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ABSTRACT

PURPOSE No biomarker capable of improving selection and monitoring of patients with rectal cancer managed by watch-and-wait (W&W) strategy is currently available. Prognostic performance of the Immunoscore biopsy (IS_B) was recently suggested in a preliminary study.

METHODS This international validation study included 249 patients with clinical complete response (cCR) managed by W&W strategy. Intratumoral CD3+ and CD8+ T cells were quantified on pretreatment rectal biopsies by digital pathology and converted to IS_B. The primary end point was time to recurrence (TTR; the time from the end of neoadjuvant treatment to the date of local regrowth or distant metastasis). Associations between IS_B and outcomes were analyzed by stratified Cox regression adjusted for confounders. Immune status of tumor-draining lymph nodes (n = 161) of 17 additional patients treated by neoadjuvant chemoradiotherapy and surgery was investigated by 3'RNA-Seq and immunofluorescence.

RESULTS Recurrence-free rates at 5 years were 91.3% (82.4%-100.0%), 62.5% (53.2%-73.3%), and 53.1% (42.4%-66.5%) with IS_B High, IS_B Intermediate, and IS_B Low, respectively (hazard ratio [HR; Low v High], 6.51; 95% CI, 1.99 to 21.28; log-rank P = .0004). IS_B was also significantly associated with disease-free survival (log-rank P = .0002), and predicted both local regrowth and distant metastasis. In multivariate analysis, IS_B was independent of patient age, sex, tumor location, cT stage (T, primary tumor; c, clinical), cN stage (N, regional lymph node; c, clinical), and was the strongest predictor for TTR (HR [IS_B High v Low], 6.93; 95% CI, 2.08 to 23.15; P = .0017). The addition of IS_B to a clinical-based model significantly improved the prediction of recurrence. Finally, B-cell proliferation and memory in draining lymph nodes was evidenced in the draining lymph nodes of patients with cCR.

CONCLUSION The IS_B is validated as a biomarker to predict both local regrowth and distant metastasis, with a gradual scaling of the risk of pejorative outcome.

INTRODUCTION

The National Comprehensive Cancer Network guidelines for locally advanced rectal cancer (LARC) recommend treatment combining neoadjuvant chemoradiotherapy (nCRT), surgical resection with total mesorectal excision (TME), and if necessary, additional adjuvant chemotherapy.[1] Rectal resection with TME is accompanied by a high risk of poorer

CONTEXT

Key Objective

Watch-and-wait (W&W) strategy is a preservative strategy for patients with rectal cancer with clinical complete response after neoadjuvant chemoradiotherapy. We conducted a multicenter international, retrospective validation cohort study of 249 W&W patients to confirm the utility of the Immunoscore biopsy (IS_B) performed on pretreatment biopsies to predict time to recurrence (TTR).

Knowledge Generated

IS_B categories are significantly associated with a gradual scaling of the risk of both local regrowth and distant metastasis (IS_B High *v* Intermediate *v* Low). IS_B is independent and superior to clinical parameters in predicting TTR and improves the predictive model when combined to them.

Relevance (E.M. O'Reilly)

This manuscript further adds to the body of evidence supporting the use of IS_B as a marker of outcome in colorectal cancers. Specifically, the potential value of IS_B as an important prognostic tool is illustrated in non-operative management of localized rectal cancer.*

*Relevance section written by *JCO* Associate Editor Eileen Mary O'Reilly, MD.

quality of life (QOL) arising from impaired anal, bowel, urinary, and sexual functions.[2] Since the rectal cancer incidence increases significantly in patients younger than 50 years, improving the QOL of patients with rectal cancer is of major importance.[3] The observed 10%-30% rate of pathologic complete response (pCR) after nCRT and more recently 28%-50% rate of pCR after additional induction or consolidation chemotherapy[4-6] have prompted the development of the so-called watch-and-wait (W&W) strategy, pioneered by Habr-Gama et al.[7] A large international registry-based study, the International Watch & Wait Database has shown satisfying long-term oncologic outcomes and superiority in terms of QOL.[8] A validated biomarker that could improve the accuracy of patients' selection, monitoring, and prognostic is awaited for the widespread adoption of such preservative strategy.[9]

We showed that intratumoral immune contexture of tumors assessed by the test Immunoscore (IS) is a dominant determinant of clinical outcome in patients with early- and advanced-stage colorectal cancer.[10-12] To our knowledge, the IS test is the first and only internationally validated[12] standardized assay[13] for quantifying the immune infiltrate. The intratumoral immune quantification has been added to the 2019 WHO classification of tumors of the digestive systems,[14] and the IS is now recommended by the European Society for Medical Oncology (ESMO) and Pan-Asian adapted ESMO Clinical Practice Guidelines for prognostic purpose in patients with localized colon cancer.[15,16]

In rectal cancer, Garcia-Aguilar and colleagues showed that a local hot immune signature in the tumor before treatment is associated with increased response to nCRT and prolonged disease-free survival (DFS).[17] Furthermore, patients with LARC deficient in mismatch repair achieved a 100% complete response rate after PD-1 blockade, strongly suggesting that the in situ immune response boosted by the treatment can eradicate the tumor and prevent recurrences.[18] We also evidenced that an IS biopsy (IS_B) performed at diagnosis predicts response to neoadjuvant treatment (nT)[19,20] and strongly complements imaging data in so doing. Clinical utility of IS_B in patients managed by W&W strategy was further suggested: IS_B was an independent prognostic factor related to time to recurrence (TTR) in a test cohort of 73 patients.[20]

The primary objective of the current study was to validate, through a large multicentric independent cohort of W&W patients, the ability of the IS_B performed on pretreatment biopsies to predict TTR. Additionally, we questioned the putative immune benefits of not removing tumor-draining lymph nodes in the W&W strategy.

