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Prognostic Effect of Ultra-Staging Node Negative Colon Cancer without Adjuvant Chemotherapy: A Prospective National Cancer Institute Clinical Trial

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Abstract

BACKGROUND—We recently reported in a prospective randomized trial that ultra-staging of patients with colon cancer is associated with significantly improved disease-free survival (DFS) compared with conventional staging. That trial did not control for lymph node (LN) number or adjuvant chemotherapy use.

STUDY DESIGN—The current international prospective multi-center cooperative group trial (NCI Clinical Trial NCT00949312), "Ultra-staging in Early Colon Cancer" (UECC), evaluates whether the 12-LN quality measure and nodal ultra-staging impact DFS in patients not receiving adjuvant chemotherapy. Eligibility criteria include: a) biopsy-proven colon adenocarcinoma; b)

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absence of metastatic disease; c) > 12 LNs staged pathologically; d) pan-cytokeratin immunohistochemistry (IHC) of H&E-negative LNs; e) no adjuvant chemotherapy.

RESULTS—Of 442 patients screened, 203 patients were eligible. The majority of patients had intermediate grade (57.7%) and T3 tumors (64.9%). At a mean follow-up of 36.8±22.1 months (range 0–97 months), 94.3% remain disease-free. Recurrence was least likely in patients with ≥12, H&E negative, and IHC negative LNs (pN0i−): 2.6% vs.16.7% in the pN0i+ group ($p<0.0001$).

CONCLUSIONS—This is the first prospective report to demonstrate that patients with optimally staged node-negative colon cancer (≥12 LNs, pN0i−) are unlikely to benefit from adjuvant chemotherapy, as 97% remain disease free after primary tumor resection. Both surgical and pathological quality measures are imperative in the planning of clinical trials in non-metastatic colon cancer.

Each year colon cancer (CC) affects approximately1.4 million people and causes more than 693,000 deaths worldwide.[1] Colorectal cancer is the third most common cause of cancer-related death in the United States (U.S.), with estimates that 132,700 new cases were diagnosed in 2014 accounting for 50,710 deaths.[2] The principal treatment modality remains surgical resection with adjuvant systemic therapy recommended primarily for node-positive patients, and possibly for high-risk node-negative patients.[3,4] Lymph node status continues to be the most important prognostic factor in CC, and determinant of American Joint Commission on Cancer (AJCC) stage.[4]

Metastasis to regional lymph nodes is the predominant distinguishing pathological feature between AJCC TNM Stage I/II (pN0) and Stage III (pN1-N2b) CC. The current AJCC staging system (version 7) recognizes the importance and prognostic impact of number of nodes involved by cancer (N1a = 1, N1b = 2–3, N2a = 4–6 and N2b = 7+ nodes), as well as the total number of nodes staged pathologically.[5–7] Adjuvant chemotherapy has demonstrated survival benefit for Stage III (pN+), but not for Stage I/II (pN0) patients with CC. [8–11] Most patients with pN0 CC diagnosed and treated early in the course of the natural history of their disease survive 5 years or more after surgical resection alone. However, up to 20% of these patients with early “node-negative” (pN0; Stage I/II) CC ultimately recur.[12,13] This fact implies that these “node-negative” patients may have one of several scenarios: [1] aggressive tumor biology, [2] been insufficiently treated by surgery alone (i.e. incomplete nodal resection), [3] extra-nodal spread of disease, or [4] occult nodal disease overlooked with conventional histopathological staging methods (*i.e.*, pathological under-staging).

