original report abstract

# Prospective Multicenter Study on the Prognostic and Predictive Impact of Tumor Budding in Stage II Colon Cancer: Results From the SACURA Trial

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**PURPOSE** The International Union Against Cancer highlighted tumor budding as a tumor-related prognostic factor. International assessment criteria for tumor budding were recently defined by the 2016 International Tumor Budding Consensus Conference (ITBCC2016). This study aimed to clarify the prognostic and predictive values of tumor budding in a randomized controlled trial evaluating the superiority of adjuvant chemotherapy with oral tegafur-uracil over surgery alone for stage II colon cancer (SACURA trial; ClinicalTrials.gov identifier: NCT00392899).

**PATIENTS AND METHODS** Between 2006 and 2010, we enrolled 991 patients from 123 institutions with stage II colon cancer. Tumor budding was diagnosed by central review on the basis of the criteria adopted in the ITBCC2016. We prospectively recorded all clinical and pathologic data, including the budding grade, and performed prognostic analyses after 5 years of completing the patients' registration.

**RESULTS** Of 991 tumors, 376, 331, and 284 were classified as BD1, BD2, and BD3, respectively; the 5-year relapse-free survival (RFS) rate was 90.9%, 85.1%, and 74.4%, respectively ($P < .001$), and ranged widely in T4 tumors (86.6% to 53.3%). The budding grade significantly correlated with recurrence in the liver, lungs, lymph nodes, and peritoneum ($P < .001$ to .01). Multivariable analysis revealed that budding and T stage exerted an independent impact on RFS, and on the basis of the Harrell concordance index, these two factors substantially contributed to the improvement of the Cox model for predicting RFS. Both the BD2 and BD3 groups demonstrated greater improvement in the 5-year recurrence rate in the adjuvant chemotherapy group than the surgery-alone group by approximately 5%, but the difference was statistically nonsignificant.

**CONCLUSION** Tumor budding grade on the basis of the ITBCC2016 criteria should be routinely evaluated in pathologic practice and could improve the benefit of adjuvant chemotherapy for stage II colon cancer.

**J Clin Oncol 37:1886-1894. © 2019 by American Society of Clinical Oncology**



## INTRODUCTION

Stage II colorectal cancer (CRC) accounts for one third of curatively resected patients with CRC, and robust decision-making factors are required for optimal postoperative adjuvant treatment because identifying patients who need chemotherapy at this stage remains debatable.[1] In April 2016, the International Tumor Budding Consensus Conference (ITBCC; Bern, Switzerland) reached a consensus on an international, evidence-based standardized scoring system for tumor budding in CRC.[2] In 2017, the Union for International Cancer Control (UICC) publication of *TNM Classification of Malignant Tumors* (8th edition) adopted tumor budding as a potential tumor-related prognostic factor.[3] Under these circumstances, tumor budding is highly expected to be a potential prognostic factor in CRC that could identify patients with stage II disease at high risk for recurrence who need postoperative adjuvant chemotherapy.[2] However, additional work is necessary to transform it from a promising to a robust decision-making factor in treatment. The prognostic impact of tumor budding has only been evaluated retrospectively, mostly in single-institution cohort studies. In addition, no study has prospectively evaluated the value of the assessment criteria for budding recommended by the ITBCC2016.

The SACURA trial is a multicenter, randomized controlled study evaluating the superiority of 1 year of adjuvant treatment with oral tegafur-uracil (UFT) compared with surgery alone for stage II colon cancer (ClinicalTrials.gov: NCT00392899). The 5-year disease-free survival (DFS) rate was 78.4% in the surgery-alone group and 80.2% in the UFT group (hazard ratio [HR], 0.91; $P = .31$), and the superiority of adjuvant treatment with UFT over surgery alone was not shown, although the recurrence rate was lower in

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on April 11, 2019 and published at jco.org on June 10, 2019: DOI https://doi.org/10.1200/JCO.18.02059

the UFT group than in the surgery-alone group (10.4% *v* 13.4%).[4] The SACURA trial projected several translational studies in which tumor budding was prospectively evaluated to determine its prognostic value in stage II colon cancer.[5] The assessment criteria for tumor budding used in the SACURA trial were subsequently adopted as the international standard criteria in the ITBCC2016.

Budding is reportedly a morphologic characteristic of the epithelial-mesenchymal transition (EMT).[6,7] Reports suggest that tumors undergoing EMT may resist conventional chemotherapy[8,9]; thus, an important clinical question to clarify would be whether high-grade budding is associated with decreased efficiency of adjuvant chemotherapy. Specifically, budding would probably not be an optimal decision-making factor if it was associated with decreased efficacy of adjuvant chemotherapy because it is not helpful for selecting patients who would benefit from postoperative adjuvant chemotherapy. Hence, this prospective clinical study attempted to validate the prognostic stratification power of tumor budding on the basis of the ITBCC criteria and clarify the predictive impact of adjuvant chemotherapy efficiency in stage II colon cancer.

## PATIENTS AND METHODS

### Patients

This study was conducted according to the Declaration of Helsinki and comparable Japanese ethical standards and was approved by the institutional review boards of each participating institution. Furthermore, we obtained written informed consent from all study patients.

Overall, 2,024 patients with stage II colon cancer and no preoperative treatment were enrolled in the SACURA trial between October 2006 and July 2010.[5] After excluding 42 ineligible patients, 1,982 were randomly assigned to the surgery-alone group or the UFT group and compared regarding DFS (primary end point) and secondary end points, including overall survival, relapse-free survival (RFS), and incidence and severity of adverse events (Appendix Fig A1, online only). Consequently, at the primary analysis after 5 years from the last patient's enrollment, results showed that there was no superiority in any of the end points in the UFT group.[4]

Of 1,982 patients, 1,003 underwent surgery at 123 separate institutions participating in the preplanned translational study for new histopathologic prognostic factors in the SACURA trial.[5] After excluding 12 patients because of noncompliance with the allocated protocol treatment, we enrolled 991 patients with curatively resected stage II colon cancer at 123 institutions (surgery-alone group, 501 patients; UFT group, 490 patients). Of these, 807 patients had colon cancer and 184 had rectosigmoid cancer. Regarding the extent of lymph node dissection per the *Japanese Classification of Colorectal Carcinoma* (2nd English edition),[10] most patients underwent D3 or D2 procedures (800 and 188 patients, respectively). Institutional pathologists diagnosed conventional factors, including venous invasion, for which positive judgment was made regardless of whether it was observed intramurally or extramurally, and elastin stains to identify vascular invasion were left to the discretion of the pathologists. The median follow-up was 69.7 months (range, 2.1 to 105.6 months), and the 5-year RFS was 84.2% in all patients, 85.3% in the UFT group, and 83.2% in the surgery-alone group ($P = .3083$).