METHODS

Study Design and Patients

This validation cohort included patients (n = 249) who had available initial biopsies of stage I-III rectal cancer, a cCR after nT, and who were managed by a W&W strategy between 1989 and 2020, in seven centers across six countries (Fig 1A; Table 1; Data Supplement, Fig S1 [online only]). Previously published clinical data from a multicenter cohort of 73 W&W patients (test cohort) with available IS_B were updated for this study.[20] The different combinations of nT are shown in Table 1, with long course CRT being the most commonly used regimen. Mean time between the end of nT and the response assessment varied from 7 to 10 weeks. Response to nT was assessed with digital rectal examination, endoscopy, and
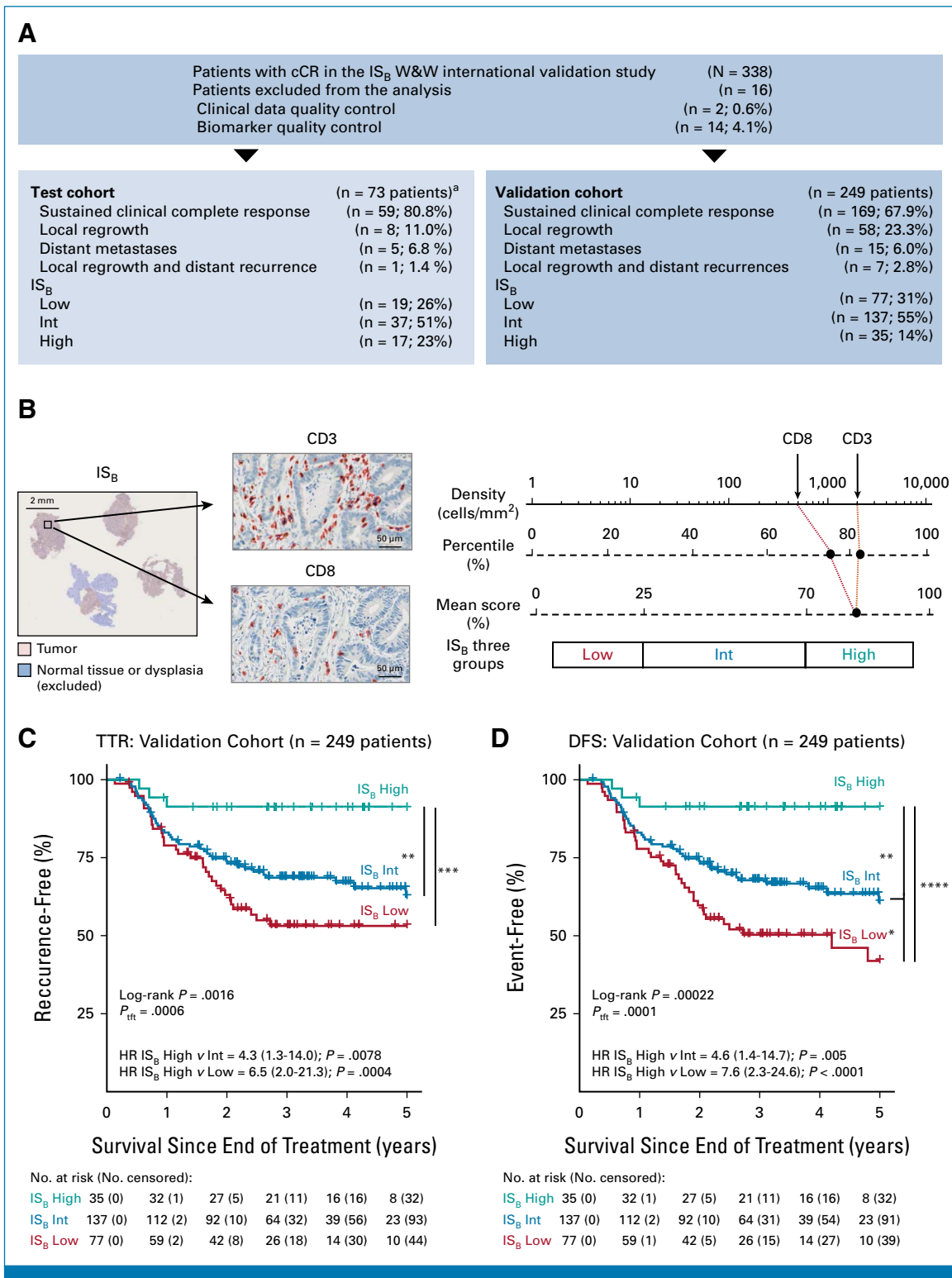
FIG 1. IS_B in patients with rectal cancer managed by the W&W strategy: study design, IS_B methodology, and prognostic value. (A) Flow chart of the IS_B multicenter study design (further details are provided in the Data Supplement). (B) Left: representative image of biopsy analysis; the tumor region is selected (pink), and normal tissue or dysplasia (blue) are excluded from the analysis. Middle: representative detection by the software of positive CD3+ and CD8+ T cells infiltrating the rectal tumor of the patient with IS_B High. Right: chart illustrating the IS_B calculation method. Densities of CD3+ and CD8+ T cells (cells/mm^2) in the tumor region are converted into percentile values using predefined cutoffs. The mean percentile of the two markers is calculated to generate IS_B mean score value, where IS_B Low, IS_B Int, and IS_B High subgroups are reflected by 0%-25%, >25% to 70%, and >70% to 100% percentile, respectively. (C) Time to recurrence and (D) DFS according to IS_B Low (red), IS_B Int (blue), and IS_B High (green) in W&W patients with cCR. Log-rank statistical test is stratified by the type of (continued on following page)

