55	71	94	176	73	0
Bouzourene et al[29]	Switzerland	123	39	32	122	99	64	123	0
Hsieh et al[30]	Taiwan	182	57	31	180	99	96	182	0
Smith[13*]	Thailand	53	NA		0		NA	0	53
Allan-Mersh et al[31]	United Kingdom	20	Not assessable‡		11	100	34	11	9
Royds et al[32]	United Kingdom	19	16	84	19	100	52	19	0
Bosari and Silverman[33]	United States	114	65	57	112	98	75	114	0
Total		3,583			2,875	80	3,474	109	

Abbreviation: NA, not available.
*No information given on primary site of tumors.
†Unpublished data.
‡This group supplied information only for patients with *p53* mutation.

Definition of TP53 *Mutation Types*

The analyses involved consideration of any *TP53* mutation, mutations specific to exons 4 to 8, and those in regions coding for the main functional and structural domains of the protein. These included the L2 loop (codons 163 to 195), L3 loop (codons 236 to 251), LSH motif (codons 271 to 286) as well as the highly conserved areas II (codons 117 to 142), III (codons 171 to 181), IV (codons 234 to 258), and V (codons 270 to 286).[34] Mutations in the hotspot codons were also examined (codons 175, 196, 213, 245, 248, 249, 273, and 282), as well as those in the denaturant codons known to have a direct effect on *TP53* stability (codons 143, 175, 245, 249, and 282), those in zinc-binding codons (codons 176, 179, 238, and 242), those involved with DNA interaction (codons 120, 241, 248, 273, 276, 277, 280, 281, and 283), and those involved in direct DNA contact (codons 248 and 282).[5,34] Analysis of point mutations (missense and nonsense), frameshift mutations (insertions and deletions), and transitions and transversions was performed. Finally, analysis of mutations that affect the following classes of amino acids was performed: polar neutral, apolar neutral, basic, and acid, together with the type of amino acid change according to the lateral group.

Statistical Analysis

Statistical analyses were performed separately for each of the three subgroups of patients classified according to the site of tumor origin. Associations between *TP53* mutations (any or specific) and clinicopathologic variables were evaluated by the χ^2 test with Yates correction, where appropriate. The relationship between different prognostic variables and overall survival (OS) was assessed univariately by the Kaplan-Meier method. Patients with no follow-up details (n = 708) were excluded from the OS analyses. Survival time was calculated from the date of surgery to the date of death (cancer-related causes) or last follow-up, with times censored for patients who died as a result of causes unrelated to CRC or perioperatively. Significant differences between survival curves were evaluated by the log-rank and Wilcoxon tests, or a test for trend where appropriate. In view of the multiple statistical analyses performed, only values where $P < .01$ were considered significant. Multivariate analysis was carried out by means of the Cox proportional hazards model, using a backward procedure.[35] Only the significant variables in univariate analysis were considered in the Cox model. All *P* values were two sided.

Table 2. Patient Characteristics According to Site of the Primary CRC

Characteristic	Proximal Colon		Distal Colon		Rectum		*P*
	No.*	%	No.*	%	No.*	%	
Total No.	1,017		426		2,031		
Age, years							
< 50	82	8	42	10	264	13	
50-75	664	66	299	71	1,364	67	
> 75	265	26	82	19	397	20	< .001
Mean	67.4		64.9		64.5		
SD	11.8		11.8		12.7		
Range	21-93		19-91		20-99		
Sex							
Male	503	49	218	51	1,153	57	
Female	512	51	207	49	877	43	< .001
Size, cm							
≤ 5	147	49	92	69	509	72	
> 5	153	51	41	31	197	28	< .001
Type							
Flat	24	15	21	23	83	19	
Polypoid	131	85	72	77	360	81	NS
Dukes' stage							
A	64	6	38	9	266	13	
B	391	39	170	40	650	32	
C	470	46	163	38	950	47	
D	90	9	55	13	154	8	< .001
Regional lymph nodes							
N0	419	48	209	54	802	49	
N1	253	29	106	28	504	31	
N2/N3	195	23	68	18	330	20	NS
Histologic grade							
Well-differentiated (G1)	139	14	79	19	283	15	
Moderately differentiated (G2)	605	62	290	70	1,398	72	
Poorly differentiated (G3)	236	24	44	11	255	13	< .001
Lymphatic invasion							
Present	115	48	54	47	249	46	
None	127	52	62	53	292	54	NS
Lymphocyte infiltration							
Prominent	43	24	48	53	124	37	
Not prominent	133	76	43	47	212	63	< .001
Vascular invasion							
Present	51	26	12	13	133	26	
None	147	74	82	87	382	74	NS
Mucinous status							
Not mucinous tumors (0%-50%)	229	84	103	88	583	93	
Mucinous tumors (> 50%)	44	16	14	12	42	7	.022
Surgical resection							
Apparently curative	817	92	271	92	1,727	94	
No resection/residual tumor	69	8	23	8	119	6	NS
Chemotherapy treatment†							
Total	470		163		950		
Yes	140	35	43	37	284	34	
No	260	65	72	63	538	66	NS

NOTE. In 109 patients, the site of primary CRC was unknown. Age was not known for 15 patients, sex was not known for 4 patients, size was not known for 2,335 patients, type was not known for 2,783 patients, Dukes' stage was not known for 13 patients, regional lymph nodes were not known for 588 patients, histologic grade was not known for 145 patients, lymphatic invasion was not known for 2,575 patients, lymphocyte infiltration was not known for 2,871 patients, vascular invasion was not known for 2,667 patients, mucinous status was not known for 1,789 patients, surgical resection was not known for 448 patients, and chemotherapy treatment in Dukes' C was not known for 246 patients.

Abbreviation: CRC, colorectal cancer.

*The percentage of clinicopathologic variables was calculated only for known patients.