### Pathologic Examination for Tumor Budding

Postoperatively, among glass slides stained with hematoxylin and eosin (HE) prepared in routine pathologic practice, only slides prepared from a whole-tumor section to include the deepest part of the tumor were collected in the study office, Tokyo Medical and Dental University, and from each institution, and submitted to National Defense Medical College, the institution responsible for the central review of new histopathologic factors and blinded to patient and tumor information. All of the HE slides collected for this study were prospectively examined by one of the authors (H.U.) to evaluate the tumor budding grade according to the criteria detailed in the following paragraph.

Tumor budding was defined as an isolated cancer cell or cluster comprising less than five cells in the invasive front and graded according to its number in a microscopic field with a ×20 objective lens (0.785 $mm^2$) in the hotspot. We classified tumors with less than five, five to nine, and 10 or more budding foci as grades BD1, BD2, and BD3, respectively. These assessment criteria were subsequently adopted in the Japanese guidelines (2009)[1] and international criteria in the ITBCC2016.[2] Category BD3 was subclassified as BD3a for tumors with 10 to 19 and BD3b for those with 20 or more budding foci in the hotspot (in a field of 0.785 $mm^2$) at the invasive front. No cytokeratin stains were used to determine the hotspots or to count the number of buds.

### Statistical Analyses

The end point definition in the SACURA trial was reported previously.[4,5] DFS was defined as the time from randomization to recurrence, second cancers, or death, whichever occurred first. Second cancers included metachronous cancers developed in both the colorectum and other organs. The SACURA trial revealed that approximately 9% of the patients experienced second cancers, comprising 40.7% of the DFS events.[4] Because we considered RFS, the time from randomization to first recurrence or death, more suitable for appraising the clinical value of the prognostic factors, we used it as a substitute end point.

The Kruskal-Wallis test was used for continuous variables and the $\chi^2$ test for categorical variables to assess

**TABLE 1.** Tumor Budding and Clinicopathologic Characteristics

| Parameters | Grade of Tumor Budding | | | P |
|---|---|---|---|---|
| | BD1 (n = 376) | BD2 (n = 331) | BD3 (n = 284) | |
| Sex | | | | .1241 |
| Male | 243 (64.6) | 195 (58.9) | 163 (57.4) | |
| Female | 133 (35.4) | 136 (41.1) | 121 (42.6) | |
| Age, years | 65.5 | 65.3 | 65.7 | .7511 |
| Tumor location | | | | .4724 |
| Right-sided colon | 150 (39.9) | 128 (38.7) | 127 (44.7) | |
| Left-sided colon | 158 (42.0) | 134 (40.5) | 110 (38.7) | |
| Rectosigmoid | 68 (18.1) | 69 (20.8) | 47 (16.5) | |
| Maximum diameter, mm | 50.1 | 50.0 | 45.8 | .0036 |
| Extent of LN dissection* | | | | .1024 |
| D1 | 0 (0.0) | 0 (0.0) | 3 (1.1) | |
| D2 | 71 (18.9) | 61 (18.4) | 56 (19.7) | |
| D3 | 305 (81.1) | 270 (81.6) | 225 (79.2) | |
| No. of examined LNs | 20.5 | 20.2 | 19.8 | .5532 |
| Tumor differentiation | | | | .0598 |
| G1 | 159 (42.3) | 156 (47.1) | 105 (37.0) | |
| G2 | 198 (52.7) | 166 (50.2) | 162 (57.0) | |
| G3 | 19 (5.1) | 9 (2.7) | 17 (6.0) | |
| T stage | | | | < .001 |
| T3 | 337 (89.6) | 278 (84.0) | 208 (73.2) | |
| T4 | 39 (10.4) | 53 (16.0) | 76 (26.8) | |
| Lymphatic invasion | | | | < .001 |
| Negative | 187 (49.7) | 136 (41.1) | 93 (32.7) | |
| Positive | 189 (50.3) | 195 (58.9) | 191 (67.3) | |
| Venous invasion | | | | .7196 |
| Negative | 152 (40.4) | 124 (37.5) | 110 (38.7) | |
| Positive | 224 (59.6) | 207 (62.5) | 174 (61.3) | |
| Preoperative CEA, ng/mL | | | | .0286 |
| ≤ 5.0 | 273 (72.6) | 214 (64.7) | 183 (64.4) | |
| > 5.0 | 87 (23.1) | 100 (30.2) | 89 (31.3) | |
| Not available | 16 (4.3) | 17 (5.1) | 12 (4.2) | |
| MSI | | | | .6076 |
| MSI-high | 26 (6.9) | 20 (6.0) | 23 (8.1) | |
| MSI-low, MSS | 340 (90.4) | 300 (90.6) | 252 (88.7) | |
| Not available | 10 (2.7) | 11 (3.3) | 9 (3.2) | |
| Treatment arm | | | | .5105 |
| Surgery alone | 193 (51.3) | 159 (48.0) | 149 (52.5) | |
| UFT | 183 (48.7) | 172 (52.0) | 135 (47.5) | |

NOTE. Data are No. (%) unless otherwise indicated.

Abbreviations: CEA, carcinoembryonic antigen; LN, lymph node; MSI, microsatellite instability; MSS, microsatellite stable; UFT, tegafur-uracil.