FIG 1. (Continued). neoadjuvant radiotherapy (standard without intensification, external intensification, or [contact] bra-chytherapy). [a]El Sissy et al.[20] *P < .05; **P < .01; ***P < .001; ****P < .0001. cCR, clinical complete response; DFS, disease-free survival; Int, Intermediate; IS$_B$, Immunoscore biopsy; P_{tft}, P test for trend; TTR, time to recurrence; W&W, watch-and-wait.

radiologic imaging modalities, detailed in the Data Supplement. Patients were categorized as clinical complete responders according to each center's modalities. Median follow-up of patients was 40.7 months (standard deviation [SD], 15 months). Local tumor regrowth was defined as any regrowth at the primary tumor site or in the regional lymph nodes. Evidence of distant metastasis was determined by computed tomography of the chest, abdomen, and pelvis. Recurrence was defined as either local tumor regrowth or the presence of distant metastasis. Seventeen additional patients with rectal cancer treated by long-course nCRT followed by surgery (ie, proctectomy with TME; nonin-clusion in W&W strategy) were further investigated. Nine patients were with cCR and eight with non-cCR (Data Supplement, Table S1). These nine patients with cCR had a very good pathologic response to nCRT (no or rare isolated tumor cells in the specimen). The immune status of draining lymph nodes from patients with cCR were de-termined and compared with that of patients with non-cCR. Ethical approval was obtained according to local authorities per participating institute.

Procedures

IS$_B$ was determined as previously described[20] in the coordi-nating center (Immunomonitoring Platform, Georges Pom-pidou European Hospital, Paris, France). Briefly, two biopsy sections of 4 μm of all available diagnostic biopsies were processed for CD3 and CD8 immunostainings. An experienced pathologist (C.L.) from the coordinating center reviewed the delimitation of the tumoral component and assessed the immunostaining quality for all cases, leading to exclusion of 14 cases. Stained cells were quantified using a previously vali-dated IS module of the image analysis software Developer XD (Definiens).[13] CD3+ and CD8+ densities were converted into percentiles using predefined cutoffs.[20] The mean percentiles were translated into IS$_B$ categories: IS$_B$ Low (0%-25%), IS$_B$ Intermediate ([Int] >25% to 70%), and IS$_B$ High (>70% to 100%; Fig 1B; Data Supplement) as established in the inter-national validation of the consensus IS.[12] IS$_B$ determination was performed blinded to the study end point.

To investigate lymph nodes, lymphatic vessels of the medulla, B lymphocytes, and proliferative cells were detected with antibodies against Lyve-1, CD20, and Ki67, respectively. The Tissue Classifier Module of Halo (Indica Labs, Albuquerque NM) was used to detect cortex area (CD20+), paracortex area (CD3+), and medulla area (Lyve1+). Multiplex immunofluo-rescence panel (CD20, CD27, CD38, DAPI) was used for B-cell memory detection and analyzed with HALO AI software.

For each patient, total RNA was isolated from 20-μm FFPE slices from highly (Ki67+) or weakly (Ki67−) proliferative

lymph nodes using the Allprep DNA/RNA FFPE kit (Qiagen Inc, Valencia, CA). PolyA-RNAseq libraries were prepared using the QuantSeq 3'mRNA-Seq Kit FWD for Illumina