†Dukes' C patients only; chemotherapy treatment was with or without radiotherapy in rectal cancer patients.

RESULTS

The 25 different groups that contributed data to this study on *TP53* mutations in CRC are listed in Table 1. Raw data from individual studies are listed in the Appendix, Supplementary Table.

Clinicopathologic Results

Clinicopathologic data were analyzed according to the site of tumor origin in the large bowel (Table 2). In line with previous studies on CRC, rectal cancer patients were younger and more often male compared with proximal cancer patients. Rectal tumors were also smaller compared with proximal colon tumors. Proximal cancers were more often poorly differentiated and mucinous but showed less lymphocyte infiltration compared with rectal cancers. No site-related differences were apparent for the frequency of nodal involvement, vascular invasion, or the use of chemotherapy.

Using proximal colon cancer as the reference group, patients with distal colon cancer showed marginally better OS (relative risk [RR] = 0.82; 95% CI 0.68 to 1.00; $P = .05$). No significant difference in OS was observed between proximal colon and rectal tumor groups. The OS of patient subgroups classified according to the site of tumor origin in the large bowel is listed in Table 3. For each tumor site, only the clinical features that show significant prognostic value are shown. As expected, advanced Dukes' stage, nodal involvement, poor histologic grade, lymphatic invasion, and noncurative resections were all associated with significantly worse survival.

Relationship Between TP53 *Mutations and Clinicopathologic Features*

The overall frequency of *TP53* mutation in this CRC series was 42% (1,449 of 3,474). A significantly higher frequency of mutations ($P < .001$) was found in distal colon and rectal tumors (both groups, 45%) compared with proximal tumors (34%; Table 4). *TP53* mutations were associated with lymphatic invasion in proximal tumors and showed trends for association with advanced Dukes' stage (all sites) and with lymphatic (rectal

Table 3. Overall Survival of the CRC Patients According to Clinicopathologic Variables (univariate analyses)

	Proximal Colon				Distal Colon				Rectum			
Clinicopathologic Variable	No.	OR*	95% CI	*P*	No.	OR	95% CI	*P*	No.	OR	95% CI	*P*
Total No. of patients	853				282				1,740			
Age, years												
< 50	65	1.18	0.82 to 1.70	NS	188	0.90	0.49 to 1.65	NS	227	0.95	0.77 to 1.18	NS
50-75	561	1.00			28	1.00			1,164	1.00		
> 75	**225**	**1.33**	**1.09 to 1.63**	**.006**	64	1.40	0.94 to 2.08	NS	**346**	**1.62**	**1.39 to 1.89**	**< .001**
Sex												
Male	413	1.00			132	1.00			972	1.00		
Female	440	1.05	0.87 to 1.27	NS	149	1.58	1.11 to 2.26	.012	768	0.85	0.74 to 0.97	.013
Dukes'stage												
A	44	1.00			23	1.00			201	1.00		
B	322	1.09	0.66 to 1.81	NS	119	1.43	0.61 to 3.38	NS	549	1.29	0.97 to 1.70	.078
C	**423**	**2.71**	**1.65 to 4.44**	**< .001**	115	2.75	1.19 to 6.38	.018	**867**	**2.60**	**2.00 to 3.37**	**< .001**
D	**62**	**7.15**	**4.12 to 12.4**	**< .001**	**25**	**7.97**	**3.20 to 19.8**	**< .001**	**113**	**6.61**	**4.70 to 9.11**	**< .001**
Regional lymph nodes												
N0	329	1.00			135	1.00			632	1.00		
N1	**222**	**2.37**	**1.83 to 3.07**	**< .001**	**65**	**1.99**	**1.26 to 3.14**	**.003**	**443**	**1.86**	**1.56 to 2.23**	**< .001**
N2/N3	**156**	**3.70**	**2.83 to 4.85**	**< .001**	**39**	**2.66**	**163 to 4.35**	**< .001**	**282**	**2.82**	**2.33 to 3.41**	**< .001**
Histologic grade												
Well-differentiated (G1)	108	1.00			47	1.00			214	1.00		
Moderately differentiated (G2)	516	1.05	0.79 to 1.41	NS	189	1.19	0.72 to 1.96	NS	1217	1.32	1.07 to 1.64	.011
Poorly differentiated (G3)	**201**	**1.57**	**1.14 to 2.15**	**.006**	**36**	**2.50**	**1.34 to 4.67**	**.004**	**228**	**2.06**	**1.60 to 2.67**	**< .001**
Lymphatic invasion												
None	110	1.00			55	1.00			254	1.00		
Present	**99**	**2.27**	**1.39 to 3.70**	**.001**	**49**	**3.12**	**1.67 to 5.88**	**< .001**	210	**2.12**	**1.56 to 2.86**	**< .001**
Mucinous status												
Not mucinous (0%-50%)	199	1.00			91	1.00			505	1.00		
Mucinous (> 50%)	41	1.76	1.12 to 2.77	.014	13	0.96	0.41 to 2.24	NS	38	1.51	0.98 to 2.31	.061
Surgical resection												
Apparently curative	765	1.00			255	1.00			1,608	1.00		
No resection/residual tumor	**62**	**4.04**	**3.00 to 5.42**	**< .001**	**20**	**6.17**	**3.57 to 10.7**	**< .001**	106	**4.48**	**3.57 to 5.62**	**< .001**

NOTE. Significant results are shown in bold type.
Abbreviations: OR, odds ratio; NS, not significant.
*The reference group value is 1.00.