*Japanese Classification of Colorectal Carcinoma (2nd English edition).[10]

differences between tumor budding in clinicopathologic characteristics and postoperative oncologic events. The RFS and recurrence rates were estimated using Kaplan-Meier analysis. We evaluated the 95% CIs at a specific time using the SE computed by the Greenwood formula and performed comparisons using the log-rank test. Univariable and multivariable analyses using the Cox proportional hazards regression model were performed to calculate HRs and 95% CIs for RFS of eight prespecified, elemental prognostic factors, including conventional factors used in the current international guidelines (number of lymph nodes examined, tumor differentiation, T stage, lymphatic and venous invasion, and microsatellite instability),[11,12] treatment arm, and tumor budding. Additional multivariable analyses were also performed as sensitivity analyses in other sets of prognostic factors. Furthermore, we compared the multivariable Cox models for the prediction of RFS to assess the prognostic power of the individual prognostic factors using the Harrell concordance index (C-index).[13] The 95% CI for the difference in Harrell C-index from the interest model was estimated using the bootstrap percentile method with resampling 10,000 times. We conducted an interaction analysis to compare the treatment effect of UFT between subgroups determined according to the three-tier tumor budding grade by using a Cox model with treatment, three-tier tumor budding (two terms), and their interaction (two terms) as covariables to have an interaction test with degrees of freedom of 2. We also estimated subgroup-specific treatment effects to inspect the profile of the interaction. All statistical analyses were performed using SAS, version 9.3 (SAS Institute, Cary, NC).

## RESULTS

### Incidence of Grades of Tumor Budding in the SACURA Trial

On the basis of the budding grade, 376, 331, and 284 tumors were classified as BD1, BD2, and BD3, respectively. The proportion of T4, positive lymphatic invasion, and preoperative serum carcinoembryonic antigen value of more than 5.0 ng/mL was higher based on the increased tumor budding grade ($P < .001$ to .03; Table 1). The budding grade was marginally associated with the tumor differentiation grade ($P = .0598$).

### Tumor Budding and Prognostic Outcomes

On the basis of the tumor budding grade, the 5-year RFS rate was 90.9% (95% CI, 87.4% to 93.5%), 85.1% (95% CI, 80.7% to 88.6%), and 74.4% (95% CI, 68.9% to 79.1%) for BD1, BD2, and BD3, respectively ($P < .001$; Fig 1). Moreover, a significant difference was observed in RFS between BD3a and BD3b ($P = .0465$); the 5-year RFS rate was 77.5% (95% CI, 71.3% to 82.6%) for BD3a and 64.6% (95% CI, 52.0% to 74.7%) for BD3b. A positive correlation was observed between the three-tier

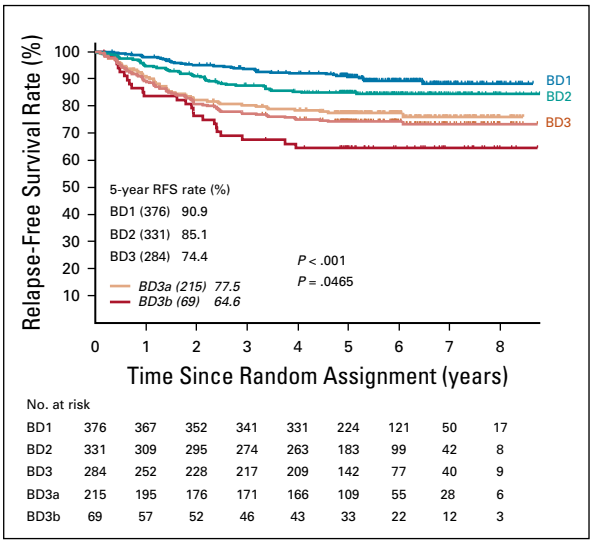


**FIG 1.** Kaplan-Meier estimates of the relapse-free survival (RFS) rate in patients with colon cancer according to grade of tumor budding.

budding grade and RFS in both T3 and T4 ($P = .0100$ and $< .001$, respectively); however, the 5-year RFS rate stratified according to the three-tier budding grade was wider in patients with T4 tumors (86.6% to 53.3%) compared with those with T3 tumors (91.4% to 82.1%).

The incidence of recurrence was 6.4%, 12.1%, and 23.6% in the BD1, BD2, and BD3 groups, respectively ($P < .001$; Table 2). Of first relapse organs, the three-tier budding grade was significantly associated with the incidence of liver, lung, lymph node, and peritoneal recurrence, respectively ($P < .001$ to .02; Table 2).

### Identification of Significant Prognostic Factors for RFS

Among eight prespecified prognostic factors, T stage, microsatellite instability status, and tumor budding correlated with RFS on the basis of univariable analysis using the Cox proportional hazards regression model (Table 3). However, other factors were not significant, including tumor differentiation, lymphatic and venous invasions, and number of lymph nodes examined.

Multivariable analysis for RFS revealed budding (BD2: HR, 1.5; 95% CI, 1.0 to 2.3; $P = .0692$; BD3: HR, 2.6; 95% CI, 1.7 to 3.9; $P < .001$) along with T stage (T4: HR, 2.5; 95% CI, 1.8 to 3.6; $P < .001$) as independently affecting the prognostic outcome (Table 3). In sensitivity analysis with the other two sets of combined prognostic factors, we had similar results in which tumor budding was selected as an independent factor for RFS (Appendix Table A1, online only).

### Value of Tumor Budding as a Prognostic Model Factor on the Basis of Harrell C-Index

Table 4 lists a comparison of multivariable Cox models for predicting RFS according to the Harrell C-index. The C-index of a prognostic model consisting of eight elemental prognostic factors was 0.6805 (full model). Among the prognostic models excluding a component factor from the full model, the reduction in C-index was the most significant in the model excluding tumor budding (0.0423), and its 95% CI did not contain zero (0.0086 to 0.0712). Similarly, T factor was associated with a substantially reduced C-index, of which 95% CI did not contain zero.