TABLE 1. Characteristics of Participants

Characteristic	Validation Cohort (n = 249)
Sex, No. (%)	
Female	80 (32.1)
Male	169 (67.9)
Age, years, mean (SD)	66.9 (10.8)
Tumor location, No. (%)	
Lower rectal (0-5 cm)[a]	174 (69.9)
Mid rectal (5-10 cm)	65 (26.1)
Upper rectal (10-15 cm)	7 (2.8)
Not reported	3 (1.20)
Pretreatment tumor (cT) stage, No. (%)	
1	12 (4.8)
2	90 (36.1)
3	134 (53.8)
4	13 (5.2)
Pretreatment nodal (cN) status, No. (%)	
0	123 (49.4)
1	79 (31.7)
2	46 (18.5)
Not reported	1 (0.4)
Type of nRT, No. (%)	
Long/short course	134/2 (54.6)
External nRT intensification	75 (30.1)
(Contact) brachytherapy	38 (15.3)
Relapse site,[b] No. (%)	
Local	58 (23.3)
Distant	15 (6.0)
Local and distant	7 (2.8)
No relapse	169 (67.9)
5-year recurrence-free rate, % (95% CI)	63.7 (57.1 to 71.1)
5-year local recurrence-free rate, % (95% CI)	71.2 (65.2 to 77.6)
5-year overall survival rate, % (95% CI)	92.0 (87.3 to 96.8)
IS$_B$, No. (%)	
Low	77 (30.9)
Int	137 (55.0)
High	35 (14.1)

NOTE. Data are No. (%), unless otherwise specified. Some totals do not add up to 100% because of rounding.

Abbreviations: cN, N, regional lymph node, c, clinical; cT (T, primary tumor; c, clinical); Int, Intermediate; IS$_B$, Immunoscore biopsy; nRT, neoadjuvant radiotherapy treatment; SD, standard deviation.

[a]From anal verge.

[b]Events occurred during the first 5 years after the end of neoadjuvant treatment.

(Lexogen, Vienna, Austria). Further details of the procedures are provided in the Data Supplement.

Outcomes

The primary end point was to evaluate the prognostic value of IS_B for TTR, defined as time from the end of nT to the first occurrence of local regrowth or distant metastasis. Additional outcome of interest was DFS, defined as the time from the end of nT to the first observation of disease recurrence or death from any cause.

Statistical Analysis and Data Visualization

The associations between IS_B and clinical characteristics were assessed through ANOVA tests for continuous variable (age), chi-squared tests (χ^2; for sex, pretreatment cTNM and cN (N, regional lymph node; c, clinical) stages, and type of nRT), or Fisher tests (for tumor location, and cT [T, primary tumor; c, clinical] stage) of independence for categorical variables. Survival univariate analyses were performed using the log-rank test and the Cox proportional hazards model. The log-rank test for trend was performed to detect ordered differences in survival curves. The corrected Harrell's C-index[21] with $1,000\times$ bootstrap resampling was used to assess IS_B discriminatory ability, accounting for potential model overfitting. Calibration accuracy was evaluated for 5-year estimates from the Cox model, using adaptive linear spline hazard regression.

Multivariate survival analyses were performed with the Cox models. The relative importance of each parameter to survival risk was assessed by the χ^2 proportion (rms R package). The accuracy of models was evaluated by the integrated area under the ROC curve (iAUC) with $1,000\times$ bootstrap resampling. The performance of risk prediction models was compared using the likelihood ratio P value. Stratification by type of neoadjuvant radiotherapy was applied to the Cox models and log-rank tests. The confounded measures of association were log-rank, Wald, and likelihood ratio P values, and hazard ratios. The Wilcoxon-Mann-Whitney test was used in lymph nodes analysis. Two-sided P values < .05 were considered statistically significant. Statistical analyses and data visualizations were performed using the R software version 4.1.2 (R foundation, Vienna, Austria).

RESULTS

Patient Population Characteristics and IS_B Determination

A multicenter validation cohort of 249 patients was investigated, from seven centers across six countries (Data Supplement, Fig S1), with cCR after nCRT and managed with the W&W strategy. This independent validation cohort complements the study conducted on a test cohort of 73 W&W patients[20] (Fig 1A) with the aim to confirm the IS_B prognostic performance. Clinical and biological characteristics of patients are provided in Table 1 and the Data Supplement (Table S2). A

mean of four biopsies (SD, 2.7) per patient was assessed for IS_B determination (Fig 1B). Mean counts of CD3+ and CD8+ T cells were 1,251 and 281 cells/mm², respectively. IS_B Low, Int, and High were observed in 30.9%, 55.0%, and 14.1% of patients, respectively (Table 1), and were similar to that observed in the previously published test cohort. No significant association was observed between IS_B categories and clinical characteristics (Data Supplement, Table S3).

