Pathology and Prognosis of Colonic Adenocarcinomas With Intermediate Primary Tumor Stage Between pT2 and pT3

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Colorectal carcinoma is the fourth most commonly diagnosed cancer in the United States, with the National Cancer Institute Surveillance, Epidemiology, and End Results Program estimating that there will be 149,500 new cases and 52,980 deaths in 2021.1 Pathologic assessment of colorectal resection specimens plays a key role in guiding treatment decisions and determining prognosis, with stage being the most powerful predictor of patient outcomes, and specifically survival.2 The most widely used stage reporting classification is the Tumor, Node, Metastasis (TNM) staging system, as depicted in the most recent edition of the American Joint Committee on Cancer (AJCC) staging manual.3 For colorectal cancer, the primary tumor (pT) category is based on depth of tumor invasion through the colon wall, with pT2 comprising “tumor that invades the muscularis propria,” pT3 defined as “tumor that invades through the muscularis propria into pericolic peritoneum,” and pT4a designating “tumor that invades through the visceral peritoneum.” Accuracy, consistency, and completeness of colorectal cancer staging across institutions provides the foundation for evidence-based patient management decisions.4

Stage-related diagnostic challenges in standardized colorectal cancer reporting have been highlighted by review articles, survey-based publications, and clinical outcome studies, with much of the focus centered on controversies and problems regarding the classification of pT3 versus pT4a tumors, in particular those tumors that approach within 1 mm of the visceral peritoneum.5-12 However, a similar staging dilemma and caveat, hitherto unexplored, also exists in distinguishing pT2 from pT3 colonic tumors. In our experience, a particular underrecognized subset of colonic tumors is inconsistently staged and poses a diagnostic reporting challenge: tumors that invade deeply into the muscularis propria without extending beyond the outer plane of the muscle layer, but yet show a lack of smooth muscle fibers beyond the deepest invasive tumor front. As far as we can tell, the only mention in the literature
addressing this issue states that “the minimal criterion for pT3 is lack of smooth muscle from the muscularis propria between the leading edge of the tumor and the pericolonic soft tissue,” suggesting that such cases should be classified as pT3. Nevertheless, this recommendation is not based on actual cases or data, and tumors meeting this criterion have not yet technically invaded into the suberosal soft tissue, their prognostic significance not having been explicitly elucidated. The issue may be more than just an academic one, given that lymph node–negative pT3 tumors belong to a higher overall prognostic stage group compared with pT2 cases (IIA versus I) and may additionally benefit from adjuvant chemotherapy, particularly if they also fulfill certain high-risk criteria.14–19

Therefore, we aimed to compare the clinicopathologic features and prognostic outcomes of this group of inconsistently staged tumors that seemingly exist as a histologic intermediary between the pT2 and pT3 categories. Our goal was to further define this diagnostically challenging subset of tumors and investigate whether their clinicopathologic characteristics and predictive behavior are more akin to pT2 or pT3 lesions, in order to further inform current staging guidelines as to their proper classification.

PATIENTS AND METHODS

Study Cases

The study was approved by our institutional review board. Colectomies with adenocarcinomas were retrieved from our surgical pathology database during a 50-month period (January 2010 to February 2014). Inclusion criteria comprised surgical resection specimens with a pT stage of pT2 or pT3. The following types of cases were excluded: (1) adenocarcinomas of the anus, rectum, or terminal ileum; (2) malignancies other than adenocarcinoma (eg, small cell, squamous cell, or neuroendocrine carcinomas); (3) patients who had received neoadjuvant therapy; (4) patients with inflammatory bowel disease or other cancer predisposition syndromes (eg, familial adenomatous polyposis, or Lynch syndrome); (5) specimens from endoscopic procedures (biopsies, endoscopic mucosal resections, or endoscopic submucosal dissections); and (6) tumors where hematoxylin–eosin–(H&E)–stained sections were not available for review. Cases wherein the anatomic site of the primary tumor had been listed (in the operative report, specimen requisition form, gross description, or prior endoscopy report) as being in the rectosigmoid colon or ileocecal valve (the latter staged as tumors of the cecum, per AJCC guidelines) were included.

Clinicopathologic Data

Data on clinicopathologic features, including patient age and sex, tumor location, tumor size, number of tissue blocks with tumor, tumor grade/differentiation, tumor histologic type, mismatch repair protein expression status (immunohistochemical [IHC] stain results, available in 139 cases), small vessel lymphovascular invasion (LVI), perineural space invasion (PNI), extramural venous invasion (EMVI), tumor deposits, tumor budding, pT, number of lymph nodes with metastatic disease, total number of lymph nodes harvested, and presence of diverticulae were obtained from surgical pathology reports and patient medical records. Staging parameters (pN and pM) and overall prognostic stage group were scored according to current (8th edition) AJCC guidelines.3 Tumor budding was recorded as the number of tumor buds present at the advancing tumor edge on H&E-stained slides and was scored as high grade if ≥10 per high-power field (×200). Tumors located from the cecum to the distal transverse colon were considered to be in the right colon, whereas those found in the splenic flexure and distally were deemed to be left-sided. Tumors with exclusively classic adenocarcinoma appearance were listed as having conventional histology, whereas tumors with any variant elements (mucinous, medullary, signet ring cell, etc), even if only focally present (ie, <50% and not the dominant pattern), were grouped separately. Patient outcomes were evaluated by reviewing medical records (including physician notes, imaging studies, etc) and included administration of adjuvant therapy (if any), disease progression (defined as locoregional recurrence and/or metachronous development of distant metastases), and disease-specific mortality. Follow-up was defined from the date of index colectomy to event (disease progression or disease-specific mortality) or to last clinical follow-up, and was measured in months.