### Impact of Adjuvant Chemotherapy on Recurrence Rate According to Tumor Budding Grade

On interaction analysis, the treatment effect of UFT was not significantly different between subgroups according to the grade of tumor budding ($P = .5733$ in the interaction test; Fig 2). Although the interaction was not significant, we observed a tendency of the beneficial effect of UFT with HRs of 0.84 (95% CI, 0.53 to 1.33) and 0.72 (95% CI, 0.41 to 1.27) in patients with BD2 tumors and those with BD3 tumors, respectively, but no such a tendency in patients with BD1 tumors with an HR of 1.14 (95% CI, 0.60 to 2.16).

## DISCUSSION

Initially, tumor budding was loosely defined as a histologic characteristic on HE glass slides.[14,15] However, from early

**TABLE 2.** Incidence of Postoperative Oncologic Events According to the Grade of Tumor Budding

| | Grade of Tumor Budding | | | |
|---|---|---|---|---|
| **Events** | **BD1 (n = 376)** | **BD2 (n = 331)** | **BD3 (n = 284)** | ***P*** |
| Recurrence | 24 (6.4) | 40 (12.1) | 217 (76.4) | < .001 |
| First relapse organs | | | | |
| Liver | 15 (4.0) | 18 (5.4) | 27 (9.5) | .0137 |
| Lungs | 6 (1.6) | 7 (2.1) | 24 (8.5) | < .001 |
| Lymph nodes | 1 (0.3) | 3 (0.9) | 13 (4.6) | < .001 |
| Peritoneum | 1 (0.3) | 8 (2.4) | 10 (3.5) | .0025 |
| Local | 4 (1.1) | 9 (2.7) | 8 (2.8) | .1741 |
| Secondary malignancy | 39 (10.4) | 27 (8.2) | 21 (7.4) | .3850 |

NOTE. Data are No. (%) unless otherwise indicated.

**TABLE 3.** Univariable and Multivariable Analyses of Relapse-Free Survival Using Cox Proportional Hazards Regression Model

| | | Univariable | | Multivariable* | |
|---|---|---|---|---|---|
| **Parameter** | **No.** | **HR (95% CI)** | ***P*** | **HR (95% CI)** | ***P*** |
| No. of LNs examined | | | | | |
| ≥ 12 | 749 | 1 | | 1 | |
| < 12 | 242 | 1.26 (0.90 to 1.78) | .1799 | 1.22 (0.85 to 1.74) | .2880 |
| Tumor differentiation | | | | | |
| G1 | 420 | 1 | | 1 | |
| G2 | 526 | 1.25 (0.91 to 1.72) | .1734 | 1.16 (0.84 to 1.62) | .3741 |
| G3 | 45 | 0.45 (0.14 to 1.44) | .1808 | 0.60 (0.18 to 2.01) | .4056 |
| T stage | | | | | |
| T3 | 823 | 1 | | 1 | |
| T4 | 168 | 2.76 (1.98 to 3.84) | < .001 | 2.53 (1.79 to 3.58) | < .001 |
| Lymphatic invasion | | | | | |
| Negative | 416 | 1 | | 1 | |
| Positive | 575 | 1.10 (0.80 to 1.51) | .5682 | 0.93 (0.67 to 1.31) | .6859 |
| Venous invasion | | | | | |
| Negative | 386 | 1 | | 1 | |
| Positive | 605 | 1.29 (0.93 to 1.79) | .1293 | 1.12 (0.79 to 1.59) | .5182 |
| MSI | | | | | |
| MSI-low, MSS | 892 | 1 | | 1 | |
| MSI-high | 69 | 0.33 (0.12 to 0.90) | .0296 | 0.41 (0.15 to 1.17) | .0944 |
| Treatment arm | | | | | |
| Surgery alone | 501 | 1 | | 1 | |
| UFT | 490 | 0.85 (0.62 to 1.16) | .3099 | 0.85 (0.61 to 1.16) | .3007 |
| Tumor budding | | | | | |
| BD1 | 376 | 1 | | 1 | |
| BD2 | 331 | 1.58 (1.03 to 2.42) | .0352 | 1.51 (0.97 to 2.34) | .0692 |
| BD3 | 284 | 2.93 (1.97 to 4.36) | < .001 | 2.57 (1.69 to 3.91) | < .001 |

Abbreviations: HR, hazard ratio; LN, lymph node; MSI, microsatellite instability; MSS, microsatellite stable; UFT, tegafur-uracil.
*Only 961 patients with MSI values were analyzed.

reports on the prognostic impact of tumor budding[15,16] until the latest edition of the UICC TNM staging system, which listed tumor budding as a tumor-associated prognostic factor in CRC,[3] several attempts were made to define the grading system and maximize its value, accounting for various internationally proposed assessment criteria.[17] First, the size of the buds needs to be characterized, because the definition of budding is not always uniform among studies (ie, not strictly defined[15]; less than five cells[16]; five or more cells[18]). Second, grading was determined according to either the subjective manner,[15] bud intensity criteria,[16,18] or percentage of the area with budding at the infiltrating margin.[19]

Third, even in studies using the intensity-based grading system, the most widely used system in prior studies, several inconsistencies in the detailed criteria exist, including field selection (eg, not specified[15]; hotspot method[16,18]; randomly determined[20]), objective lens magnification for counting buds (×20[21,22]; ×25[16,23]; ×40[18,24]), and number of fields assessed (one per case[16]; five per slide[20]; 10 per case[25]). Finally, perhaps a more important issue in the intensity-based grading system, cytokeratin staining was applied in some studies to improve the diagnostic accuracy of isolated cancer cells, and an issue was raised regarding whether either HE or cytokeratin should be used for bud scoring.[23,24,26]

Although these multidirectional approaches have substantially contributed to establishing the value of tumor budding, its routine implementation has been hindered by inconsistencies in the assessment criteria. In the ITBCC2016, consensus was reached in the following four assessment criteria for tumor budding[2]: (1) tumor budding is defined as a single tumor cell or a cell cluster comprising four or fewer tumor cells; (2) tumor budding is

  