IS_B and Clinical Outcomes in Patients From the Validation Cohort

After a 5-year follow-up, 58 (23.3%) patients had local regrowth, 15 (6.0%) experienced distant metastasis, and 7 (2.8%) patients had both events (Table 1). Thirteen (5.2%) patients died. IS_B identified three populations with significantly different survival profiles for TTR in univariate analysis stratified by treatment (IS_B High v IS_B Int: unadjusted hazard ratio [HR], 4.3 [95% CI, 1.3 to 14.0]; IS_B High v IS_B Low: HR, 6.5 [95% CI, 2.0 to 21.3]; log-rank P = .0016; P test for trend (P_{tft}) = .0006; bootstrap C-index, 0.61; Fig 1C; Data Supplement, Table S4). Recurrence-free rates at 5 years were 91.3% (95% CI, 82.4 to 100.0), 62.5% (95% CI, 53.2 to 73.3), and 53.1% (95% CI, 42.4 to 66.5) for IS_B High, Int, and Low, respectively (Data Supplement, Table S4). Similar results were found for the predictive performance of IS_B for DFS (log-rank P = .0002, bootstrap C-index, 0.62; Fig 1D; Data Supplement, Table S5). The relative restricted mean survival time analysis evidenced significant differences in the survival months without recurrence gained according to IS_B categories (all P < .001; Data Supplement, Tables S4 and S5). In accordance, immune densities expressed as a continuous variable (mean score of percentiles for CD3+ and CD8+ densities) illustrated a progressive risk of recurrence with decreasing immune densities (Wald test P = .0017; Data Supplement, Fig S2 and Table S4).

The Cox multivariable analysis stratified by treatment for TTR and DFS (Data Supplement, Fig S3), adjusted for IS_B categories, age, sex, cT stage, and cN stage, showed that age, cT4, and IS_B were significantly associated with clinical outcomes (TTR: IS_B High v Int and v Low HR, 4.6 [95% CI, 1.4 to 15] and 6.9 [95% CI, 2.1 to 23.1], respectively; all P < .05; bootstrap C-index, 0.63; DFS: all P < .01; bootstrap C-index, 0.63). IS_B in three categories (the primary objective) was confirmed in the validation cohort as an independent prognostic factor for TTR in patients with cCR managed by W&W strategy.

Overall Clinical Performance of the IS_B in the Whole Cohort of Patients Managed by a W&W Strategy

To ensure a comprehensive assessment of the predictive performance of IS_B with increased statistical power and to allow for subgroup analyses, we pooled patients from both the test and validation cohorts (n = 322; Data Supplement, Fig S4). A calibration plot showed a good correlation of IS_B

prediction and actual observation for 5-year TTR (Data Supplement, Fig S5). Multivariable analysis confirmed the independent prognostic value of IS_B for TTR (IS_B High v Int and v Low HR, 4.3 [95% CI, 1.5 to 12.1] and HR, 6.7 [95% CI, 2.4 to 19.2], respectively; all $P < .01$; bootstrap C-index, 0.64; Fig 2) and for DFS (all $P < .005$; bootstrap C-index, 0.63; Data Supplement, Fig S3), together with age and cT4.

IS_B categorization further predicts both local regrowth and distant metastasis with a bootstrap C-index of 0.60 for each subgroup (Figs 3A and 3B). The HR between IS_B Low versus IS_B High for local regrowth and distant metastasis was 6.3 (95% CI, 1.9 to 20.7; log-rank $P_{tft} = .0004$) and 6.7 (95% CI, 0.87 to 51.7; log-rank $P_{tft} = .029$), respectively. Organ preservation rates at 3 years and 5 years also differed significantly between IS_B categories (IS_B High v Low, all $P < .01$; Fig 3C). The predictive accuracy for TTR of all parameters on the basis of iAUC with 1,000× bootstrap resampling showed that IS_B was superior to clinical parameters (Fig 3D). The relative contribution for TTR prediction of IS_B was 49% compared with 31% for age and 18% for cT stage. Importantly, the addition of IS_B to a model including all clinical variables significantly improved the prediction for TTR (likelihood ratio test; $P < .0001$; Fig 3D).

Immune Status of the Draining Lymph Nodes

The observed inverse correlation between IS_B and recurrence suggests the involvement of the immune response in the control of the disease after nT. We hypothesized that the drainage lymph nodes left in place by a W&W strategy are the site of immune education. The immune status of draining lymph nodes from nine patients with cCR (potentially eligible to W&W strategy) treated by proctectomy after nCRT were compared with eight patients with non-cCR, for a total of 161 lymph nodes. Lymph nodes from patients with cCR exhibited signs of activation, with an increase of proliferating lymphocytes (KI67+) in the cortex, paracortex, and medulla (all $P < .01$; Figs 4A and 4B), compared with patients with non-cCR. Differences were particularly pronounced in the cortex, the site of B lymphocyte development, and the medulla where lymphocytes circulate. In accordance, an increased expression of B-cell genes (18/30) and of memory B-cell densities (CD20+, CD27+, and CD38−) was detected in proliferative lymph nodes of patients with cCR (Figs 4C and 4D). A significantly higher density of proliferative B lymphocytes (CD20+ and Ki67+) was also detected in lymph nodes of patients with cCR compared with patients with non-cCR (Fig 4D). Thus, by using a unique cohort of patients with cCR taken to surgery and confirmed to have no or rare isolated tumor cells in the final resected specimen, we observed B-cell activation and increased proliferating lymphocytes in the draining lymph nodes.