Table 4. Associations Between *TP53* Mutation and Clinicopathologic Features According to Site of the Primary CRC

Clinicopathologic Variable	Proximal Colon			Distal Colon			Rectum		
	TP53 Mutation	%	*P*	*TP53* Mutation	%	*P*	*TP53* Mutation	%	*P*
All patients	350 of 1,017	34		191 of 426	45		908 of 2031	45	
Dukes' stage									
A	15 of 64	23		11 of 38	29		109 of 266	41	
B	133 of 391	34		72 of 170	42		288 of 650	44	
C	163 of 470	35		79 of 163	49		423 of 950	45	
D	38 of 90	42	< .05	29 of 55	53	< .05	84 of 154	55	< .05
Lymphatic invasion									
None	32 of 127	25		22 of 62	35		136 of 292	47	
Present	49 of 115	43	< .01	27 of 54	50	NS	140 of 249	56	< .05
Vascular invasion									
None	18 of 147	12		36 of 82	44		182 of 382	48	
Present	22 of 51	43	NS	9 of 12	75	< .05	79 of 133	59	< .05

Abbreviations: CRC, colorectal cancer; NS, not significant.

tumors) or vascular invasion (distal and rectal tumors; Table 4). None of the other clinicopathologic features (age, sex, size, or grade) showed significant associations with *TP53* mutation frequency. Frameshift mutations were associated with lymphatic invasion in proximal tumors ($P < .01$; data not shown), and in rectal tumors frameshift mutations showed trends for association with advanced Dukes' stage, and lymphatic and vascular invasion ($P < .05$; data not shown).

Mutation Analysis of the TP53 *Gene*

The different types of *TP53* mutations in this CRC cohort are shown in Table 5. Three hundred fifty (34%) of the 1,017 patients with a proximal colon cancer had *TP53* mutations, with 28 showing more than one mutation. Of these, seven had two mutations in one exon, 18 had two mutations in two exons, and three had three mutations, producing a total of 381 mutations identified. One hundred ninety-one (45%) of the 426 patients with distal colon cancer had *TP53* mutations, with 11 showing more then one mutation. Of these, seven had two mutations in one exon, three had two mutations in two exons, and one had three mutations in two different exons, for a total of 203 mutations identified. Finally, 908 (45%) of the 2,031 patients with rectal cancer had a mutation in *TP53*, with 46 patients showing more than one mutation. Of these, 14 patients had two mutations in one exon, 29 had two mutations in two exons, and three had three mutations in two different exons, producing a total of 957 mutations identified. A remarkably similar profile for the type of *TP53* mutation was observed for tumors from the three different sites, with no significant differences between sites observed for the frequency of any individual *TP53* mutation type examined (Table 5).

TP53 *Mutations and Clinical Outcome*

TP53 mutations in the overall CRC cohort or in the three different tumor site groups did not show significant prognostic value (Table 6). Investigation of different types of *TP53* mutations revealed some interesting associations, however, particularly for distal colon tumors. In this group, worse outcome compared with tumors with wild-type *TP53* was observed for mutations in the LSH region, denaturing mutations, multiple mutations, or mutations yielding the same amino acid side group or an amino acid loss (Table 6). For proximal colon tumors, only *TP53* mutations in exon 5 were significantly associated with worse survival; for rectal tumors, only those giving rise to an amino acid loss were significantly associated with worse survival. In multivariate analysis adjusted for Dukes' stage, nodal status, histologic grade, and lymphatic invasion, only *TP53* mutation associated with an amino acid loss in distal colon tumors was an independent factor for worse survival (RR = 2.52; 95% CI, 1.28 to 4.93; $P = .007$). A trend toward statistical significance for worse outcome was also observed for exon 5 mutations in proximal colon tumors (RR = 1.36; 95% CI, 1.03 to 1.79; $P = .03$). Adjustment for study center revealed no significant differences in the odds ratio for survival for either *TP53* mutation or Dukes' stage (results not shown).

TP53 *Mutations and Adjuvant Treatment*

The predictive significance of *TP53* mutation in Dukes' C patients treated with or without adjuvant chemotherapy is listed in Table 7. For patients with wild-type *TP53*, those treated with chemotherapy showed significantly better survival in proximal colon and rectal tumor groups, whereas a trend toward statistical significance ($P = .022$) was observed for the distal colon tumors. For patients with

Table 5. Frequency of *TP53* Mutations in CRC According to Tumor Site and Type of Mutation

TP53 Mutation	Total		Proximal Colon		Distal Colon		Rectum	
	No.	%	No.	%	No.	%	No.	%
Any mutation	1,541		381		203		957	
Mutations in functional domains								
L2	249	20	53	19	26	16	170	22
L3	318	26	64	22	45	29	209	27
LSH	251	21	57	20	39	24	155	20
Outside L2-L3-LSH	396	33	113	39	50	31	233	31
Exons								
4	44	3	15	4	3	2	26	3
5	454	30	121	32	55	27	278	29
6	203	13	56	15	20	10	127	13
7	438	29	93	24	62	31	283	30
8	350	22	82	21	54	27	214	23
Other	41	3	14	4	7	3	20	2
Areas								
Conserved	845	69	193	67	115	72	537	70
Nonconserved	372	31	94	33	45	28	233	30
Codons								
175	139	9	30	8	19	9	90	9
196	11	1	1	0	0	0	10	1
213	42	3	17	4	6	3	19	2
245	68	4	9	2	5	2	54	6
248	138	9	31	8	20	10	87	9
249	21	1	2	0	7	3	12	1
273	116	8	28	7	16	8	72	8
282	76	5	15	4	13	6	48	5
All hot-spot	609	40	131	34	86	42	392	41
All denaturing	305	20	56	15	45	22	204	21
All directly contact DNA	275	18	64	17	39	19	172	18
All bind zinc	36	2	9	2	4	2	23	2
All severe contact	214	14	46	12	33	16	135	14
Other	548	36	138	36	65	32	345	36
Mutations distribution								
One mutation in one exon	1,364	94	322	92	180	94	862	94
Two mutations in one exon	28	2	7	2	7	4	15	2
Two mutations in two exons	50	4	18	5	3	2	28	3
Three mutations	7	0	3	1	1	0	3	1
Amino acid change								
Same side group	478	40	105	37	59	38	314	41
Different side group	507	42	125	44	71	45	311	41
Amino acid loss	217	18	52	18	27	17	138	12
Amino acid pH								
Basic	632	53	147	52	95	65	390	51
Acid	40	3	15	5	5	3	20	2
Polar neutral	321	27	70	25	33	21	218	29
Apolar neutral	209	17	50	18	24	15	135	18
Type of mutation								
Frameshift*	140	12	34	12	19	12	87	11
Point mutation†	1,071	88	250	88	139	88	682	89
Transition	855	80	199	80	114	82	542	80
Transversion	216	20	51	20	25	18	140	20