Assignment of pT Stage

All H&E-stained sections with tumor from every case were independently and blindly reviewed by both authors to confirm depth of tumor invasion. Cases with disagreement were jointly reviewed on a multiheaded microscope for consensus diagnosis. Each case was classified into 1 of 3 groups as follows: pT2, bona fide T2 tumors with unequivocal presence of smooth muscle fibers beyond the leading tumor edge at the deepest point of invasion; pT3, bona fide T3 tumors with unequivocal invasion beyond the muscularis propria into pericolonic soft tissue; and intermediate cases (termed pT2int), tumors without unequivocal advancement beyond the deepest border of muscularis propria in any of the tumor sections, but showing apparent lack of smooth muscle fibers between the leading tumor edge and surrounding soft tissue. All pT2int tumors were confirmed with IHC staining for desmin (prediluted mouse monoclonal antibody DE-R-11, Leica Biosystems, Wetzlar, Germany). An additional case where the presence of smooth muscle fibers beyond the leading tumor edge was considered ambiguous was stained with desmin and eventually classified as pT3.

Statistical Analysis

Continuous variables (age, tumor size, number of tumor blocks, number of positive and harvested lymph nodes, percentage of lymph nodes with metastases) were compared using analysis of variance. Categorical variables (sex; tumor site; grade; histology; presence of lymphovascular, perineural, and extramural venous invasion; pT, pN, pM, and TNM stage; tumor deposits; high-grade tumor budding; microsatellite instability [MSI] status; presence of diverticulae) were compared using the Pearson χ² or 2-sided Fisher exact test (the latter if 1 or more cells had expected counts <5). Multivariate logistic regression incorporated all parameters (categorical and continuous) as independent variables with calculated odds ratio (OR) and 95% CIs. Cohen k coefficient was used to evaluate concordance between pathologists’ reads in the determination of pT stage. Cox proportional hazards regression was used to test for the effect of listed variables on disease progression (disease-free survival [DFS]) and cancer-related mortality (disease-specific survival [DSS]). Kaplan-Meier analysis was used to test for differences in progression-free survival (PFS) and DSS, and log-rank test was employed for statistical significance. All
analysis was carried out using Statistical Package for the Social Sciences software (SPSS, build 1.0.0.1327, copyright 2019, IBM), with \( P < .05 \) considered significant.

**RESULTS**

**Clinicopathologic Characteristics**

Resection specimens from 168 patients with primary colonic adenocarcinomas invading into the muscularis propria (pT2) or subserosal soft tissues (pT3) fulfilled criteria and were included in the study (Supplemental Table 1, see supplemental digital content containing 2 tables at https://meridian.allenpress.com/aplm in the May 2022 table of contents). Rectal and any colonic carcinomas that had received neoadjuvant chemotherapy prior to surgical resection were excluded, as per study criteria, because treatment effect (including mucin pools) would interfere with proper evaluation of pT stage and the relationship of the tumor with the border between muscularis propria and subserosal soft tissue. The patients, 91 of whom (54.2%) were female, had a mean age of 70.3 ± 14.2 years and a median age of 72 years (range, 33–94 years). Diverticulosis in the same colon segment as the tumor was present in 16 patients (9.5%). The tumors were right-sided in 90 of the 168 cases (53.6%), had a mean size of 4.67 ± 2.04 cm, and had been histologically sampled with an average of 5.04 ± 1.87 tissue sections per case. Carcinomas were low grade (well or moderately differentiated) in 133 cases (79.2%), with conventional adenocarcinoma histology present in 119 (70.8%). High-grade tumor budding and MSI (all with MLH1/PMS2 loss) were present in 21 (12.5%) and 18 (12.9%) cases, respectively. Lymphovascular invasion, PNI, and EMVI were present in 55 (32.7%), 30 (17.9%), and 21 (12.5%) cases, respectively. The tumors had been initially staged as pT2 in 39 (23.2%) and pT3 in 129 (76.8%) cases. Interestingly, of 53 pT3 cases with LVI in our study, small vessel invasion was identified in the submucosa in 37 cases (69.8%), the muscularis propria in 19 cases (35.8%), and the subserosa in 39 cases (73.6%; some cases had LVI identified in more than 1 or even all 3 colonic wall layers). A total of 60 cases (35.7%) had lymph node metastasis, with a mean of 19.40 ± 7.97 lymph nodes harvested per case overall and 1.17 ± 2.82 of those being positive for metastatic carcinoma, on average. The average percentage of lymph nodes being positive per case was 7.0% ± 14.85%. Discontinuous subserosal tumor deposits were identified in 25 cases (14.9%). Lymph node stage was negative (pN0) in 100 (59.5%), pN1 in 54 (32.1%), and pN2 in 14 (8.3%). Distant metastases (pM1) were present at the time of resection in 15 cases (8.9%), and the overall stage was localized (stage groups I and II) in 97 cases (57.7%) and advanced (stage group III/IV) in 71 cases (42.3%).