DISCUSSION

In rectal cancer, the W&W strategy offers patients with cCR after nCRT the possibility of organ preservation. However,

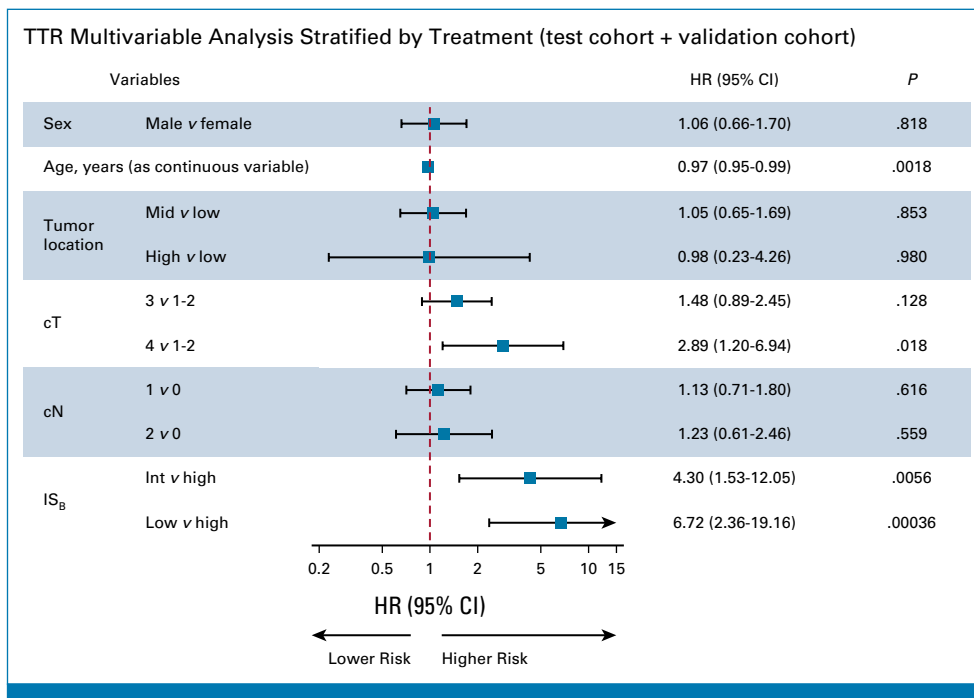**FIG 2.** Multivariable time to recurrence survival analysis in the test and validation cohorts (n = 317). The model is stratified by neoadjuvant radiotherapy type (standard without intensification, external intensification, or [contact] brachytherapy). c, clinical; HR, hazard ratio; Int, Intermediate; IS_B, Immunoscore biopsy; N, regional lymph node; T, primary tumor; TTR, time to recurrence.
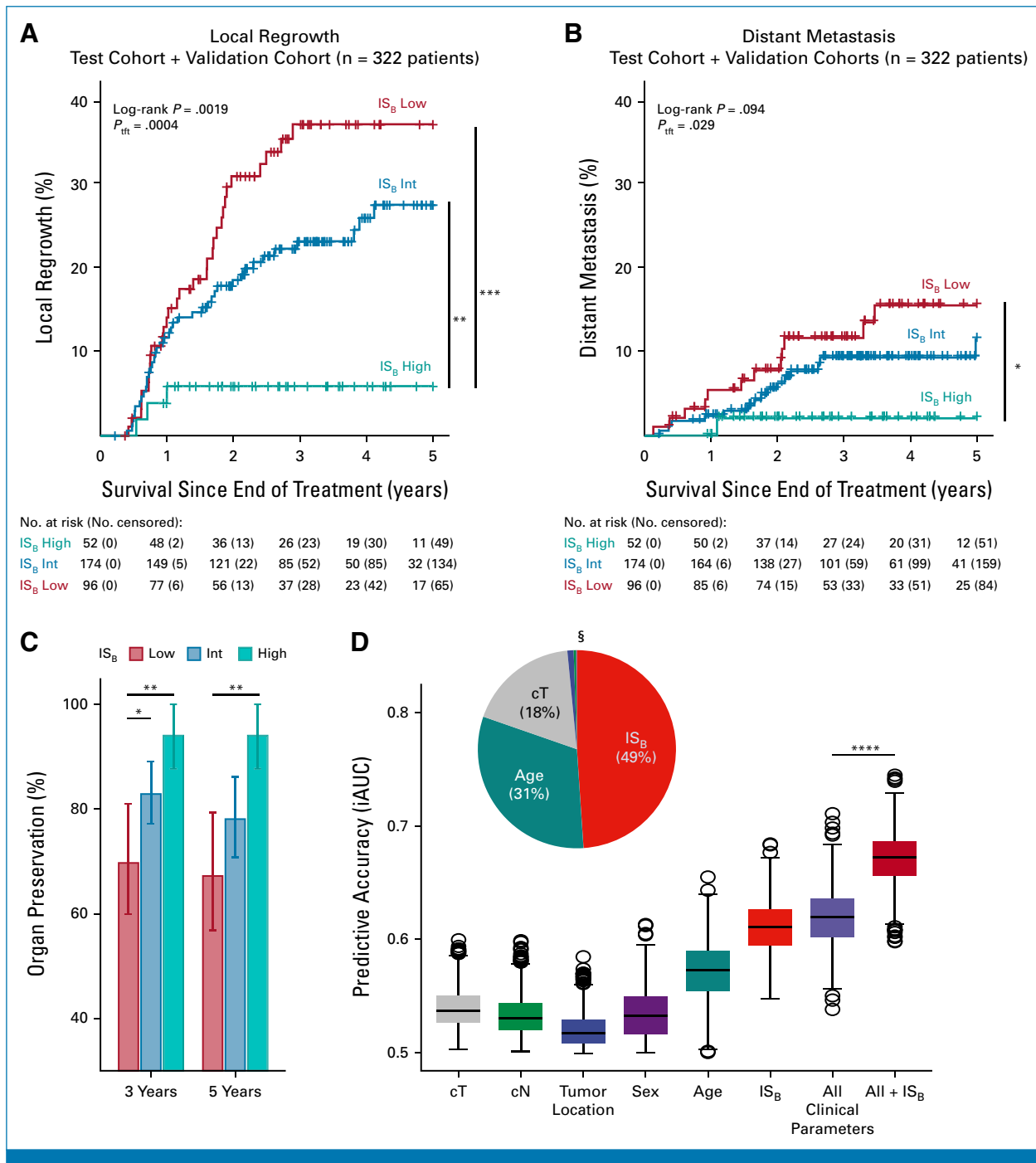
FIG 3. IS_B and clinical outcome. Comparison with clinicopathologic parameters. Cumulative incidence of (A) local regrowth and (B) distant metastasis in W&W patients from the test and the validation cohorts (n = 322 patients), according to IS_B categories. (C) The 3- and 5-year organ-preservation rates in W&W patients with cCR according to IS_B categories. (D) Box plot of the predictive accuracy for TTR on the basis of the incremental area under the curve with 1,000× bootstrap resampling for each parameter. The log likelihood ratio test between clinical model and clinical model plus IS_B is shown. The relative importance of each risk parameter to recurrence risk using the χ^2 proportion test is shown in the pie chart. §cN, tumor location, and sex represent <2% of the relative contribution. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$. c, clinical; cCR, clinical complete response; iAUC, integrated area under the ROC curve; Int, Intermediate; IS_B, immunoscore biopsy; N, regional lymph node; P_{tft}, P test for trend; T, primary tumor; TTR, time to recurrence; W&W, watch-and-wait.