NOTE. Mutations could not be ascribed to functional groups in 246 patients, to exons in 11 patients, to conserved areas in 324 patients, to codons in 324 patients, to amino acid change in 339 patients, to amino acid pH in 339 patients, and to type of mutation in 330 patients.
*Deletion plus insertion.
†Missense plus nonsense.

Table 6. Prognostic Significance (univariate overall survival analysis) of Different Types of *TP53* Mutation According to Tumor Site

Type of *TP53* Mutation	Proximal Colon				Distal Colon*				Rectum†			
	No.	OR	95% CI	*P*	No.	OR	95% CI	*P*	No.	OR	95% CI	*P*
Total	853				282				1,740			
TP53 mutations												
WT	563	1.00			164	1.00			968	1.00		
Any mutations	290	1.19	0.98 to 1.44	.073	118	1.29	0.91 to 1.83	NS	772	0.97	0.85 to 1.11	NS
Functional domains												
WT	563	1.00			164	1.00			968	1.00		
Mutation in L2	76	1.18	0.85 to 1.63	NS	21	0.96	0.44 to 2.10	NS	167	1.09	0.87 to 1.37	NS
Mutation in L3	38	1.50	0.99 to 2.27	.058	15	1.29	0.62 to 2.70	NS	141	0.88	0.68 to 1.13	NS
Mutation in LSH	51	1.08	0.72 to 1.61	NS	20	1.87	1.05 to 3.33	.033	161	0.95	0.76 to 1.20	NS
Outside L2-L3-LSH	46	0.80	0.51 to 1.26	NS	23	1.26	0.67 to 2.38	NS	136	0.88	0.69 to 1.13	NS
TP53 Exons												
WT	563	1.00			164	1.00			968	1.00		
Exon 5	**91**	**1.47**	**1.11 to 1.93**	**.007**	31	1.42	0.84 to 2.38	NS	224	0.98	0.80 to 1.19	NS
Other exons (4, 6, 7, 8)	199	1.07	0.86 to 1.34	NS	86	1.24	0.84 to 1.83	NS	540	0.97	0.83 to 1.12	NS
Site of *TP53* mutations												
WT	563	1.00			164	1.00			968	1.00		
Denaturing	47	1.16	0.76 to 1.76	NS	21	**2.22**	**1.27 to 3.90**	**.005**	177	0.94	0.75 to 1.18	NS
Other mutations	163	1.10	0.86 to 1.40	NS	58	1.83	0.69 to 1.72	NS	439	0.96	0.82 to 1.12	NS
TP53 mutation distribution												
WT	563	1.00			164	1.00			968	1.00		
One mutation in one exon	264	1.19	0.98 to 1.46	.082	107	1.18	0.82 to 1.69	NS	731	0.98	0.86 to 1.12	NS
Two mutations in one exon	7	0.96	0.39 to 2.32	NS	7	**3.56**	**1.63 to 7.78**	**.001**	14	1.10	0.55 to 2.22	NS
Two mutations in two exons	19	1.29	0.74 to 2.26	NS	4	1.52	0.37 to 6.19	NS	27	0.75	0.44 to 1.28	NS
Amino acid change												
WT	563	1.00			164	1.00			968	1.00		
Same side group	70	0.99	0.69 to 1.41	NS	28	1.77	1.04 to 3.01	.035	243	0.92	0.75 to 1.12	NS
Different side group	95	1.20	0.89 to 1.62	NS	36	0.77	0.42 to 1.42	NS	248	0.87	0.71 to 1.06	NS
Amino acid loss	43	1.12	0.74 to 1.71	NS	14	2.35	1.21 to 4.59	0.012	111	1.30	1.01 to 1.67	0.045
Amino acid type												
WT	563	1.00			164	1.00			968	1.00		
Polar neutral	55	1.25	0.87 to 1.79	NS	22	2.03	1.14 to 3.61	.016	180	1.00	0.81 to 1.24	NS
Other type (apolar neutral, basic, acid)	151	1.04	0.81 to 1.34	NS	55	1.11	4.89 to 1.76	NS	422	0.93	0.79 to 1.09	NS

NOTE. Significant results are shown in bold type. Reference group was WT (risk ratio, 1.00).
Abbreviations: OR, odds ratio; WT, wild type; NS, not significant.
*Mutations could not be ascribed to functional groups in 39 patients, to site of mutations in 39 patients, to amino acid change in 40 patients, and to amino acid type in 41 patients.
†Mutations could not be ascribed to amino acid change in 170 patients.

mutated *TP53*, better survival with chemotherapy was only observed for the proximal colon tumor group ($P < .001$). *TP53* mutation had no predictive value within Dukes' C patient groups treated by surgery alone or within those treated by surgery and chemotherapy (results not shown).