It is also well known that some subgroups of patients with Stage I/II (pN0) CC may benefit from adjuvant chemotherapy,[14–17] including those with limited number of lymph nodes retrieved, lymphovascular invasion, T4 disease or under-staging by conventional histopathology assessment. Typically, standard histopathological assessment of lymph nodes (LNs) involves one or two sections of each LN stained by hematoxylin and eosin (H&E). In 2006 the AJCC 6th edition modified the description for LN staging according to nodal tumor volume. Single or small clusters of distinct tumor cells smaller than 0.2 mm identified by IHC or molecular staging were classified pN0i+; micro-metastases ranging in size from 0.2–2.0 mm were staged pN1mi; macro-metastases larger than 2 mm were in the pN1-2b staging

category. Isolated tumor cells (ITCs), cell clusters and often micro-metastases (mi) are generally detected by immunohistochemistry (IHC) or molecular tumor-cell detection (reverse transcriptase polymerase chain reaction, RT-PCR). Hence, standard histopathological nodal evaluation which involves H&E examination of a limited nodal volume is generally inadequate for the detection of small aggregates of tumor cells.

Initiatives to improve staging accuracy in CC were recently undertaken, aimed at identifying node-negative (pN0) patients at risk of recurrence that might benefit from adjuvant systemic therapy. This aim was further emphasized in a meta-analysis of 39 studies including over 4,000 patients.[18] The meta-analysis demonstrated that, independent of the type of detection method utilized (IHC or RT-PCR) to identify small tumor-cell aggregates, the presence of occult nodal disease (pN0i+ or pN0mi) in patients with CC is an independent predictor of disease recurrence, disease-specific and overall mortality. Ten of these 39 studies included patients with Stage I/II CC without adjuvant chemotherapy, but no study stratified patients according to number of examined LNs in the analysis of CC outcomes.[18]

We previously conducted a randomized trial in patients with non-metastatic CC. Our intent was to determine if standard histopathology combined with targeted nodal assessment and ultra-staging [*ex vivo* lymphatic mapping and step sectioning of the sentinel node(s) followed by pan-cytokeratin IHC (targeted nodal assessment and ultra-staging, TNA-us)] improves nodal staging accuracy through the identification of small tumor-cell aggregates (pN0i+ or pN0mi+) over standard histopathology alone. In that study, we identified a significantly increased nodal upstaging with TNA-us compared to standard histopathology alone (57.3% vs. 38.7%, respectively; p= 0.019).[19] Patients with TNA-us in our trial had better 5-year DFS than those in the control group (control 71% vs. TNA-us 86%; P = 0.04). This significant difference in DFS between groups was even more pronounced for Stage II CC patients (control 65% vs. TNA-us 83%; P = 0.03). That study, however, did not stratify patients according to LN number, and adjuvant chemotherapy was given at the discretion of the treating oncologist.[20] The purpose of the present trial was to evaluate DFS in patients with CC whereby both surgical (≥12 LNs) and pathological (ultra-staging) quality measures are applied without the use of adjuvant chemotherapy.

METHODS

This report complies with the reporting standards established by the revised Consolidated Standards of Reporting Trials consensus statement.[21]

Trial Design

This clinical trial was planned as an International Prospective Trial (Prospective NCI Clinical Trial NCT00949312).

Participants

Adult patients (18 years of age or older) with biopsy-proven, primary, AJCC Stage I/II, colon adenocarcinoma with ≥12 LNs staged pathologically were eligible for study. Those with metastatic or recurrent colon adenocarcinoma were excluded, along with patients having undergone systemic therapy or radiation, or those with tumor histology other than

adenocarcinoma. This study was conducted from April 2001 to June 2014 at academic medical centers located within the United States, Serbia and Israel as part of the United States Military Cancer Institute (USMCI) Clinical Trials Group. Institutional Review Board (IRB) approval for this study was provided by all participating study sites.