**Intermediate Primary Tumor Stage**

All cases were reassigned pT based on microscopic review of all original H&E-stained tumor sections (Figure 1, A through C). Bona fide pT2 cases with confirmed tumor invasion into, but not through, the muscularis propria comprised 29 of the 168 cases (17.3%; Supplemental Table 1). Bona fide pT3 primary tumor stage with unequivocal
| Characteristic                                      | T2 (n = 29) | T2 Versus T2_int | T2_int (n = 21) | T2_int Versus T3 | P Value |
|---------------------------------------------------|-------------|-----------------|-----------------|-----------------|---------|
| **Patient sex, No. (%)**                          |             |                 |                 |                 |         |
| Female                                            | 20 (69.0)   | 10 (47.6)       | 61 (51.7)       |                 | .13     |
| Male                                              | 9 (31.0)    | 11 (52.4)       | 57 (48.3)       |                 | .73     |
| **Patient age, y**                                |             |                 |                 |                 | .87     |
| Mean ± SD                                         | 71.7 ± 13.7 | 71.0 ± 14.1     | 69.9 ± 14.5     |                 | .73     |
| Median (range)                                    | 73 (39–91)  | 72 (33–92)      | 71 (35–94)      |                 |         |
| **Diverticulosis, presence, No. (%)**              |             | >.99            | 2 (9.5)         | >.99            | 10 (8.5) |
| Tumor location, No. (%)                           |             |                 |                 |                 | .70     |
| Right colon                                       | 14 (48.3)   | 9 (42.9)        | 67 (56.8)       |                 | .24     |
| Left colon                                        | 15 (51.7)   | 12 (57.1)       | 51 (43.2)       |                 |         |
| **Tumor size, cm**                                |             | >.99            | 2 (9.5)         | >.99            | 10 (8.5) |
| Mean ± SD                                         | 3.37 ± 1.41 | 4.39 ± 1.68     | 5.05 ± 2.10     |                 | .03     |
| Median (range)                                    | 3.3 (0.7–7.0)| 4.0 (1.8–8.0)  | 4.8 (1.4–12.5)  |                 |         |
| **Tumor tissue blocks, No.**                       |             | .81             |                 |                 | .41     |
| Mean ± SD                                         | 4.69 ± 1.98 | 4.81 ± 1.21     | 5.17 ± 1.93     |                 |         |
| Median (range)                                    | 4 (1–11)    | 4 (3–8)         | 5 (2–13)        |                 |         |
| **Tumor grade, No. (%)**                          |             | >.99            |                 |                 |         |
| Low grade                                         | 28 (96.6)   | 20 (95.2)       | 85 (72.0)       |                 | .03     |
| High grade                                        | 1 (3.4)     | 1 (4.8)         | 33 (28.0)       |                 |         |
| **Tumor histology, No. (%)**                      |             | .34             |                 |                 | .29     |
| Conventional                                      | 20 (69.0)   | 17 (81.0)       | 82 (69.5)       |                 |         |
| Mucinous/medullary/SRC                            | 9 (31.0)    | 4 (19.0)        | 36 (30.5)       |                 |         |
| **Tumor budding, No. (%)**                        |             | .22             |                 |                 | .49     |
| Absent or low grade                               | 27 (93.1)   | 17 (81.0)       | 103 (87.3)      |                 |         |
| Present, high grade                               | 2 (6.9)     | 4 (19.0)        | 15 (12.7)       |                 |         |
| **MSI status by IHC, No. (%)**                    |             | .63             |                 |                 | .69     |
| MSS (retained expression)                         | 23 (85.2)   | 16 (94.1)       | 82 (68.3)       |                 |         |
| MSI (all MLH1/PMS2 loss)                          | 4 (14.8)    | 1 (5.9)         | 13 (13.7)       |                 |         |
| **Lymphovascular invasion, No. (%)**              |             | >.99            |                 |                 | .001    |
| Absent                                            | 28 (96.6)   | 21 (100)        | 65 (55.1)       |                 |         |
| Present                                           | 1 (3.4)     | 0 (0)           | 53 (44.9)       |                 |         |
| **Perineural invasion, No. (%)**                  |             | .07             |                 |                 | .57     |
| Absent                                            | 29 (100)    | 18 (85.7)       | 91 (77.1)       |                 |         |
| Present                                           | 0 (0)       | 3 (14.3)        | 27 (22.9)       |                 |         |
| **Extramural venous invasion, No. (%)**           |             | >.99            |                 |                 | .04     |
| Absent                                            | 28 (96.6)   | 21 (100)        | 98 (83.1)       |                 |         |
| Present                                           | 1 (3.4)     | 0 (0)           | 20 (16.9)       |                 |         |
| **TDs, No. (%)**                                  |             | .50             |                 |                 | .02     |
| Absent                                            | 27 (93.1)   | 21 (100)        | 95 (80.5)       |                 |         |
| Present                                           | 2 (6.9)     | 0 (0)           | 23 (19.5)       |                 |         |
| **Lymph nodes, No.**                              |             |                 |                 |                 |         |
| Total sampled, mean ± SD                          | 19.52 ± 8.68| 20.43 ± 9.94    | 19.19 ± 7.45    |                 |         |
| Positive, mean ± SD                               | 0.17 ± 0.66 | 0.10 ± 0.30     | 1.60 ± 3.25     |                 |         |
| % positive, mean ± SD                             | 1.01 ± 4.20 | 1.56 ± 5.63     | 9.44 ± 16.88    |                 |         |
| **Lymph node metastasis, No. (%)**                |             | >.99            |                 |                 | .001    |
| Absent                                            | 27 (93.1)   | 19 (90.5)       | 62 (52.5)       |                 |         |
| Present                                           | 2 (6.9)     | 2 (9.5)         | 56 (47.5)       |                 |         |
Colon Carcinomas With Intermediate pT2-pT3 Stage—Paulsen & Polydorides

Table 1. Continued

| Parameter                           | T2 (n = 29) | T2 Versus T2int | T2int (n = 21) | T2int Versus T3 | T3 (n = 118) |
|-------------------------------------|-------------|----------------|----------------|----------------|--------------|
| Regional lymph modes (pN), No. (%)  |             |                |                |                |              |
| Negative (pN0)                      | 26 (89.7)   | > .99          | 19 (90.5)      | .001*          | 55 (46.6)    |
| Positive (pN1 or pN2)               | 3 (10.3)    |                | 2 (9.5)        |                | 63 (53.4)    |
| 1–3 nodes or TD (pN1)               | 3 (10.3)    |                | 2 (9.5)        |                | 49 (41.5)    |
| 4 or more (pN2)                     | 0 (0)       |                | 0 (0)          |                | 14 (11.9)    |
| Distant metastasis (pM), No. (%)    |             |                |                |                |              |
| Negative (pM0)                      | 29 (100)    | .42            | 20 (95.2)      | .47            | 104 (88.1)   |
| Positive (pM1)                      | 0 (0)       | .08*           | 1 (4.8)        | .04*           | 14 (11.9)    |
| AJCC/TNM stage group, No. (%)       |             | .69            | .001*          |                |              |
| Local (stage I/II)                  | 26 (89.7)   |                | 18 (85.7)      |                | 53 (44.9)    |
| Advanced (stage III/IV)             | 3 (10.3)    |                | 3 (14.3)       |                | 65 (55.1)    |