DISCUSSION

Although a large number of research groups have studied *TP53* gene mutations in CRC, controversy still exists regarding the prognostic significance of this alteration.[36] The likely explanation for this is the insufficient statistical power of the various individual studies. Another reason might be that most studies have considered the prognostic significance of all mutations combined. These are usually within the conserved region spanning exons 5 to 8 (codons 130 to 286). However, several authors have suggested that mutations that affect certain functionally important regions of the TP53 protein may have a stronger prognostic impact.[7,9] Another reason for discordance in the literature may be that CRC has often been considered a single disease. There is increasing evidence to suggest that different pathways of tumor progression exist within different anatomic regions of the colon,[2,37,38] and hence, *TP53* mutations may have a different prognostic impact depending on the site of tumor origin. Finally, the prognostic significance of *TP53* mutation may also depend on the adjuvant treatment status of the patient group being studied.

The TP53-CRC International Collaborative Study is the largest study to date on the prognostic value of *TP53*

Table 7. Overall Survival of Dukes' C Patients Treated With or Without Chemotherapy and According to *TP53* Mutation Status (univariate analyses)

	Proximal Colon				Distal Colon				Rectum*			
Treatment	No.	RR	95% CI	*P*	No.	RR	95% CI	*P*	No.	RR	95% CI	*P*
Wild-type *TP53*												
No chemotherapy	164	1.00			39	1.00			305	1.00		
Chemotherapy	98	**0.61**	**0.43 to 0.87**	**.006**	23	0.35	0.14 to 0.86	.022	**163**	**0.55**	**0.43 to 0.71**	**< .001**
Mutated *TP53*												
No chemotherapy	96	1.00			30	1.00			233	1.00		
Chemotherapy	**42**	**0.39**	**0.22 to 0.68**	**< .001**	20	1.15	0.49 to 2.70	NS	121	0.78	0.57 to 1.06	NS

NOTE. Significant results are shown in bold type.
Abbreviations: RR, relative risk; NS, not significant.
*Chemotherapy was or was not associated with radiotherapy in rectal cancer patients.

mutations in colorectal cancer. The large sample size has allowed investigation of factors that might influence the prognostic significance of this genetic alteration, including tumor site, type of mutation, and adjuvant therapy status. In accordance with other reports,[12,37] distal colon and rectal tumors were found to have significantly more mutations than proximal colon tumors (45%, 45%, and 34%, respectively; Table 4). Other genetic and epigenetic alterations also differ in frequency according to tumor site and include microsatellite instability, the cytosine phosphate guanine island methylator phenotype, aneuploidy, and loss of heterozygosity.[39,40] In agreement with the proposal that *TP53* mutation occurs late in tumor progression,[11] the frequency of this alteration increased with advancing tumor stage (Table 4).

Regarding other clinical features of CRC, *TP53* mutations were associated with lymphatic invasion in proximal colon and showed trends toward statistical significance for associations with lymphatic (rectal tumors) and vascular invasion (distal and rectal tumors). We found no associations between specific *TP53* mutations and clinicopathologic variables other than that frameshift mutations were strongly associated with lymphatic invasion in the proximal colon. These data suggest that *TP53* frameshift mutations might be a useful marker of more advanced and aggressive cancer arising at this site.

Our study also demonstrated some interesting trends ($P < .05$) for associations in rectal cancer among any *TP53* mutation or frameshift *TP53* mutation and advanced Dukes' stage, lymphatic invasion, and vascular invasion. This might be explained by different biologic effects of mutagenic agents (eg, alkylating agents) depending on site in the large bowel. There have been reports that certain dietary-associated risks are strongest in the distal colon.[41] In the rectum, the presence of these mutagenic agents for a longer period might have more pronounced effects on *TP53* mutation and cause the observed associations with more aggressive clinicopathologic features. At present there are no other reports enabling confirmation of our hypothesis; no studies have been conducted on the effect of these mutagenic agents on rectal cancer and on specific *TP53* mutations at this site.

As expected, the conventional clinicopathologic variables (Dukes' stage, histological grade, mucinous status, node status, lymphatic invasion, tumor type, and surgical resection) each showed prognostic value in this cohort of CRC patients (Table 3). Small deletions of the *TP53* gene causing amino acid loss were also found to be an independent prognostic factor in distal colon tumors in our study (Table 6). The prognostic significance of this type of *TP53* mutation has not been reported previously for any tumor type. It is generally recognized that chromosomal region 17p13.1 containing the *TP53* gene is subjected frequently to allelic deletions in human CRC.[42-45]

Kern et al[43] have found that analysis of allelic deletions may be an efficient means to identify subsets of CRC patients at higher risk for distant metastases and cancer-related death, especially with regard to left-sided tumors. Not all studies have been able to confirm the prognostic significance of 17p allelic loss, however.[45] The current findings suggest that small deletions in the *TP53* gene in distal colon tumors leading to loss of amino acids might provide more valuable prognostic information than allelic loss. In addition, *TP53* mutations in exon 5 showed a trend toward statistical significance when OS was considered in patients with proximal colon tumors (Table 6). Other authors have reported previously that mutations in specific *TP53* exons are factors for poor prognosis in colorectal and lung cancers[6,46] and Vega et al[47] have reported that mutations in exon 5 are associated with shortened survival in non–small-cell lung cancer.

None of the different *TP53* mutation types evaluated in this study showed independent prognostic value in rectal tumors. The different behavior of rectal tumors compared with tumors from other anatomic regions of the colon may have been masked in previous studies by the grouping of all colorectal tumors together. Furthermore, in rectal cancers the quality of surgery is an important factor in outcome,

particularly whether total mesorectal excision is carried out, and hence it will not be possible to clarify the role of individual prognostic factors at this site until standardized surgery is performed.[48] Until recently, there have been few studies dealing with biologic differences between tumors of the colon and those of the rectum,[48] and few authors have investigated the prognostic role of *TP53* in rectal carcinoma. The present results agree with some previous reports[49,50] but do not support those of a recent study by Rebischung et al,[51] in which they state that *TP53* status is an independent prognostic factor of survival in rectal carcinoma.