Interventions

Patients were screened pre-operatively with serum biochemistry and carcinoembryonic antigen (CEA), chest radiographs / computed tomography, endoscopy with biopsy. All patients signed an informed consent form consistent with IRB guidelines. Study participants were then considered screen failures if they were found to have metastatic disease, had <12 LNs retrieved, or had received adjuvant chemotherapy. All patients underwent standard colon resection based on standard oncological principles.[22] After the peritoneal cavity was explored to rule out distant metastases, the position of the colon tumor was determined and the extent of resection defined. Carcinoma of the right colon was treated by right hemicolectomy, which included approximately 5–10 centimeters of the terminal ileum. The ileocolic artery, right colic artery, and right branch of the middle colic artery were ligated and divided, including the adjacent mesentery. If the tumor involved the right hepatic flexure, the resection included the left branch of the middle colic artery. Mid-transverse CCs were treated by an extended right hemicolectomy to include the splenic flexure and both branches of the middle colic artery, and an anastomosis between the ileum and the descending colon. Carcinomas of the left colon were treated with left hemicolectomy including resection of the inferior mesenteric artery and its branches as well as the proximal rectum. Cases of mid or distal sigmoid cancer were treated with sigmoidectomy where the left branch of the middle colic artery was spared.[22]

Immediately after removal of the colon, a specimen of primary tumor was frozen in liquid nitrogen for molecular analyses. The remaining resected colon and attached mesentery were fixed in formalin for standard histopathological evaluation. Primary tumor sections were obtained for histopathological evaluation, one per cm of tumor. The deepest extent of primary tumor invasion, and resection margins, including radial margin, were determined. Instruments were changed prior to lymph node retrieval to help avoid contamination with displaced epithelium. All lymph node tissue was embedded in paraffin for sectioning, unless a node was grossly positive, in which case one section was sufficient for histopathological analysis. If fewer than 12 LNs were retrieved, re-examination of the specimen was performed to identify any remaining nodes. Fat-clearing fixative use was optional. Histopathological examination of retrieved LNs was conducted with H&E-stained 4-μm nodal sections. The LN was split longitudinally if >3mm in size and two 4-μm nodal sections were stained and examined microscopically.

Standard pathological assessment was performed on all retrieved LNs, and pan-cytokeratin (CK) immunohistochemistry (IHC) was performed on all H&E-negative LNs. All LNs were measured and bisected along their longitudinal axis. Paraffin-embedded nodes (single face if node <3 mm, and 2 faces in bi-valved nodes 3+mm in size) underwent step sectioning at 40-μm intervals and at 4 node levels, yielding LN sections approximately 4-μm thick. All 4 LN sections were stained with H&E. Two unstained slides were prepared at the second and

fourth levels of the LN block for IHC staining. Lymph node ultra-staging was defined as step sectioning of the LNs, followed by microscopic evaluation of 4 H&E-stained and 2 pan-CK IHC-stained sections of each LN. IHC was performed on formalin-fixed paraffin-embedded sections of the LNs using the avidin-biotin-peroxidase complex method incorporating a commercially obtained pan-CK antibody cocktail (Ventana Medical Systems, Tucson, AZ) as previously detailed.[19] A total of 4 H&E- and 2 CK-IHC stained sections were examined for each LN block. A CK immunostain was considered positive if strongly positive individual tumor cells or cell clusters (≤0.2mm; pN0i+) or micro-metastases (>0.2–2.0mm; pN1mi) were identified that demonstrated anatomical and cytological features of CC cells.

Patients were followed per protocol by annual CT scan, colonoscopy at 1 and 4 years post-surgery and by semi-annual serum CEA levels for a minimum of 4 years. The pre-specified primary outcome variable was 4-year DFS, which was recorded from time of study entry to time of first local, regional nodal, and/or distant disease recurrence.

Statistical Methodology

The Fisher exact test or chi-square test was used to compare categorical variables between groups. Analysis of covariance was used to compare means, and the Wilcoxon rank sum test was used to compare medians. The rate of development of the primary outcome measure (DFS) was estimated using the Kaplan Meier product limit method and log-rank test. Statistical analyses were performed using SPSS v17.0 (SPSS, Inc, Chicago IL). All tests were two-sided, and $p<0.05$ was considered statistically significant.