**TABLE 4.** Comparison of Multivariable Cox Models for Relapse-Free Survival to Estimate the Contribution of Individual Prognostic Factors According to the Harrell C-Index

| Combinations of Prognostic Factors | Harrell C-Index | Difference (reduction) of Harrell C-Index (*v* full model) | 95% CI of Difference |
|---|---|---|---|
| Full model* | 0.6805 | — | — |
| No. of LN examined (< 12, ≥ 12) | 0.6815 | −0.0010 | −0.0044 to 0.0093 |
| Tumor differentiation (G1, G2, G3) | 0.6792 | 0.0013 | −0.0036 to 0.0166 |
| T stage (T3, T4) | **0.6520** | **0.0284** | **0.0068 to 0.0514** |
| Lymphatic invasion (negative, positive) | 0.6793 | 0.0012 | −0.0025 to 0.0112 |
| Venous invasion (negative, negative) | 0.6804 | 0.0000 | −0.0039 to 0.0108 |
| MSI (MSI-low/MSS, MSI-high) | 0.6745 | 0.0060 | −0.0012 to 0.0201 |
| Treatment arm (surgery alone, UFT) | 0.6792 | 0.0013 | −0.0024 to 0.0158 |
| Tumor budding (BD1, BD2, BD3) | **0.6382** | **0.0423** | **0.0086 to 0.0712** |

NOTE. Bold type indicates the factors associated with a substantially reduced C-index, of which 95% CI did not contain zero. Only 961 patients with microsatellite instability (MSI) values were analyzed.

Abbreviations: C-index, concordance index; LN, lymph node; MSI, microsatellite instability; MSS, microsatellite stable; UFT, tegafur-uracil.

*Prognostic model consisting of eight elemental prognostic factors (number of lymph nodes examined, tumor differentiation, T stage, lymphatic invasion, venous invasion, MSI, treatment arm, and tumor budding).

counted on HE; (3) tumor budding is assessed in one hotspot (in a field measuring 0.785 $mm^2$) at the invasive front; and (4) a three-tier system should be used along with budding count to facilitate risk stratification in CRC.

In the SACURA trial (2006 to 2010), the tumor budding status of stage II colon cancer was prospectively assessed based on the criteria later adopted in the ITBCC2016. This study revealed that RFS decreased according to an increase in the three-tier grade. Also, the SACURA trial assigned subgrades to the BD3 category on the basis of the number of budding foci in a field measuring 0.785 $mm^2$ (ie, BD3a, 10 to 19; BD3b, ≥ 20) and revealed a marked difference in RFS between BD3a and BD3b, resulting in statistically different 5-year RFS rates, with BD1, BD2, BD3a, and BD3b decreasing in this order. Zlobec et al[27] argued that one of the essential properties of budding is that it is a continuous variable affecting the event of metastases, that is, the higher the number of buds, the higher the risk of lymph node and distant metastases. Although tumor budding was an intensity-dependent prognostic factor that potentially facilitated patient stratification into four groups with different survival outcomes in 638 patients with stage I to III rectal cancer at St Mark's Hospital,[16] all prior studies reporting the prognostic value of tumor budding in stage II CRC demonstrated that the entire population was successfully stratified into only two different prognosis groups.[19,20,28-32] Notably, the ITBCC2016 criteria resolved the intrastage prognostic heterogeneity, enabling the stratification of patients with stage II colon cancer into four groups with wide-ranging differences in 5-year RFS rates (65% to 91%). We believe our results show that the hotspot method for tumor budding adopted in the ITBCC2016 criteria is practical and allows us to make the most of its property, although additional studies are needed to confirm the clinical benefit of the subclassification categories for BD3.

In this study, we evaluated the prognostic value of tumor budding on the basis of two statistical methods. First, we evaluated whether tumor budding was an independent prognostic factor using a Cox proportional hazard model together with other prespecified factors. Consequently, tumor budding was shown to be an independent factor, along with T stage, and the result was similar in other sensitivity analyses. Second, we compared the Harrell C-index between Cox models, in which tumor budding was shown to be essential for improving the performance of prognostic prediction, as well as T factor.

Conversely, our study shows that some of the prognostic factors in stage II CRC that have been adopted in clinical guidelines are worthy of reconsideration, for example, tumor differentiation grade is listed in the National Comprehensive Cancer Network guidelines[11] and the European Society for Medical Oncology guidelines,[12] but that has no impact on this study. A conclusion reached by the ITBCC2016 is that tumor budding is not the same as tumor grade,[2] which is well supported in this study, presenting only marginal correlation and a different survival impact. Another factor for reevaluation is whether vascular invasion is a reliable treatment indicator for adjuvant therapy in stage II colon cancer.[33]

A retrospective analysis of 979 patients in the QUASAR (Quick And Simple And Reliable) trial (majority stage II) revealed a nonsignificant trend toward increased chemotherapy efficacy with increased bud counts,[34] which is consistent with our study, that is, although time to recurrence was similar in BD1 tumors, adjuvant chemotherapy with UFT