One of the most important clinical applications of this study involves the possibility for improved selection of patients to receive chemotherapy. Molecular profiling may serve as a complement to established morphologic parameters for the improved identification of chemotherapy-responsive patients. The response to most drugs, including FU, is complex and therefore unlikely to be explained by any single genetic alteration. However, in vitro studies have shown that disruption of *TP53* causes colorectal cancer cells to be more resistant to the apoptotic effects of FU.[52] In agreement with these observations, we found that colorectal cancer patients with wild-type *TP53* have significantly better survival when treated with chemotherapy compared with those treated with surgery alone, regardless of tumor site (Table 7). In contrast, for patients with mutated *TP53*, only those with proximal colon cancers showed significantly better survival when treated with chemotherapy compared with those treated by surgery alone. These results should be interpreted with caution because of the nonrandomized nature of the chemotherapy treatment. In addition, we grouped all FU-based treatment regimens into one group, even though *TP53* mutation may show different predictive values according to the exact type of treatment used. Nevertheless, our results suggest that use of chemotherapy can influence survival depending on *TP53* mutation status; this may also be dependent on tumor site. Previous studies showing site-related differences in the frequency of *TP53* mutations and other genetic or epigenetic alterations have also suggested that these findings could translate into differential survival benefits from chemotherapy.[37,53]

This study also investigated the effect of *TP53* mutations in patients with Dukes' stage C rectal cancer who underwent adjuvant chemotherapy with or without radiotherapy. In vitro studies have demonstrated that cells with *TP53* mutations show reduced radiation-induced growth arrest and increased radioresistance,[54,55] although ionizing radiation may induce apoptosis through *TP53*-independent mechanisms.[56] Our results and other studies[57,58] show that rectal tumor patients with wild-type *TP53* derived significant survival benefit from the use of FU-based chemotherapy, whether combined with radiotherapy or not. Because the current study was retrospective, not all groups that contributed data were able to provide information about treatment modality. Moreover, in the period before 1991, few patients received adjuvant treatment. These results should therefore be considered as preliminary only.

In conclusion, the results of the TP53-CRC International Collaborative Study demonstrate the importance of primary tumor site when analyzing the prognostic value of *TP53* mutations in CRC. In addition, different types of *TP53* mutation might play a pivotal role in determining the biologic behavior of CRC from different sites and hence the prognosis of patients. This meta-analysis found evidence for interesting tumor site differences in the predictive value of *TP53* mutation for survival benefit from FU chemotherapy. We believe that additional trials on the prognostic value of *TP53* mutation are probably not warranted in view of the relatively weak associations observed here (Table 6) and the emergence of newer technologies that investigate genome-wide markers.[59] Additional trials to evaluate the predictive significance of *TP53* mutation are justified, however, in light of the present findings (Table 7). These would require sufficient patient numbers to allow multivariate analysis, and preferably would involve homogenous treatment regimens and standardized *TP53* mutation screening techniques.

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Appendix

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

Appendix: Supplementary Table. Patient and Tumor Characteristics From Individual Study Centers That Contributed Data to the TP53-CRC Meta-Analysis

Characteristic	No. Proximal Colon	No. Distal Colon	No. Rectum
Iacopetta et al[14]: Australia			
Total	342	99	680
Age, years			
< 50	22	9	78
50-75	215	65	424
> 75	105	25	178
Sex			
Male	150	48	353
Female	192	51	327
Dukes' stage			
A	5	2	11
B	83	21	167
C	247	75	493
D	7	1	9
Histologic grade			
G1	32	17	82
G2	211	63	445
G3	95	17	115
Chemotherapy treatment (Dukes' C stage)*			
Yes	77	27	149
No	170	48	344
Kandioler et al[15]: Austria			
Total	10	1	62
Age, years			
< 50	0	0	12
50-75	9	1	45
> 75	1	0	4
Sex			
Male	6	1	39
Female	4	0	23
Dukes' stage			
A	0	0	13
B	0	0	18
C	10	1	26
D	0	0	4
Histologic grade			
G1	32	0	1
G2	211	0	33
G3	95	0	17
Chemotherapy treatment (Dukes' C stage)*			
Yes	9	1	16
No	1	0	10
Lung et al[16]: China			
Total	23	10	64
Age, years			
< 50	4	0	15
50-75	15	6	34
> 75	4	4	15
Sex			
Male	15	4	38
Female	8	6	26
Dukes' stage			
A	1	0	3
B	14	8	33
C	5	1	17
D	3	1	11

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Appendix: Supplementary Table. Patient and Tumor Characteristics From Individual Study Centers That Contributed Data to the TP53-CRC Meta-Analysis (continued)

Characteristic	No. Proximal Colon	No. Distal Colon	No. Rectum
Histologic grade			
G1	2	2	9
G2	15	6	50
G3	5	1	1
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	1
No	5	1	16
Yuen et al[17]: China			
Total	6	4	57
Age, years			
< 50	2	1	17
50-75	3	1	21
> 75	1	2	19
Sex			
Male	4	3	32
Female	2	1	25
Dukes' stage			
A	0	1	13
B	2	1	19
C	2	2	21
D	2	0	4
Histologic grade			
G1	1	1	10
G2	4	2	44
G3	0	1	3
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	3
No	2	2	18
Crapez et al: France			
Total	30	10	51
Age, years			
< 50	2	4	4
50-75	12	4	31
> 75	16	2	16
Sex			
Male	11	6	23
Female	19	4	28
Dukes' stage			
A	2	2	13
B	9	5	16
C	8	0	10
D	11	3	12
Histologic grade			
G1	11	5	32
G2	13	4	17
G3	5	1	1
Chemotherapy treatment (Dukes' C stage)*			
Yes	5	0	5
No	3	0	5
Leahy et al[18]: Ireland			
Total	17	2	47
Age, years			
< 50	3	0	2
50-75	3	1	37
> 75	11	1	8
Sex			
Male	0	1	32
Female	6	1	15
Dukes' stage			
A	2	0	12
B	6	1	16