RESULTS

Participant flow

Of the 445 patients screened preoperatively, 203 patients had ≥ 12 H&E-negative LNs (pN0) stained with pan-CK-IHC. 242 patients were excluded because they were found to be node positive by H&E (pN1), had <12 LNs retrieved or received adjuvant chemotherapy for pN0 disease. Nine of the patients were upstaged to pN1mi (0.2–2 mm metastases) by focused analysis and were therefore excluded from the analysis, leaving194 evaluable patients (pN0i−).

Recruitment

The study recruitment period extended from April 2001 to June 2014. Follow-up was continued through September 2014.

Baseline data and numbers analyzed

Baseline demographic and clinical characteristics of the 194 patients with AJCC Stage I/II colon adenocarcinoma are shown in Table 1. The pN0i− (n=152) group was comparable to the pN0i+ group (n=42), except for lympho-vascular invasion (6.4% vs 17.1%; p=0.045) and pT3-pT4 Stage (62.3% vs 85.7%; p=0.007).

Outcomes analysis

At a median (IQ range) follow-up of 37.6±22.0 months, eleven patients (5.7%) recurred. Mean DFS for the entire group was 89.9 months. Seven had distant recurrences, two local and two undefined recurrences. The patients with local recurrence had resection and the others systemic therapy. Six patients died of disease. The disease recurrence incidence for the pN0i− group was 2.6% compared with 16.7% for the pN0i+group ($p<0.001$), as shown in Table 2. DFS was 92.9 months for the pN0i− group and 71.8 months for the pN0i+ group (Figure 1; $p<0.0001$).

DISCUSSION

Despite technological advances to improve staging accuracy in CC, LN analysis continues to be an essential prognostic factor. It has been an ongoing challenge to identify the “high risk” group of patients that recur and may benefit from adjuvant systemic therapy, while at the same time avoiding chemotherapy in those likely cured by surgery alone.[23]

Standard pathological examination of resected CC-bearing specimens and regional nodes consists primarily of identifying and examining a sufficient number of LNs. There is controversy regarding the definition of what constitutes a sufficient number of LNs. Chang *et al*. [24] analyzed more than 60,000 patients from seven studies and found that in Stage II and III CC 5-year overall survival was directly dependent on number of LNs examined. The minimum number of recommended LNs to be examined, according to current standards, is 12 nodes.[25–27] It is considered highly unlikely to overlook or under-stage the node-positive patient if adherence to the 12-LN minimum standard is maintained, but others have recommended an even larger number of LNs be examined, especially for advanced T stage and pN0 patients.[28,29] Joseph *et al*. [30] suggested that up to 40 or more LNs should be examined in pT1 and pT2 CC, and that at least 40 LNs for pT3 disease be examined in order to achieve a staging accuracy of 85%. Gonen and colleagues[31] found that the number of examined LNs depends on the T-stage and recommended a minimum of 25 LNs for pT3, and more than 30 LNs for pT4 disease to achieve a 95% probability of accurate histopathological staging.

Because the number of surgically resected and pathologically examined LNs are significant prognostic factors in CC, we established the 12-LN minimum as a strict eligibility criterion and quality standard for the UECC Trial. The prognostic relevance of the volume of nodal disease in CC however remains controversial. According to the 7th edition of the American Joint Committee on Cancer Staging Manual, LN+ status is considered to exist if nodal macrometastases are identified on standard histopathological examination (clusters of tumor cells greater than 2 mm in size).[26] The AJCC version 7 nodal stage depends on the number of involved nodes. The prognostic importance of the presence of nodal macrometastasis for overall survival has been repeatedly demonstrated in patients with Stage III CC. Accordingly, current guidelines for the treatment of patients with CC underscore the importance of adjuvant chemotherapy for LN+ disease.[5,27,32–35]

There is a growing body of literature supporting the prognostic impact of not only nodal micrometastases, that is tumor cell clusters between 0.2 and 2 mm in size (pN0mi+) detected

by IHC, but also isolated tumor cells/cell clusters (ITC/CC) smaller than 0.2 mm in size (pN0i+).[20,23,36–39] A recently published meta-analysis supports the prognostic role of tumor cells identified at the molecular level by RT-PCR in patients found to be node negative by standard histopathology (pN0mol +).[18] Notwithstanding, the clinical significance of the presence of so-called 'occult nodal metastasis' remains controversial, as the benefit of adjuvant systemic therapy in these patients has yet to be demonstrated.