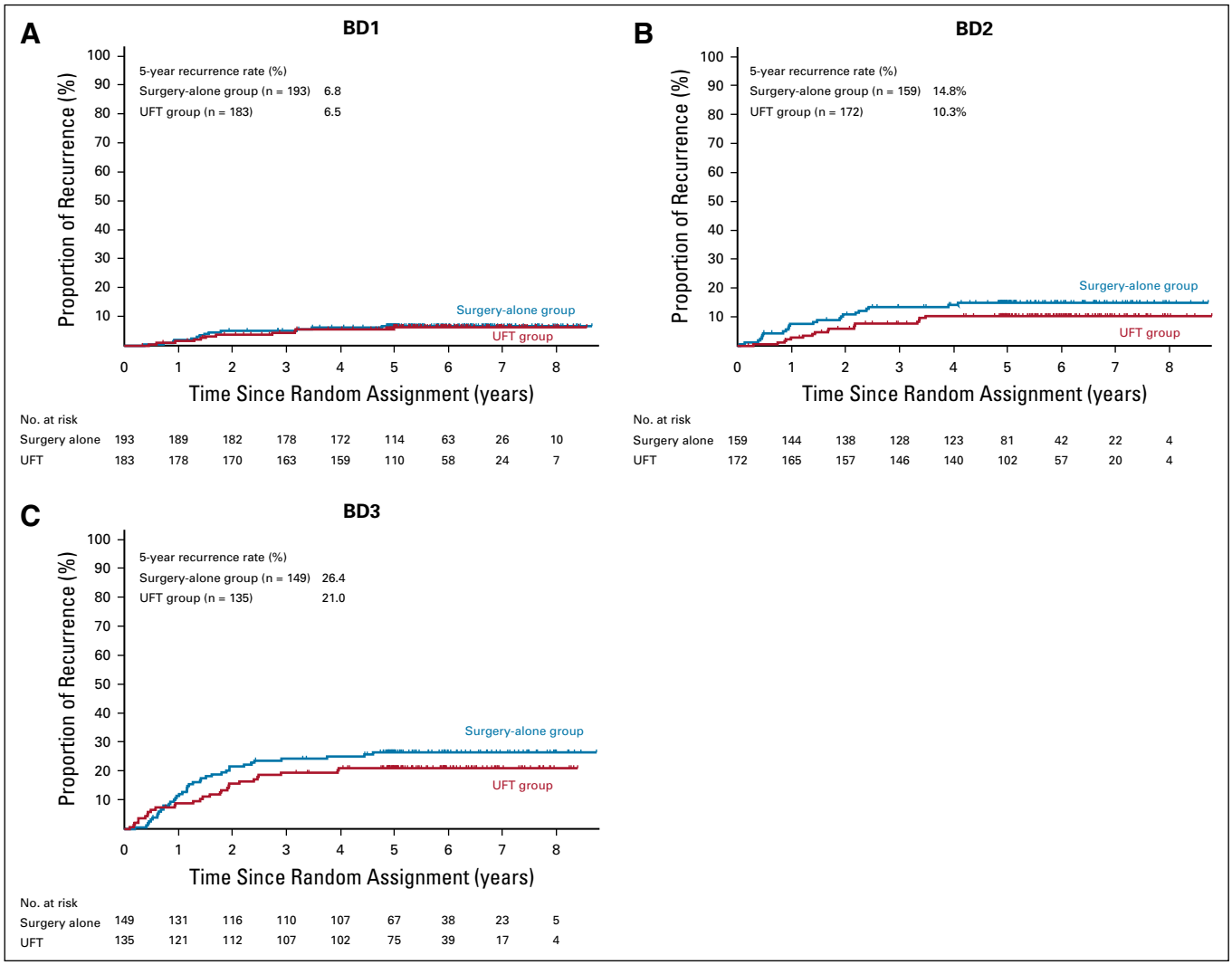


**FIG 2.** Comparison of time to recurrence between the surgery-alone group and the chemotherapy group in patients with (A) BD1, (B) BD2, and (C) BD3 tumors. The 5-year recurrence rates (95% CI) for the surgery-alone and tegafur-uracil (UFT) group were 6.8% (4.0% to 11.4%) and 6.5% (3.6% to 11.5%) in BD1, 10.3% (6.5% to 16.0%) and 14.8% (10.1% to 21.5%) in BD2, and 21.0% (15.0% to 29.0%) and 26.4% (20.1% to 34.4%) in BD3, respectively. Log-rank test: BD1, $P = .8124$; BD2, $P = .1889$; BD3, $P = .2954$.

for 1 year seemed to be associated with an improved 5-year recurrence rate by approximately 5% in both BD2 and BD3 tumors. Although the *P* values were statistically insignificant, perhaps because of the small number of patients enrolled in the individual subgroups and the limited adjuvant power of UFT, the results of two discrete randomized controlled studies suggest that the proportional reduction in recurrence with chemotherapy in patients with higher bud counts might seem at least equivalent in those with low counts.

Budding is reportedly a morphologic characteristic of the EMT,[6,7] and tumors undergoing EMT may resist conventional chemotherapy.[8,9] However, the results of the QUASAR and SACURA trials indicate that the value of tumor budding as a decision-making factor for adjuvant chemotherapy was upheld in terms of its predictive effect in patients with stage II colon cancer, for whom none have been confirmed as predictive markers to direct use of adjuvant therapy. Because this study has limitations regarding the small sample size and single-agent UFT as an adjuvant therapy as stated previously, additional validation will be required, including perhaps a prospective randomized trial using tumor budding as a stratification factor and more effective adjuvant therapy for the high-risk groups, that is, oxaliplatin combination therapy.

In conclusion, the clinical value of the definition and grading system for tumor budding adopted in the Japanese guidelines and ITBCC2016 criteria is well validated in this multicenter prospective study for stage II colon cancer. The role of tumor budding, a tumor-related prognostic factor adopted in the UICC TNM classification, should be emphasized in the adjuvant treatment setting.

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## PRIOR PRESENTATION

Presented in part at the ASCO 2017 Annual Meeting, Chicago, IL, June 2 to 6, 2017.

## SUPPORT

Supported by the Foundation for Biomedical Research and Innovation at Translational Research Center for Medical Innovation at Kobe under the funding contract with Taiho Pharmaceutical, Tokyo, Japan. The funding sources had no role in the design of the study; collection, analysis, or interpretation of the data; writing of the report; or in the decision to submit for publication. The corresponding author had full access to all of the study data and final responsibility for the decision to submit the report for publication.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

Disclosures provided by the authors and data availability statement (if applicable) are available with this article at DOI https://doi.org/10.1200/JCO.18.02059.

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## ACKNOWLEDGMENT

We are grateful to all the patients and the co-investigators for their cooperation in the SACURA trial. A list of participating institutions is given in the Appendix (online only). The authors also thank the following collaborators for their contributions to this trial: Kenichi Kono, Satoshi Nakagawa, Yasuyo Kusunoki, Fumie Kinoshita, and Naoko Kashiwagi in data management; Tasuku Inaji, Hayami Tsumura, Akinori Ogasawara, Yuri Ueda, and Syuichiro Sugimoto as the project office staff; Yoshihiro Matsubara, Satomi Sakabayashi, Yoko Nakagawa, and Tatsuo Kagimura as the statistical staff; Yoko Takagi, Aiko Saito, Sachiko Kishiro, and Yasuyo Okamoto as the translational study office staff; and Masanori Fukushima, MD, PhD, as a director of the Translational Research Center for Medical Innovation.