Abbreviations: AJCC, American Joint Committee on Cancer; IHC, immunohistochemistry; MSI, microsatellite instability; MSS, microsatellite stable; N/A, not applicable; SRC, signet ring cell; TD, tumor deposit; TNM, Tumor Node Metastasis classification of malignant tumors.

* Statistically significant P values (ie, < .05).

pT2 versus (pT2int and pT3).

pT2 and pT2int versus pT3.

...staged from pT3 to pT2int, there were no cases with high-grade histology, LVI, or EMVI. A total of 3 of 11 patients in the pT3-to-pT2int group received adjuvant chemotherapy, including 1 case with lymph node metastasis (pN1a), 1 case with distant (liver) metastasis (pM1a), and 1 case with PNI, suggesting that these patients would have most likely received treatment regardless of final pT stage.

pN and pM

There were no significant differences between pT2 and pT2int cases in terms of the presence of lymph node metastasis, the number and percentage of lymph nodes with metastatic carcinoma, pN stage, pM, and the overall AJCC stage group (Table 1). In contrast, pT2int tumors were significantly less likely to have lymph node metastasis (P = .001) and had a smaller mean number (P < .001) and percentage (P < .001) of positive lymph nodes, compared with pT3 tumors, with similar numbers of lymph nodes having been harvested among the different groups. Importantly, pT2int carcinomas were more likely to be of lower pN stage (P = .001) and lower AJCC stage group (P = .001). Although PM was not significantly different between pT2int and pT3 cases per se (probably because of the small number of events), it was significant when pT2 and pT2int tumors together were compared to pT3 (P = .04); however, it was not when pT2int and pT3 tumors together were compared to pT2. These data would suggest that for staging purposes, pT2int cases are more similar to pT2 and achieve better statistical separation when grouped with them, as opposed to with pT3 tumors.

To test this hypothesis, we examined the clinicopathologic characteristics that predict pN and pM stages in these cases (Table 2). In total, 68 of the 168 cases (40.5%) had positive lymph node status (pN1 and pN2), and in univariate analysis this was associated with high tumor grade (P = .008) and the presence of high-grade tumor budding (P = .03), LVI (P < .001), PNI (P < .001), and EMVI (P < .001), but not patient age and sex or tumor location, size, histology, and MSI status. When pT2int cases were grouped with pT2 (as proposed herein) they were significantly less likely to be associated with lymph node disease compared...
with pT3 tumors \((P < .001)\). Multivariate logistic regression identified the presence of LVI \((P = .02; \text{OR}, 5.59; \text{CI}, 1.84–17.04)\), EMVI \((P = .003; \text{OR}, 14.74; \text{CI}, 2.47–88.11)\), and pT3 (versus combined pT2 and pT2int) stage \((P = .04; \text{OR}, 3.96; \text{CI}, 1.09–14.42)\) as independently associated with positive pN stage. Importantly, when using the currently recommended sorting of pT2int with pT3 cases, pT stage was no longer independently associated in multivariate analysis with pN stage, whereas other variables (LVI and EMVI) remained so.

Overall, 15 of the 168 cases (8.9%) were positive for distant metastases at the time of colectomy, and in univariate analysis this was associated with younger patient age \((P = .007)\), high tumor grade \((P = .02)\), and the presence of LVI \((P < .001)\) and PNI \((P = .03)\), whereas it was not associated with patient sex and tumor location, size, histology, and MSI status and the presence of high-grade tumor budding. Grouped pT2 and pT2int cases together were significantly associated with fewer distant metastases in univariate analysis compared with pT3 \((P = .04)\). Multivariate logistic regression showed that only larger tumor size was independently associated with the presence of distant metastases \((P = .04; \text{OR}, 1.55; \text{CI}, 1.03–2.35)\). Similar to pN, when pT2int were combined with pT3 tumors, they were not associated with a significant risk of distant metastases, in univariate or multivariate analysis.

**Disease Progression and Patient Outcomes**

We next examined the clinicopathologic features associated with disease progression, defined as tumor recurrence or distant metastasis at any time after surgical resection (Table 3). A total of 38 of the 168 patients (22.6%) exhibited disease progression, whereas the remaining 130 patients (77.4%) did not, during a mean clinical and pathologic follow-up of 44.9 months. In univariate analysis, disease progression was significantly associated with the presence of LVI \((P = .001)\), PNI \((P < .001)\), and EMVI \((P = .03)\) and with higher pN status (pN1 and pN2; \(P < .001)\). Multivariate logistic regression identified only the presence of PNI as remaining independently associated with disease progression \((P = .04; \text{OR}, 3.54; \text{CI}, 1.06–11.89)\). Using our proposed grouping of pT2 and pT2int cases showed a significantly
Table 2. Clinicopathologic Characteristics of Colon Carcinomas Associated With Lymph Node Status (pN) and Distant Metastases (pM)