A debate has been on-going for more than 20 years over the prognostic significance of occult nodal metastases. Oberg *et al.* [40] studied 147 node-negative patients with colorectal cancer, and found that 47 (32%) had nodal micrometastasis and the disease-specific mortality was 17% and 15%, respectively, for patients with and without nodal micrometastasis. On that basis, the authors concluded that the presence of nodal micrometastases is not a significant prognostic factor for survival in patients with colorectal cancer. One important point for consideration in this study was the low number of examined LNs: only one patient had 12 or more lymph nodes examined. Furthermore these authors did not report or analyze the effect of other potentially prognostic clinical and pathological factors for survival, in particular the administration of adjuvant chemotherapy. Liefers *et al.* [41] studied the potential prognostic impact of low volume nodal disease utilizing IHC and RT-PCR for nodal analysis amongst 26 patients with Stage II CC. In 14 of the 26 (54 %) occult nodal disease was identified. Fifty percent of Stage II patients with nodal micrometastasis died of recurrent CC, whereas only one patient (8%) with Stage II CC who had no nodal micrometastasis identified, died of disease ($p = 0.02$). The five-year overall survival was 36% and 75%, respectively, for those with and without nodal micrometastasis ($p = 0.03$). In that study most patients had an inadequate number of examined LNs, as only five (19.2 %) had 12 or more surgically resected and histopathologically staged nodes.[41]

More recently, Rahbari *et al.* [18] conducted a meta-analysis of 39 studies including over 4,000 patients, and found that molecular detection of occult nodal disease in patients with CC staged as pN0 using standard histopathology, was associated with a significantly increased risk of disease recurrence, disease-specific and overall mortality. In this meta-analysis, 13 of the 39 studies had examined < 12 LNs, and 10 series did not report the actual number of nodes examined. Only 9 of the 39 studies analyzed included patients with pN0 CC who did not receive adjuvant systemic therapy.[18]

To our knowledge there are only two prospective studies in which an average of 12 or more nodes were examined and in which patients with pN0 CC found to have occult nodal micro-metastasis did not receive adjuvant systemic therapy.[42,43] The finding of occult nodal metastasis by CK20 RT-PCR amongst patients conventionally staged as pN0 was associated with a statistically significant worse survival. [42] However, in these prospective trials, the exclusion of patients failing to meet the 12-node quality benchmark, as well as those receiving adjuvant systemic therapy, was not pre-specified. Furthermore, Fearden *et al.* [43] included patients with less than 12 nodes, as evidenced by an average nodal count of 12 LNs for the entire study group.

In our opinion, it is imperative in prospective CC trials to have consistent inclusion criteria, particularly pertaining to the use of adjuvant systemic therapy and the number of LNs

examined. Using these criteria, other potentially prognostically relevant factors can then be examined. In our study we evaluated prospectively a homogeneous population of patients with conventionally staged pN0 CC treated and followed according to a standardized clinical pathway, whilst minimizing the impact of the number of examined nodes by adhering to the 12-node minimum quality standard. Patients screened preoperatively were excluded if found to be node positive by H&E (pN1) or were upstaged to pN1mi (0.2–2mm). Furthermore the variable of adjuvant systemic chemotherapy was removed since patients with >12 LNs staged pN0 were also excluded if chemotherapy was administered during the period of the study. We believe that this is a sufficiently rigorous approach to objectively interpret the oncological significance of occult nodal micro-metastases in Stage I and II CC.