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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

**Prospective Multicenter Study on the Prognostic and Predictive Impact of Tumor Budding in Stage II Colon Cancer: Results From the SACURA Trial**

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/site/ifc.

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No other potential conflicts of interest were reported.

## APPENDIX

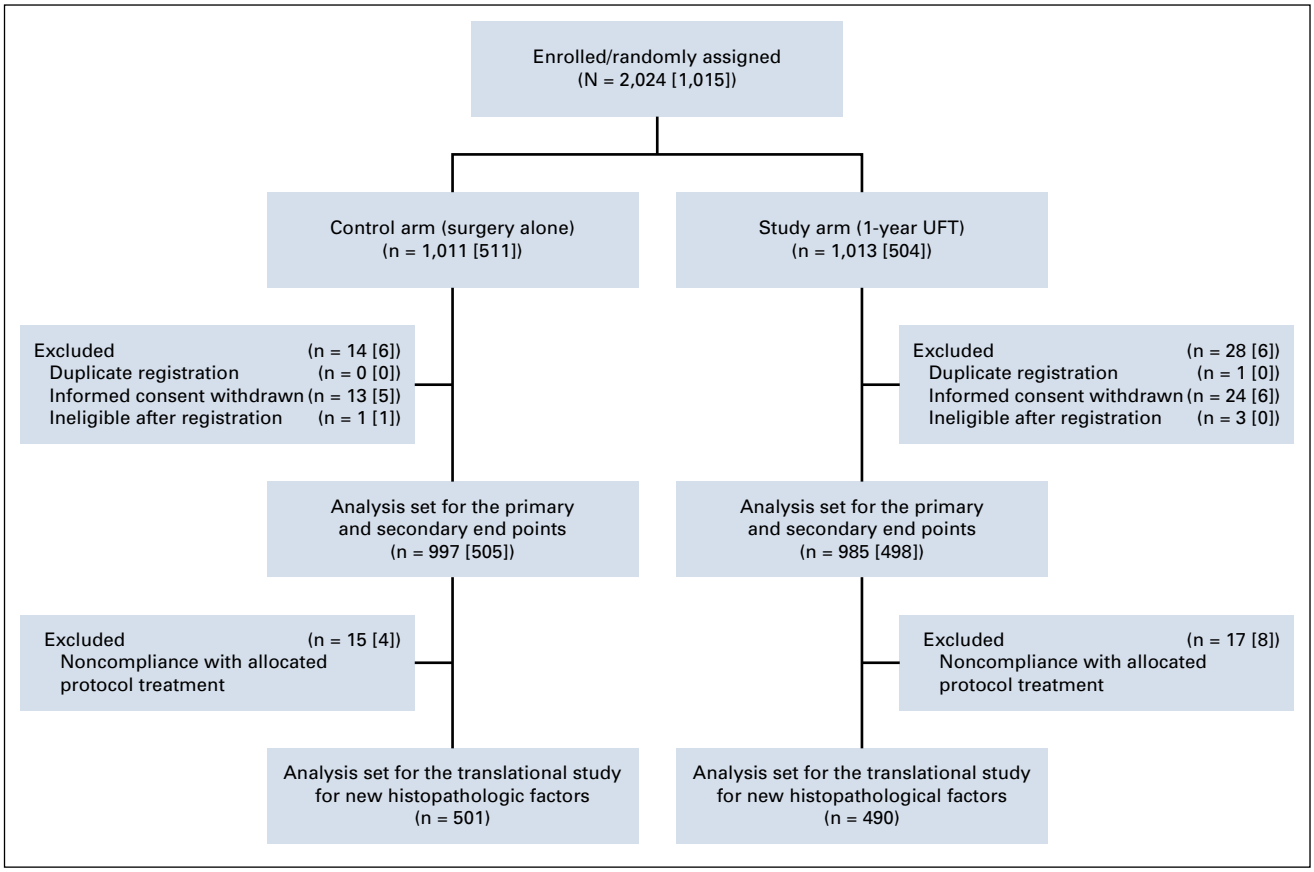


**FIG A1.** CONSORT diagram. Numbers in brackets indicate the number of patients in the translational study for new histopathologic factors. UFT, tegafur-uracil.

**TABLE A1.** Multivariable Analyses for Relapse-Free Survival as Sensitivity Analysis

| | | Selected Prognostic Factors | | Full Prognostic Factors* | |
|---|---|---|---|---|---|
| **Parameter** | **No.** | **HR (95% CI)** | ***P*** | **HR (95% CI)** | ***P*** |
| Sex | | | | | |
| Female | 390 | | | 1 | |
| Male | 601 | | | 0.87 (0.61 to 1.25) | .4541 |
| Age, years | | | | | |
| ≤ 70 | 674 | | | 1 | |
| 71 to 80 | 317 | | | 1.42 (1.00 to 2.02) | .0482 |
| Tumor location | | | | | |
| Right-sided colon | 405 | | | 1 | |
| Left-sided colon | 402 | | | 1.10 (0.76 to 1.61) | .6119 |
| Rectosigmoid | 184 | | | 1.23 (0.77 to 1.97) | .3964 |
| Extent of LN dissection† | | | | | |
| D3 | 800 | | | 1 | |
| D1, D2 | 191 | | | 1.57 (0.76 to 3.23) | .2222 |
| No. of LN examined | | | | | |
| ≥ 12 | 749 | 1 | | 1 | |
| < 12 | 242 | 1.29 (0.92 to 1.83) | .1442 | 1.20 (0.82 to 1.75) | .3401 |
| Tumor differentiation | | | | | |
| G1 | 420 | 1 | | 1 | |
| G2 | 526 | 1.23 (0.89 to 1.7) | .2045 | 1.19 (0.84 to 1.67) | .3332 |
| G3 | 45 | 0.42 (0.13 to 1.35) | .1462 | 0.77 (0.23 to 2.6) | .6765 |
| T stage | | | | | |
| T3 | 823 | 1 | | 1 | |
| T4 | 168 | 2.46 (1.75 to 3.46) | < .001 | 2.60 (1.82 to 3.74) | < .001 |
| Lymphatic invasion | | | | | |
| Negative | 416 | 1 | | 1 | |
| Positive | 575 | 0.92 (0.66 to 1.28) | .6149 | 0.83 (0.54 to 1.28) | .4003 |
| Venous invasion | | | | | |
| Negative | 386 | 1 | | 1 | |
| Positive | 605 | 1.18 (0.84 to 1.66) | .3448 | 0.85 (0.53 to 1.37) | .4975 |
| Preoperative CEA, ng/mL | | | | | |
| ≤ 5.0 | 670 | | | 1 | |
| > 5.0 | 276 | | | 1.62 (0.92 to 2.86) | .0942 |
| MSI* | | | | | |
| MSI-low, MSS | 892 | | | 1 | |
| MSI-high | 69 | | | 0.33 (0.1 to 1.09) | .0695 |
| Treatment arm | | | | | |
| Surgery alone | 501 | 1 | | 1 | |
| UFT | 490 | 0.83 (0.61 to 1.14) | .2497 | 0.87 (0.63 to 1.22) | .4219 |
| Tumor budding | | | | | |
| BD1 | 376 | 1 | | 1 | |
| BD2 | 331 | 1.47 (0.96 to 2.27) | .0772 | 1.46 (0.92 to 2.32) | .1072 |
| BD3 | 284 | 2.51 (1.67 to 3.77) | < .001 | 2.52 (1.63 to 3.89) | < .001 |