|                  | pN                        | pM                        |
|------------------|---------------------------|---------------------------|
|                  | Negative (n = 100)        | Positive (n = 68)         | Absent (n = 153) | Present (n = 15) |
| **P Value**      | Univariate | Multivariate | Univariate | Multivariate |
| **Patient sex, No. (%)** | .71   | .22       | .95 | .68 |
| Female           | 53 (53.0)                  | 38 (55.9)                  | 83 (54.2)       | 8 (53.3)        |
| Male             | 47 (47.0)                  | 30 (44.1)                  | 70 (45.8)       | 7 (46.7)        |
| **Patient age, y** | .10   | .28       | .007* | .13 |
| Mean ± SD        | 71.8 ± 13.4                | 68.1 ± 15.2                | 71.3 ± 14.1     | 60.9 ± 12.6     |
| Median (range)   | 73 (33–94)                 | 69 (35–94)                 | 73 (33–94)      | 60 (35–82)      |
| **Tumor location, No. (%)** | .65 | .64 | .29 | .26 |
| Right colon      | 55 (55.0)                  | 35 (51.5)                  | 80 (52.3)       | 10 (66.7)       |
| Left colon       | 45 (45.0)                  | 33 (48.5)                  | 73 (47.7)       | 5 (33.3)        |
| **Tumor Size, cm** | .52   | .63       | .07 | .04* |
| Mean ± SD        | 4.76 ± 2.04                | 4.55 ± 2.04                | 4.59 ± 2.03     | 5.57 ± 1.94     |
| Median (range)   | 4.5 (0.7–11.0)             | 4 (1.4–12.5)               | 4.2 (0.7–12.5)  | 5.2 (3–9.7)     |
| **Tumor grade, No. (%)** | .008* | .36       | .02* | .81 |
| Low grade        | 86 (86.0)                  | 47 (69.1)                  | 125 (81.7)      | 8 (53.3)        |
| High grade       | 14 (14.0)                  | 21 (30.9)                  | 28 (18.3)       | 7 (46.7)        |
| **Tumor histology, No. (%)** | .53   | .54       | .56 | .65 |
| Conventional     | 69 (69.0)                  | 50 (73.5)                  | 107 (69.9)      | 12 (80.0)       |
| Mucinous/medullary/SRC | 31 (31.0) | 18 (26.5) | 46 (30.1) | 3 (20.0) |
| **Tumor budding, No. (%)** | .03*  | .05       | .41 | .24 |
| Absent           | 92 (92.0)                  | 55 (80.9)                  | 135 (88.2)      | 12 (80.0)       |
| Present (high grade) | 8 (8.0)   | 13 (19.1) | 18 (11.8) | 3 (20.0) |
| **MSI status by IHC, No. (%)** | .19   | .72       | .61 | .99 |
| MSS (retained expression) | 75 (84.3) | 46 (92.0) | 112 (86.2) | 9 (100) |
| MSI (MLH1/PMS2 loss) | 14 (15.7) | 4 (8.0)  | 18 (13.8) | 0 (0) |
| **Lymphovascular invasion, No. (%)** | <.001* | .002* | <.001* | .46 |
| Absent           | 89 (89.0)                  | 25 (36.8)                  | 111 (72.5)      | 3 (20.0)        |
| Present          | 11 (11.0)                  | 43 (63.2)                  | 42 (27.5)       | 12 (80.0)       |
| **Perineural invasion, No. (%)** | <.001* | .95       | .03* | .37 |
| Absent           | 91 (91.0)                  | 47 (69.1)                  | 129 (84.3)      | 9 (60.0)        |
| Present          | 9 (9.0)                    | 21 (30.9)                  | 24 (15.7)       | 6 (40.0)        |
| **Extramural venous invasion, No. (%)** | <.001* | .003* | .10 | .42 |
| Absent           | 98 (98.0)                  | 49 (72.1)                  | 136 (88.9)      | 11 (73.3)       |
| Present          | 2 (2.0)                    | 19 (27.9)                  | 17 (11.1)       | 4 (26.7)        |
| **Proposed pT, No. (%)** | <.001* | .04*       | .04* | .97 |
| pT2 (including pT2int) | 45 (45.0) | 5 (7.4) | 49 (32.0) | 1 (6.7) |
| pT3 (strictly defined) | 55 (55.0) | 63 (92.6) | 104 (68.0) | 14 (93.3) |
| **Current pT, No. (%)** | <.001* | .12       | .08 | .99 |
| pT2 (strictly defined) | 26 (26.0) | 3 (4.4) | 29 (19.0) | 0 (0) |
| pT3 (including pT2int) | 74 (74.0) | 65 (95.6) | 124 (81.0) | 15 (100) |

Abbreviations: IHC, immunohistochemistry; MSI, microsatellite instability; MSS, microsatellite stable; pT, primary tumor stage; pT2int, intermediate tumor stage between pT2 and pT3; SRC, signet ring cell.

*Statistically significant P values (ie, <.05).