The significance of the number of examined nodes in patients with CC having only occult nodal metastasis speaks to our previous work in which we showed that only 3 of 108 (3%) of patients with CC having 12+ nodes examined and staged with H&E/IHC as pN0i− developed recurrent disease, whereas 6 of 33 (18%) similarly ultra-staged patients (pN0i−) who had less than 12 nodes examined had relapse of disease during the same study period ($p = 0.0015$).[44] Although there is no randomized trial showing the benefit of adjuvant therapy in patients with Stage I and II CC with nodal micro-metastasis, there is one study that suggests a benefit.[45] In this study of 109 patients who underwent sentinel lymphatic mapping and nodal ultra-staging with pan-CK IHC, there were 14 pN0i+ patients who received adjuvant chemotherapy. All of these patients survived 5 years without recurrence (5-year DFS, 100%), while in the group of 60 patients found to have sentinel nodes ultra-staged as pN0i−, the 5-year DFS was 96.2% even though some of these patients had false-negative sentinel lymph nodes.

For these reasons, we felt it was necessary in our prospective trial to establish inclusion criteria with established minimum number of surgically resected and pathologically staged nodes according to standard methodology, in the absence of adjuvant chemotherapy delivery. Mescoli et al. [23] published a detailed evaluation of 312 patients with CC staged as pN0 who were ultra-staged with CK-IHC, in which they found a statistically significant difference in outcome according to volume of nodal disease.[23] There was an ~10% absolute increase in recurrence amongst patients with pN0i+ CC: 14 % (25/185) pN0i+ vs. 4.7 % (6/127) pN0i− patients developed recurrent disease ($p = 0.013$). The absolute difference in recurrence rates of 16.7% in the pN0i+ group and 2.63 % in the pN0i− patients with Stage I and II CC ($p<0.0001$) in our study are similar to those published by Mescoli et al. [23] A pronounced difference in DFS was reported by Faerden et al. with 23% (9/39), and 7% (6/87) recurrence, respectively, amongst pN0i+ and pN0i− ultra-staged patients with CC ($p = 0.01$).[43]

CONCLUSIONS

This is the first multicenter prospective report to demonstrate that in patients with optimally staged pN0 CC (≥12 LNs, pN0i−), adjuvant chemotherapy is unlikely to reduce disease recurrence, as 97.4% of these patients are disease free. The survival benefit seen in some previous clinical trials might reflect inclusion of understaged patients for whom stage

migration is likely to be a factor because of limited lymph node retrieval. Further follow-up in this prospective trial is anticipated to yield a definitive statement as to the biological significance of nodal micro-metastasis. Both surgical and pathological quality measures are strongly advised in the planning of clinical trials in non-metastatic colon cancer.

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Abbreviations and Acronyms

CC	Colon Cancer
CEA	Carcino-embryonic antigen
CORI	California Oncology Research Institute
DFS	Disease-free survival
H&E	Hematoxylin & eosin
IHC	Immunohistochemistry
IRB	Institutional review board
ITCs	Isolated tumor cells
LN	Lymph node
N0	Node negative
N1	Node positive
NCI	National Cancer Institute
panCK	pan-Cytokeratin
pN+	pathologically node positive
pN0	pathologically node negative
pN0i−	pathologically node negative and negative pancytokeratin immunohistochemistry
pN0i+	Single or small clusters of distinct tumor cells < 0.2 mm identified by IHC or molecular staging
pN1mi	micro-metastases ranging in size from 0.2–2.0 mm
pN1-N2b	macro-metastases >2.0 mm in size
PRT	prospective randomized trial
RT-PCR	Reverse transcriptase polymerase chain reaction
TNA	targeted nodal assessment
TNA-us	targeted nodal assessment and ultra-staging

UECC	Ultra-staging in Early Colon Cancer
USMCI	United States Military Cancer Institute