(continued on following page)

Appendix: Supplementary Table. Patient and Tumor Characteristics From Individual Study Centers That Contributed Data to the TP53-CRC Meta-Analysis (continued)

Characteristic	No. Proximal Colon	No. Distal Colon	No. Rectum
C	9	1	19
D	0	0	0
Histologic grade			
G1	0	0	0
G2	12	2	42
G3	5	0	5
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	0
No	9	1	19
Chieco-Bianchi et al[19]: Italy			
Total	87	119	123
Age, years			
< 50	7	13	19
50-75	55	94	87
> 75	24	12	17
Sex			
Male	48	75	81
Female	39	44	42
Dukes' stage			
A	12	13	33
B	32	40	37
C	23	37	24
D	20	29	29
Histologic grade			
G1	14	28	25
G2	50	86	80
G3	18	3	14
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	0
No	0	0	0
Giaretti et al[20]: Italy			
Total	19	7	30
Age, years			
< 50	2	0	3
50-75	10	2	20
> 75	2	2	2
Sex			
Male	8	3	16
Female	9	3	13
Dukes' stage			
A	2	0	10
B	11	3	7
C	6	4	12
D	0	0	0
Histologic grade			
G1	1	2	5
G2	15	4	17
G3	1	1	4
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	0
No	0	0	0
Ricevuto et al: Italy			
Total	15	6	22
Age, years			
< 50	1	0	3
50-75	13	6	19
> 75	1	0	0
Sex			
Male	8	4	14
Female	7	2	8

(continued on following page)

Appendix: Supplementary Table. Patient and Tumor Characteristics From Individual Study Centers That Contributed Data to the TP53-CRC Meta-Analysis (continued)

Characteristic	No. Proximal Colon	No. Distal Colon	No. Rectum
Dukes' stage			
A	3	0	5
B	5	4	9
C	5	2	6
D	2	0	1
Histologic grade			
G1	1	1	2
G2	12	4	17
G3	2	0	1
Chemotherapy treatment (Dukes' C stage)*			
Yes	3	2	5
No	2	0	1
Russo et al[9]: Italy			
Total	31	52	77
Age, years			
< 50	2	3	9
50-75	20	39	52
> 75	9	10	16
Sex			
Male	12	20	44
Female	19	32	33
Dukes' stage			
A	6	11	23
B	11	15	25
C	10	13	18
D	4	13	11
Histologic grade			
G1	21	10	10
G2	7	37	46
G3	0	5	21
Chemotherapy treatment (Dukes' C stage)*			
Yes	5	4	4
No	5	9	14
Miyachi et al[21]: Japan			
Total	14	4	39
Age, years			
< 50	2	0	3
50-75	9	3	30
> 75	3	1	6
Sex			
Male	10	1	22
Female	4	3	17
Dukes' stage			
A	4	0	12
B	7	1	11
C	3	3	16
D	0	0	0
Histologic grade			
G1	9	2	27
G2	2	2	10
G3	1	0	0
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	0
No	0	0	0
Onda et al[22]: Japan			
Total	14	0	31
Age, years			
< 50	0	0	6
50-75	9	0	23
> 75	5	0	2

(continued on following page)

Appendix: Supplementary Table. Patient and Tumor Characteristics From Individual Study Centers That Contributed Data to the TP53-CRC Meta-Analysis (continued)

Characteristic	No. Proximal Colon	No. Distal Colon	No. Rectum
Sex			
Male	6	0	18
Female	8	0	13
Dukes' stage			
A	5	0	6
B	4	0	10
C	5	0	13
D	0	0	1
Histologic grade			
G1	7	0	10
G2	7	0	18
G3	0	0	1
Chemotherapy treatment (Dukes' C stage)*			
Yes	5	0	11
No	0	0	2
Kampman et al[23]: Netherlands			
Total	77	21	74
Age, years			
< 50	13	1	12
50-75	59	20	58
> 75	5	0	4
Sex			
Male	40	14	46
Female	37	7	28
Dukes' stage			
A	4	4	17
B	41	13	28
C	21	4	22
D	10	0	7
Histologic grade			
G1	14	2	11
G2	33	12	49
G3	29	5	12
Chemotherapy treatment (Dukes' C stage)*			
Yes	5	1	5
No	16	3	16
Lothe et al[24]: Norway			
Total	67	11	143
Age, years			
< 50	3	2	14
50-75	41	7	88
> 75	23	2	41
Sex			
Male	36	5	64
Female	31	6	79
Dukes' stage			
A	6	1	25
B	29	7	63
C	23	2	42
D	9	1	13
Histologic grade			
G1	18	2	11
G2	45	9	122
G3	4	0	10
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	0
No	0	0	0
Guzinska et al: Poland			
Total	6	1	40
Age, years			
< 50	1	0	6
50-75	5	1	27
> 75	0	0	7

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Appendix: Supplementary Table. Patient and Tumor Characteristics From Individual Study Centers That Contributed Data to the TP53-CRC Meta-Analysis (continued)