25% of these patients will eventually relapse,[8] partly because of the limitations of the consensus criteria used to anticipate pCR at the tumor site[22] and assess residual lymph node involvement.[23] In addition, even pCR patients (5%–15%) can experience local recurrence or distant metastasis.[24] Thus, W&W is proposed for patients who potentially harbor tumoral islands not visible by standard clinical means at the primary tumor site and/or distant subclinical metastasis.
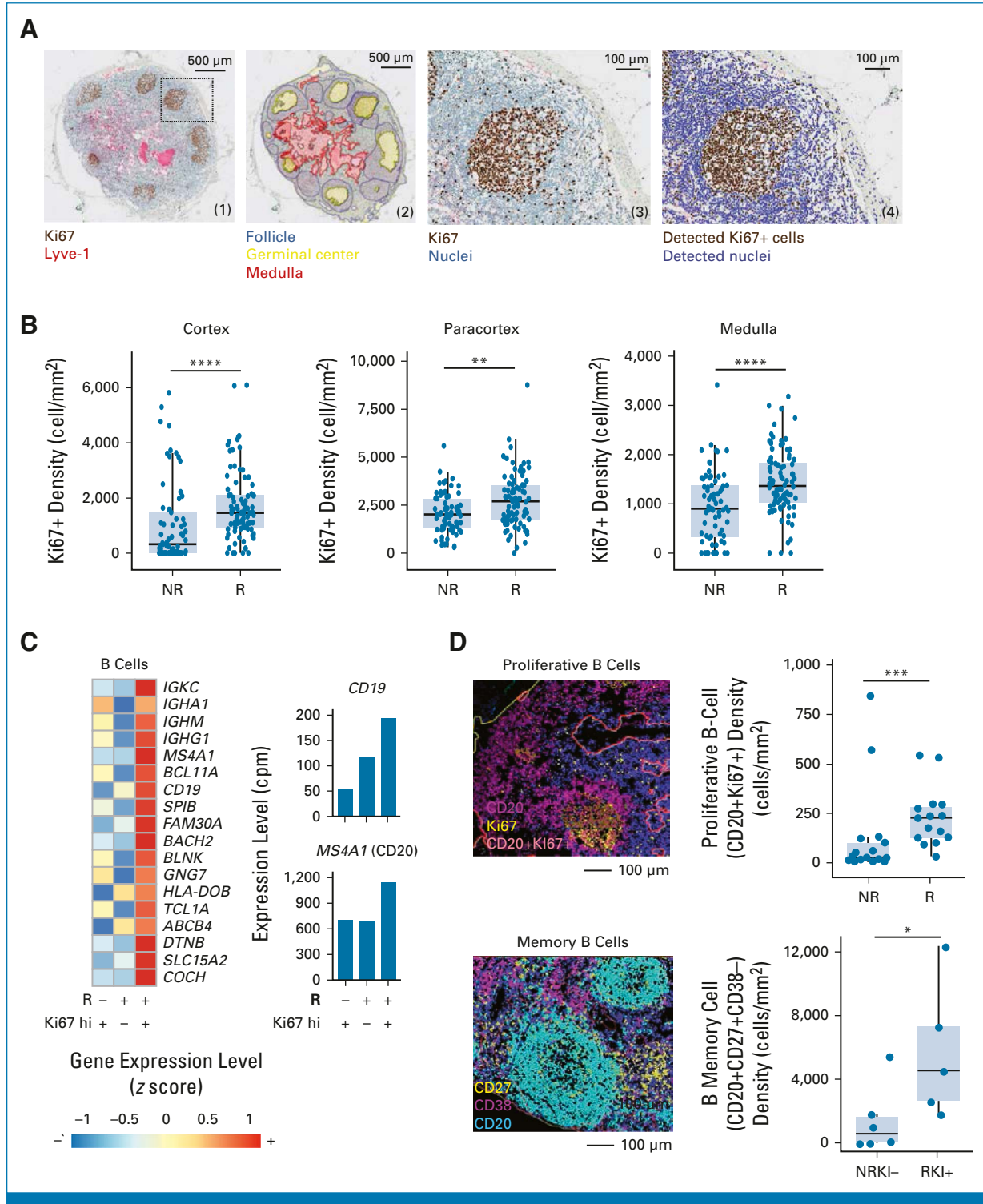
FIG 4. Immune status of the draining lymph nodes of rectal cancer patients with or without cCR to neoadjuvant chemoradiotherapy. Illustrations from the left to the right of (1) double immunostaining of Ki-67 (brown) and Lyve-1 (red) in lymph nodes, (2) recognition of regions of interests: follicle (blue), germinal center (yellow), and medulla (red) by the software (Classifier module, Halo), (3) focus on a follicle with Ki-67+ cells, and (4) detection of nuclei (blue) and Ki-67+ cells (brown). (B) Ki67+ cell densities in lymph node regions (cortex, paracortex, and medulla) of cCR (R) or non-cCR (NR) to neoadjuvant chemoradiotherapy. (C) Heatmaps of genes associated with B cells according to proliferative (Ki67) status of lymph nodes and response status (R) of the patients to nCRT. Gene expression level is represented as a color gradient from low (blue) to high (red) intensity. Only overexpressed genes among pathologic complete response proliferative (Ki67 hi) lymph nodes in responders are shown (18/30 expressed genes). (D) Upper left: immunofluorescence staining for proliferative B-cell panel (Ki67 in yellow, CD20 in pink, and double positive in orange) in a draining lymph node. Lower left: immunofluorescence staining for memory B-cell panel (CD27 in yellow, (continued on following page)