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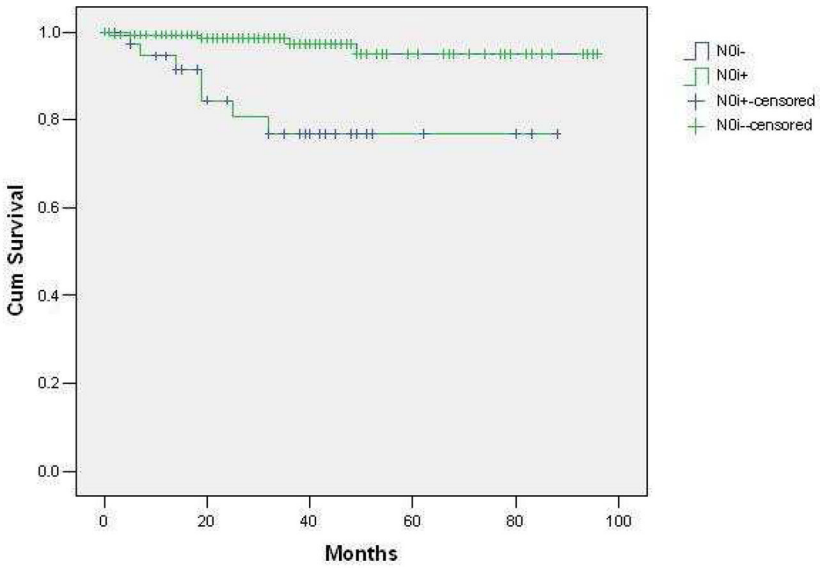

Figure 1.

Disease-free survival (n = 194) was 92.9 months for the pathologically node negative (pN0i −) group and 71.8 months for the group with single or small clusters of distinct tumor cells < 0.2 mm identified by immunohistochemistry or molecular staging (pN0i+), $p < 0.0001$.

Table 1

Baseline Demographic and Clinical Characteristics of the 194 Patients with American Joint Commission on Cancer Stage I and II Colon Adenocarcinoma

Variable	Lymph node status		p Value	Total
	pN0i−	pN0i+		
Sex, n (%)			0.88	
Female	78 (51.3)	21 (50.0)		99 (51.0)
Male	74 (48.7)	21 (50.0)		57 (49.0)
Age, y, mean±SD	68.9±13.1	64.8±16.5	0.97	68.0±13.9
BMI, kg/m^2, mean±SD	26.2±4.7	24.7±5.0	0.10	25.9±4.89
BMI, kg/m^2, n (%)	22 (14.5)	8 (19.0)	0.32	30 (15.5)
<18.5	2 (1.3)	2 (4.8)		4 (2.1)
18.5 to25	55 (36.2)	18 (42.9)		73 (37.6)
25.1 to <30	48 (31.8)	7 (16.7)		55 (28.3)
30+	23 (15.1)	7 (16.7)		30 (15.5)
Tumor location, n (%)			0.31	
Left colon	14 (9.2)	2 (4.8)		16 (8.24)
Rectum	8 (9.2)	0 (0.0)		8 (4.12)
Right colon	72 (47.4)	26 (61.9)		98 (50.5)
Sigmoid colon	41 (27.0)	12 (28.6)		53 (27.3)
Synchronous colon	2 (1.3)	0 (0.0)		2 (1.03)
Transverse colon	15 (9.9)	2 (4.8)		17 (8.76)
Location, colon vs rectum, n (%)			0.23	
Colon	142 (93.4)	42 (100.0)		184 (94.8)
Rectum	8 (5.26)	0 (0.0)		8 (4.12)
Rectum/Synchronous	2 (1.3)	0 (0.0)		2 (1.03)
Operation category, n (%)			0.51	
Segment+	16 (10.5)	3 (7.1)		19 (9.79)
Segmental	136 (89.5)	39 (92.9)		175 (90.2)
Open vs laparoscopic resection, n (%)			0.61	
Missing	0 (0.0)	1 (2.4)		1 (0.51)
Laparoscopic	29 (19.1)	4 (9.5)		33 (17.0)
Open	123 (80.9)	37 (88.1)		160 (83.0)
Operation			0.62	
Left hemicolectomy	15 (9.9)	1 (2.4)		16 (8.25)
Low anterior resection	11 (7.2)	3 (7.1)		14 (7.21)
Right hemicolectomy	78 (51.3)	25 (59.5)		103 (53.1)
Sigmoid colectomy	31 (20.4)	10 (23.8)		41 (21.1)
Total colectomy	16 (10.5)	3 (7.1)		19 (9.79)
Total mesorectal excision	0 (0.0)	1 (2.4)		1 (0.51)