Characteristic	No. Proximal Colon	No. Distal Colon	No. Rectum
Sex			
Male	4	1	23
Female	2	0	17
Dukes' stage			
A	0	0	1
B	0	0	4
C	6	0	35
D	0	1	0
Histologic grade			
G1	0	0	2
G2	3	0	29
G3	2	1	4
Chemotherapy treatment (Dukes' C stage)*			
Yes	3	0	12
No	3	1	23
Ostrowski et al[25]: Poland			
Total	11	2	37
Age, years			
< 50	1	0	9
50-75	8	1	27
> 75	2	1	1
Sex			
Male	4	0	23
Female	7	2	14
Dukes' stage			
A	0	0	6
B	4	2	11
C	5	0	12
D	2	0	6
Histologic grade			
G1	1	0	4
G2	8	2	29
G3	2	0	1
Chemotherapy treatment (Dukes' C stage)*			
Yes	4	0	9
No	1	0	3
Capellà et al[26]: Spain			
Total	48	16	99
Age, years			
< 50	5	1	7
50-75	32	11	65
> 75	11	4	27
Sex			
Male	32	6	63
Female	16	10	36
Dukes' stage			
A	2	2	21
B	22	8	28
C	16	3	34
D	7	3	14
Histologic grade			
G1	0	0	0
G2	40	14	91
G3	8	1	7
Chemotherapy treatment (Dukes' C stage)*			
Yes	9	2	17
No	7	1	17
Lonnroth et al[27]: Sweden			
Total	41	1	52

(continued on following page)

Appendix: Supplementary Table. Patient and Tumor Characteristics From Individual Study Centers That Contributed Data to the TP53-CRC Meta-Analysis (continued)

Characteristic	No. Proximal Colon	No. Distal Colon	No. Rectum
Age, years			
< 50	1	0	8
50-75	28	1	42
> 75	12	0	2
Sex			
Male	15	0	36
Female	26	1	16
Dukes' stage			
A	3	0	1
B	16	1	22
C	21	0	25
D	1	0	4
Histologic grade			
G1	1	1	2
G2	21	0	45
G3	18	0	4
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	0
No	21	0	25
Sun et al[28]: Sweden			
Total	25	1	47
Age, years			
< 50	0	0	1
50-75	15	1	27
> 75	10	0	19
Sex			
Male	9	0	25
Female	16	1	22
Dukes' stage			
A	2	0	11
B	7	0	9
C	9	1	15
D	7	0	12
Histologic grade			
G1	1	0	4
G2	16	1	31
G3	2	0	5
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	0
No	9	1	15
Bouzourene et al[29]: Switzerland			
Total	61	27	35
Age, years			
< 50	2	0	4
50-75	35	15	26
> 75	24	12	5
Sex			
Male	33	13	21
Female	28	14	14
Dukes' stage			
A	0	0	0
B	61	27	35
C	0	0	0
D	0	0	0
Histologic grade			
G1	13	2	3
G2	36	23	29
G3	12	1	3
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	0
No	0	0	0

(continued on following page)

Appendix: Supplementary Table. Patient and Tumor Characteristics From Individual Study Centers That Contributed Data to the TP53-CRC Meta-Analysis (continued)

Characteristic	No. Proximal Colon	No. Distal Colon	No. Rectum
Hsieh et al[30]: Taiwan			
Total	35	18	129
Age, years			
< 50	5	5	30
50-75	27	11	87
> 75	3	2	12
Sex			
Male	19	7	79
Female	16	11	50
Dukes' stage			
A	1	1	15
B	14	9	50
C	19	8	60
D	0	0	4
Histologic grade			
G1	8	3	27
G2	21	11	94
G3	6	4	7
Chemotherapy treatment (Dukes' C stage)*			
Yes	13	4	44
No	6	4	16
Allan-Mersh et al[31]: United Kingdom			
Total	2	2	7
Age, years			
< 50	1	0	1
50-75	1	2	6
> 75	0	0	0
Sex			
Male	2	0	5
Female	0	2	2
Dukes' stage			
A	0	0	0
B	0	0	0
C	2	2	5
D	0	0	2
Histologic grade			
G1	0	0	0
G2	0	0	0
G3	0	0	0
Chemotherapy treatment (Dukes' C stage)*			
Yes	2	2	5
No	0	0	0
Royds et al[32]: United Kingdom			
Total	4	0	15
Age, years			
< 50	0	0	0
50-75	2	0	12
> 75	2	0	3
Sex			
Male	3	0	8
Female	1	0	7
Dukes' stage			
A	0	0	2
B	1	0	7
C	1	0	1
D	2	0	5
Histologic grade			
G1	0	0	1
G2	1	0	10
G3	3	0	4

(continued on following page)

Appendix: Supplementary Table. Patient and Tumor Characteristics From Individual Study Centers That Contributed Data to the TP53-CRC Meta-Analysis (continued)

Characteristic	No. Proximal Colon	No. Distal Colon	No. Rectum
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	0
No	1	0	1
Bosari and Silverman[33]: United States			
Total	32	12	70
Age, years			
< 50	3	2	5
50-75	20	6	59
> 75	9	4	6
Sex			
Male	22	6	48
Female	10	6	22
Dukes' stage			
A	4	1	13
B	12	4	25
C	14	4	24
D	2	3	7
Histologic grade			
G1	2	1	5
G2	19	8	50
G3	11	3	15
Chemotherapy treatment (Dukes' C stage)*			
Yes	0	0	0
No	14	4	24

NOTE. In 109 patients the site of primary CRC was unknown. Age was not known for 15 patients, sex was not known for 4 patients, size was not known for 2,335 patients, type was not known for 2,783 patients, Dukes' stage was not known for 13 patients, regional lymph nodes was not known for 588 patients, histologic grade was not known for 145 patients, lymphatic invasion was not known for 2,575 patients, lymphocyte infiltration was not known for 2,871 patients, vascular invasion was not known for 2,667 patients, mucinous status was not known for 1,789 patients, surgical resection was not known for 448 patients, and chemotherapy treatment in Dukes' C was not known for 246 patients.

*Chemotherapy treatment was or was not associated with radiotherapy in rectal cancer patients.

†G1, well-differentiated; G2, moderately differentiated; G3, poorly differentiated.

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