a Multivariate logistic regression using current (instead of proposed) pT stage designations did not appreciably change P values for the remaining variables (not shown).
|                          | Disease Progression |     |     |     |     |     |     |
|--------------------------|---------------------|-----|-----|-----|-----|-----|-----|
|                          | Absent (n = 130)    |     |     |     |     |     |     |
|                          | Present (n = 38)    |     |     |     |     |     |     |
| **Patient sex, No. (%)** |                     |     |     |     |     |     |     |
| Female                   | 73 (56.2)           | 18  | 47.4| .34 | .51 |
| Male                     | 57 (43.8)           | 20  | 52.6|     |     |
| **Patient age, y**       |                     |     |     |     |     |     |     |
| Mean ± SD                | 71.3 ± 13.6         | 67.1± 15.8 | .11 | .59 |
| Median (range)           | 73 (33-94)          | 66  | (35-94)|     |     |
| **Tumor location, No. (%)** |                   |     |     |     |     |     |     |
| Right colon              | 70 (53.8)           | 20  | 52.6| .90 | .86 |
| Left colon               | 60 (46.2)           | 18  | 47.4|     |     |
| **Tumor size, cm**       |                     |     |     |     |     |     |     |
| Mean ± SD                | 4.60 ± 2.04         | 4.92± 2.04 | .40 | .17 |
| Median (range)           | 4.25 (0.7-12.5)     | 4.55| (1.6-9.7)|     |     |
| **Tumor grade, No. (%)** |                     |     |     |     |     |     |     |
| Low grade                | 107 (82.3)          | 26  | 68.4| .06 | .76 |
| High grade               | 23 (17.7)           | 12  | 31.6|     |     |
| **Tumor histology, No. (%)** |                   |     |     |     |     |     |     |
| Conventional             | 89 (68.5)           | 30  | (78.9)| .21 | .45 |
| Mucinous/medullary/SRC   | 41 (31.5)           | 8   | (21.1)|     |     |
| **Tumor budding, No. (%)** |                   |     |     |     |     |     |     |
| Absent                   | 114 (87.7)          | 33  | (86.8)| >.99| .83 |
| Present (high grade)     | 16 (12.3)           | 5   | (13.2)|     |     |
| **MSI status by IHC, No. (%)** |               |     |     |     |     |     |     |
| MSS (retained expression)| 94 (84.7)           | 27  | (96.4)| .12 | .25 |
| MSI (MLH1/PMS2 loss)     | 17 (15.3)           | 1   | (3.6)|     |     |
| **Lymphovascular invasion, No. (%)** |               |     |     |     |     |     |     |
| Absent                   | 97 (74.6)           | 17  | (44.7)| .001a| .89 |
| Present                  | 33 (25.4)           | 21  | (55.3)|     |     |
| **Perineural invasion, No. (%)** |                 |     |     |     |     |     |     |
| Absent                   | 115 (88.5)          | 23  | (60.5)| <.001a| .04a|
| Present                  | 15 (11.5)           | 15  | (39.5)|     |     |
| **Extramural venous invasion, No. (%)** |               |     |     |     |     |     |     |
| Absent                   | 118 (90.8)          | 29  | (76.3)| .03a| .84 |
| Present                  | 12 (9.2)            | 9   | (23.7)|     |     |
| **TDs, No. (%)**         |                     |     |     |     |     |     |     |
| Absent                   | 114 (87.7)          | 29  | (76.3)| .08 | .83 |
| Present                  | 16 (12.3)           | 9   | (23.7)|     |     |
| **Proposed pT, No. (%)** |                     |     |     |     |     |     |     |
| pT2 (including pT2_intermediate) | 47 (36.2) | 3 | (7.9)| .001a| .13 |
| pT3 (strictly defined)   | 83 (63.8)           | 35  | (92.1)|     |     |
| **Regional Lymph Nodes (pN), No. (%)** |               |     |     |     |     |     |     |
| Negative (pN0)           | 88 (67.7)           | 12  | (31.6)| <.001a| .16 |
| Positive (pN1 or pN2)    | 42 (32.3)           | 26  | (68.4)|     |     |
| 1-3 nodes or TD (pN1)    | 35 (26.9)           | 19  | (50.0)|     |     |
| 4 or more (pN2)          | 7 (5.4)             | 7   | (18.4)|     |     |
| **Current pT, No. (%)**  |                     |     |     |     |     |     |     |
| pT2 (strictly defined)   | 27 (20.8)           | 2   | (5.3)| .03a| .66 |
| pT3 (including pT2_intermediate) | 103 (79.2) | 36 | (94.7)|     |     |

Abbreviations: IHC, immunohistochemistry; MSI, microsatellite instability; MSS, microsatellite stable; N/A, not applicable; pT, primary tumor stage; pT2_intermediate, intermediate tumor stage between pT2 and pT3; SRC, signet ring cell; TD, tumor deposit.

a Statistically significant P values (ie, <.05).
b Multivariate logistic regression using current (instead of proposed) pT stage designations did not appreciably change P values for the other variables (not shown).
lower risk of disease progression compared with pT3 cases in univariate analysis ($P = .001$), but when pT2int cases were combined with pT3, as currently recommended, the difference in disease progression compared with pT2, albeit still significant, was much less so ($P = .03$).

The effect of age, tumor size, pT, and pN status (parameters that were associated with disease progression) on patient outcomes was determined by evaluating DFS and DSS using proportional hazards regression during the patient follow-up period (Table 4). Grouping pT2 and pT2int cases together as proposed identified pT ($P = .04$; hazard ratio [HR], 1.36; CI, 1.05–1.72) and pN ($P = .01$; HR, 1.82; CI, 1.15–2.88) as independently associated with DFS, whereas tumor size and patient age were not. Although pN was also associated with DSS ($P = .04$; HR, 2.16; CI, 1.03–4.54), pT was not. Importantly, when grouping pT2int with pT3 tumors, only pN remained significantly associated with DFS and DSS, whereas primary tumor stage, sorted as currently suggested, did not.

Finally, to demonstrate the significance of pT stage on disease progression, Kaplan-Meier survival analysis was employed (Figure 2). When examined individually, patients with pT2, pT2int, and pT3 tumors exhibited significantly different rates of disease progression ($P = .01$, log-rank test), with pT2int cases showing the best PFS. However, the differences were not significantly different when looking at disease-related mortality, even though pT2int tumors again showed the best DSS. Importantly, when patients with pT2 and pT2int tumors were grouped together, they had significantly better PFS ($P = .02$) compared with patients with pT3 carcinomas and also showed an almost statistically significant trend toward better DSS ($P = .06$). In contrast, when grouped with pT3 cases, patients with pT2int tumors did not show significantly better PFS and DSS compared with pT2 cases. These results would suggest that pT2int sort better with pT2 as opposed to pT3 tumors, in terms of predicting disease progression and disease-related mortality.