Variable	Lymph node status		p Value	Total
	pN0i−	pN0i+		
Tumor size, cm, mean±SD	4.0±2.1	4.7 ±2.0	0.79	4.2±2.1
Tumor grade, n (%)			0.60	
Missing	6 (3.9)	1 (2.4)		7 (3.6)
High	16 (10.5)	7 (16.7)		23 (11.9)
Intermediate	87 (57.2)	25 (59.5)		112 (57.7)
Low	43 (28.3)	9 (21.4)		52 (26.8)
AJCC primary tumor stage, n (%)			0.24	
Missing	6 (3.9)	0 (0.0)		6 (3.1)
T1	25 (16.4)	1 (2.4)		26 (13.4)
T2	29 (19.1)	5 (11.9)		34 (17.5)
T3	91 (59.9)	35 (83.3)		126 (64.9)
T4	1 (0.7)	1 (2.4)		2 (1.03)
AJCC T stage category, n (%)			0.007	
Missing	6 (3.9)	0 (0.0)		6 (3.1)
T1/T2	55 (36.2)	6 (14.3)		61 (31.4)
T3/T4	92 (59.7)	37 (85.7)		127 (65.5)
AJCC stage, n (%)			0.001	
I	60 (39.5)	5 (11.9)		65 (33.5)
II	92 (60.5)	37 (88.1)		129 (66.5)
Lympho-vascular invasion			0.045	
Missing	12 (7.9)	1 (2.4)		13 (6.7)
Absent	131 (86.2)	34 (80.9)		165 (85.0)
Present	9 (5.92)	7 (16.7)		16 (8.3)
No. of lymph nodes, mean±SD	21.2±7.6	19.9±6.37	0.46	20.9±8.2

pN0i−, pathologically node negative and negative pancytokeratin immunohistochemistry; pN0i+, Single or small clusters of distinct tumor cells < 0.2 mm identified by immunohistochemistry or molecular staging. BMI, body mass index; SD, standard deviation; AJCC, American Joint Commission on Cancer.

Table 2

Baseline characteristics for 194 patients with Stage I and II colon cancer

Variable	pN0i−		pN0i+		p Value	Total	
	n	%	n	%		n	%
Recurred					<0.001		
Missing	0	0.0	1	2.38		1	0.52
No	148	97.4	34	80.9		182	93.8
Yes	4	2.6	7	16.7		11	5.67
Died	0	0.0	1	2.38	<0.001	1	0.51
No	147	96.7	30	71.4		177	91.2
Yes	5	3.3	11	26.2		16	8.3
Status					<0.001		
Unknown	0	0.0	1	2.4		1	0.5
AWD	3	2.0	1	2.4		4	2.1
DOC	4	2.63	6	14.3		10	51.5
DOD	1	0.7	5	11.9		6	3.1
NED	144	94.7	29	69.0		173	89.2

pN0i−, pathologically node negative and negative pancytokeratin immunohistochemistry; pN0i+, single or small clusters of distinct tumor cells < 0.2 mm identified by immunohistochemistry or molecular staging; AWD, alive with disease; DOC, died of unrelated causes; DOD, dead of disease; NED, no evidence of disease.