Abbreviations: CEA, carcinoembryonic antigen; HR, hazard ratio; LN, lymph node; MSI, microsatellite instability; MSS, microsatellite stable; UFT, tegafur-uracil.

*Japanese Classification of Colorectal Carcinoma (2nd English edition).[10]

†Only 901 patients with CEA and MSI values were analyzed.

| List of Institutions That Participated in the Translational Study for New Histopathologic Prognostic Factors in the SACURA Trial |
|---|
| Osaka General Medical Center |
| National Defense Medical College |
| Kobe City Hospital Organization Kobe City Medical Center West Hospital |
| Koseiren Takaoka Hospital |
| Hamamatsu University School of Medicine |
| Saiseikai Yokohamashi Tobu Hospital |
| Tokyo Medical and Dental University |
| Japan Community Health Care Organization Osaka Hospital |
| Suita Municipal Hospital |
| Japanese Red Cross Kyoto Daini Hospital |
| Nippon Medical School Chiba Hokusoh Hospital |
| Shizuoka City Shimizu Hospital |
| Sano Hospital |
| Saiseikai Tondabayashi Hospital |
| Fukui-ken Saiseikai Hospital |
| National Hospital Organization Kyoto Medical Center |
| St Mary's Hospital |
| Sakai City Medical Center |
| Ogaki Municipal Hospital |
| Tokyo Metropolitan Tama Medical Center |
| Social Insurance Tagawa Hospital |
| Chugoku Central Hospital |
| Teikyo University School of Medicine |
| National Hospital Organization Kobe Medical Center |
| Hakodate Goryoukaku Hospital |
| Gunma Prefectural Cancer Center |
| Hyogo College of Medicine |
| Kagawa Prefectural Central Hospital |
| International Goodwill Hospital |
| Kobe University Graduate School of Medicine |
| Rinku General Medical Center |
| Kyorin University |
| Niigata Cancer Center Hospital |
| Osaka Police Hospital |
| Kansai Rosai Hospital |
| National Center for Global Health and Medicine |
| Fukushima Medical University |
| Osaka Rosai Hospital |
| Sapporo Medical University |
| Miyoshi Central Hospital |
| Nagoya University Hospital |
| Osaka City General Hospital |
| Higashi Takarazuka Satoh Hospital |

(continued in next column)

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| List of Institutions That Participated in the Translational Study for New Histopathologic Prognostic Factors in the SACURA Trial |
|---|
| Tokyo Yamate Medical Center |
| Takarazuka City Hospital |
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| Tochigi Cancer Center |
| Kitakyushu Municipal Medical Center |
| Hashima City Hospital |
| Kure Medical Center and Chugoku Cancer Center |
| Hokushin General Hospital |
| Miyagi Cancer Center |
| Yamagata University Hospital |
| Yamaguchi University Graduate School of Medicine |
| Oita University Graduate School of Medicine |
| Nagano Municipal Hospital |
| Shimonoseki Medical Center |
| Himeji St Mary's Hospital |
| Nagoya Ekisaikai Hospital |
| Tokushima University Hospital |
| Anan Kyoei Hospital |
| Kumamoto University |
| National Hospital Organization Shikoku Cancer Center |
| Kanagawa Cancer Center |
| Matsunami General Hospital |
| Otemae Hospital |
| Japanese Red Cross Osaka Hospital |
| Hyogo Cancer Center |
| Aichi Cancer Center Aichi Hospital |
| University of Yamanashi Hospital |
| Jichi Medical University Hospital |
| Otsu City Hospital |
| Oita Red Cross Hospital |
| Teikyo University Chiba Medical Center |
| Tohoku Rosai Hospital |
| Kitasato University East Hospital |
| Minoh City Hospital |
| Asahikawa Medical University |
| Kyushu University Graduate School of Medical Sciences |
| Nissay Hospital |
| Gunma University Graduate School of Medicine |
| Nagahama City Hospital |
| Shinshu University Hospital |
| Japanese Red Cross Society Nagano Hospital |

(continued on following page)

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| List of Institutions That Participated in the Translational Study for New Histopathologic Prognostic Factors in the SACURA Trial |
|---|
| Tottori University Hospital |
| Kyoritsu General hospital |
| Saitama Medical University International Medical Center |
| Numazu City Hospital |
| Nishijin Hospital |
| Shizuoka City Shizuoka Hospital |
| Fujita Health University Banbuntane Hotokukai Hospital |
| Hokkaido Chuo Rosai Hospital |
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| Chigasaki Municipal Hospital |
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