**DISCUSSION**

The data presented in this study suggest that colonic adenocarcinomas with an intermediate primary tumor stage between pT2 and pT3 (herein termed pT2int), that consist of cases with no smooth muscle fibers remaining between the advancing tumor edge and the subserosal soft tissue, but that have no invasion beyond the apparent outer border of the muscularis propria, are more similar to pT2 tumors in terms of clinicopathologic characteristics and patient outcomes, compared with pT3 cases. These results, especially if confirmed in subsequent or larger studies, would indicate that, despite some recommendations to the opposite, pT2int tumors should be grouped with pT2 cases during the pathologic staging of colon carcinomas in surgical resections.

The above-described pT2int primary tumor stage category was relatively common in our study, comprising 12.5% of all pT2 and pT3 cases and 42% of all eventually pT2-designated tumors (21 of 50), of which we believe they should be considered a subset. Furthermore, the dilemma faced during diagnostic staging as to the proper category to which these cases belong is genuine, given that they were initially evenly assigned between pT2 and pT3 categories by the original pathologist (47.6% and 52.4%, respectively). Nevertheless, once specific diagnostic criteria had been established for these cases, the interobserver agreement among the study authors for their designation was excellent ($k = .84$), and a desmin IHC stain was confirmatory in every single case. However, it would be important to avoid upstaging these cases, given the outcomes observed in this study, because patients with pT3 tumors may be subjected to adjuvant chemotherapy (single-agent fluorouracil) even if average risk (defined as lacking lymph node metastasis [ie, stage II] and in the absence of other risk factors), which may have been unnecessary in this setting. A total of 58 patients in our study received adjuvant chemotherapy, and the vast majority of them (90%) had bona fide pT3 tumors, so we were not able to control for the effect of therapy in DFS and DSS. If anything, because treated tumors were mostly of pT3 stage, therapy could have potentially improved outcomes in these patients, who were nevertheless already found to have worse survival rates compared with patients with pT2 and pT2int tumors.

Conversely, an important consideration concerns the possibility that certain cases would be denied appropriate adjuvant treatment by being downstaged to pT2int. In our data, cases that had been originally designated as pT3 and restaged as pT2int were similar in all examined parameters to pT2int cases that had been initially diagnosed as pT2, suggesting that downstaging would not necessarily have altered the rate or types of cases receiving additional treatment. There were no cases with high-grade histology, LVI, or EMVI among the pT3-to-pT2int cases, and the rates of high-grade tumor budding and PNI, although a little bit

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**Table 4. Cox Regression Analysis of Disease-Free and Disease-Specific Survival When Intermediate Tumor Stage (pT2int) Cases Are Grouped Together With pT2 Versus pT3**

| Proposed pT groups | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) |
|---------------------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|
| pT2 and pT2int versus pT3 |         |             |         |             |         |             |         |             |
| Disease-free survival | .49     | .99 (0.97–1.02) | .85     | .99 (0.84–1.16) | .04*    | 3.65 (1.05–12.70) | .01*    | 1.82 (1.15–2.88) |
| Disease-specific survival | .50     | 1.01 (0.98–1.05) | .84     | 1.03 (0.79–1.34) | .25     | 3.54 (0.41–30.43) | .04*    | 2.16 (1.03–4.54) |
| Current pT groups |         |             |         |             |         |             |         |             |
| pT2 versus (pT2int and pT3) |         |             |         |             |         |             |         |             |
| Disease-free survival | .53     | .99 (0.97–1.02) | .87     | 1.01 (0.86–1.19) | .27     | 2.32 (0.53–10.20) | .001*   | 2.08 (1.33–3.23) |
| Disease-specific survival | .47     | 1.01 (0.98–1.05) | .61     | 1.07 (0.83–1.39) | .81     | 1.31 (0.15–11.47) | .01*    | 2.55 (1.23–5.30) |

Abbreviations: CI, confidence interval; HR, hazard ratio.

* Statistically significant $P$ values (ie, $<.05$).
higher, were not significantly different compared with pT2-to-pT2\textsubscript{int} tumors. The patients within the pT3-to-pT2\textsubscript{int} group who received chemotherapy had nodal or distant metastases (therefore belonging to a higher overall prognostic stage group) and would have most likely been thusly treated even if they had not been originally staged as pT3. Related is the fact that higher pT stage is correlated with the presence of LVI, which is, in turn, associated with lower survival and better response to chemotherapy in stage II colon cancer.\textsuperscript{25–28} In this study’s pT3 cases, LVI was present in all 3 wall layers, not just the subserosa, even though it was most common there. This would suggest that even though higher pT stage may signify more aggressive tumors with increased propensity for vascular invasion, LVI may nevertheless occur in any/all layers of the colon wall.

Interestingly, the one characteristic where pT2\textsubscript{int} cases differed from pT2 tumors was size, with pT2\textsubscript{int} tumors being significantly larger. This was to be expected, given that carcinomas invading deeper into the colonic wall, and therefore being of higher pT stage, would also be predicted to be of larger size, since depth of invasion is one of the dimensions commonly measured during gross examination. In fact, a large analysis of the National Cancer Database found that tumor size in colon cancer is positively correlated to be of larger size, since depth of invasion is one of the dimensions commonly measured during gross examination.

In this relationship may be limited to colonic as opposed to rectal cancer.\textsuperscript{29–32} There was also a trend for pT2\textsubscript{int} tumors in our study to be associated with higher rates of PNI compared with pT2 carcinomas, although this did not reach statistical significance. This is also unsurprising, given that deeper invading tumors within the pT2 category would be expected to have better access to the myenteric (Auerbach) nerve plexus, a major source of PNI in colon cancer.\textsuperscript{29–31} Indeed, many studies have found that PNI in colon cancer is closely correlated with higher pT stage.\textsuperscript{32–34} Nevertheless, for almost all other clinicopathologic features in our data, and particularly staging parameters, such as pN and pM, pT2\textsubscript{int} tumors were similar to pT2 and significantly different from pT3 carcinomas.

The extent to which smooth muscle layers of the colon wall provide a specific, protective, and effective mechanical or biophysical barrier to tumor invasion is under debate.\textsuperscript{35,36} Recent studies have also challenged the linear paradigm of a stepwise accumulation of driver mutations that enable metastasis, and DFS and overall survival.\textsuperscript{65} However, a separate study of both colonic as well as rectal carcinomas attempting the same subdivision of pT2 tumors found no significant differences between them, including in terms of grade, angioinvasion, lymph node involvement, and prognosis.\textsuperscript{46} Despite these contradicting data, it is tempting to speculate that drawing lines that further separate the colon wall layers into discrete subsections may impart even more granularity in terms of predicting outcomes. On the other hand, given differences in prognosis, it would be important to be confident about assigning accurate pT3 stage. Some studies have shown that the depth of invasion within the pT3 stage category correlates with nodal and distant metastasis as well as recurrence and survival, being less risky for tumors with only minimal invasion into pericolic soft tissue (less than 1 mm, also called pT3a).\textsuperscript{52,55,67}

Our results would indicate that, at least for the distinction between T2 and T3 tumors, this line should reflect the apparent outer border of muscularis propria, rather than the absence of histologic evidence of that layer’s remnants (ie, smooth muscle fibers). We did not examine deeper H&E sections (levels) or their contribution in accurate pT staging in these cases. However, desmin immunohistochemical stains confirmed that all pT2\textsubscript{int} cases in our study had no residual muscle fibers beyond the tumor edge, while at the same time showing these tumors not extending beyond the outer border of muscularis propria (as defined by a conceptual line connecting the outer borders of existing muscularis propria at the 2 lateral edges of the tumor front). Our study specifically compared and found statistically significant differences when comparing pT2\textsubscript{int} to pT3 cases, so we can only draw conclusions and make recommendations based on these groups as defined. Thus, we would advise that pathologists first determine where the outer border of the muscularis propria lies (and accordingly use a desmin stain for this step, if it helps with this visualization) and subsequently decide whether the tumor has crossed it, thus qualifying for pT3 stage. Finally, a comment in the pathology report indicating closeness to the subserosal soft tissue may be reasonable in pT2\textsubscript{int} tumors (as it is sometimes done in pT3 cases that are close to the serosa) and may help oncologists who are otherwise debating the use of adjuvant chemotherapy in these cases. In addition, it may be important to confirm the presence or absence of other...
prognostic parameters (such as LVI, PNI, EMVI, etc) in these cases.

Problematic distinctions exist within other pT categories in colorectal carcinoma staging, most notably in the separation between subserosal (pT3) tumors and those truly penetrating the peritoneal surface (pT4a). In this regard, guidelines recommend that cases where tumor cells are continuous with the peritoneal surface through inflammation be upstaged to the pT4a category, presumably because the presence of an inflammatory response betrays an actual breach of the visceral peritoneum with ensuing ulceration. Studies have found that it may be the close distance to the serosal surface (ie, less than 1 mm) that most closely approximates clinical behavior similar to that of tumors that have actually perforated the visceral peritoneum. To aid in this determination, special stains that highlight the peritoneal elastic lamina have been used, with reasonable success. In this context, the tumor microenvironment associated with peritoneal surface invasion is thought to involve fibroblasts and inflammatory cells, which may in turn play a role in further tumor progression and metastasis. It may thus make biologic sense to regard carcinomas with tumors continuous to the serosal surface through a fibroinflammatory response as similar in prognosis to bona fide pT4a tumors. In contrast, destruction of the colonic wall muscle layer does not lead to ulceration or as robust an inflammatory response (there is no penetration into the peritoneal space). Therefore, the lack of muscle fibers with only fibroinflammatory cells between the advancing tumor edge and the subserosal soft tissue may not achieve equal significance in upstaging these tumors to the next level (ie, pT3). Furthermore, desmin immunohistochemical stains may only be helpful in highlighting the outer border of the muscularis propria layer and tumor extension beyond it, without being as necessary in proving a break into the next colon wall layer.

This single-institution retrospective study faces some limitations, including the possibility of the results being limited to our particular patient population, however large and diverse. Selection bias was minimized by formulating and following strict inclusion and exclusion criteria and by incorporating in the study all sequential cases identified during review. Sampling bias was ameliorated by ensuring that all cases were uniformly handled according to our institution’s gross examination protocols. As a result, variables dependent on the quality of grossing and sampling, such as the number of tissue blocks with tumor and the total number of lymph nodes harvested per tumor, were remarkably similar across all cases in the study. Chronology and reporting bias were lessened by reviewing and classifying every case according to current staging criteria and guidelines. Primary tumor stage for every pT2\textsuperscript{int} case as well as equivocal pT2 and pT3 tumors was confirmed with IHC staining for desmin with independent review by both study pathologists. Adequate patient follow-up ensured little transfer bias, and vigorous statistical analysis with multivariate logistic, and proportional hazards regression allowed confident interpretation of the data. Nevertheless, confirmation of these results by others would be important.

In conclusion, we found that colon carcinomas that invade through the muscularis propria but without extending beyond its outer border (ie, intermediate in tumor stage between pT2 and pT3 categories) have similar clinicopathologic characteristics and patient prognosis as bona fide pT2 tumors and should therefore be grouped together during the pathologic staging of these neoplasms. Additionally, the absence of any remaining smooth muscle fibers beyond the advancing tumor edge, even when confirmed with adjunct IHC stains (ie, for desmin), is not a sufficient criterion to upstage these tumors to pT3, compared to when they exhibit significantly better long-term patient outcomes and prognosis.

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