FIG 4. (Continued). CD38 in pink, and CD20 in blue) in a draining lymph node. Upper right: proliferative B-cell densities in lymph nodes of responders compared with nonresponders. Lower right: memory B-cell densities in lymph nodes of responders Ki67 high compared with control (nonresponders Ki67 low). *P < .05; **P < .01; ***P < .001; ****P < .0001. cCR, clinical complete response; cpm, read counts per million of reads; nCRT, neoadjuvant chemoradiotherapy; NR, nonresponders; R, responders.

A large spectrum of clinical and histopathologic features, molecular markers, and tumor environment−derived factors have been shown to predict pCR.[25] However, their clinical utility remains unclear as their validation in independent cohorts is lacking. Furthermore, except IS_B,[20] the prognostic performance of these biomarkers in W&W patients have yet not been tested. Only baseline cT stage[26,27] and total radiotherapy dose[27] could predict local regrowth during the first year of follow-up.[27] The results from our previous study showed that IS_B determination improves pCR prediction and could provide a prognostic biomarker in W&W patients.[20] This study confirms through a multicenter validation cohort that IS_B in three categories (the primary objective) is an independent parameter predicting TTR. The 5-year risk of recurrence for IS_B High patients was almost null, with adverse events occurring only during the first year after nCRT. Conversely, IS_B Low patients had an almost 50% risk for recurrence with events occurring during the 5 years after nCRT.

Refining patient selection and lowering local regrowth rates would make the W&W approach more generalizable.[9] IS_B could fulfill these two objectives by providing a good risk scale for local regrowth, allowing adaptation of monitoring and/or complementary treatments. Shared decision making in cancer treatment, especially in rectal cancer with organ preservation, is likely to play an increasing role.[28] IS_B could be a decision aid to help the physician and the patient make an informed choice by providing an improved accuracy of relapse probability, hence improving decision quality. Alternative proposals could be considered for patients with a high risk of recurrence, for example, (1) noninclusion in the W&W strategy, (2) local tumor resection,[29] (3) intensification of the monitoring,[30] (4) consolidation chemotherapy,[6] (5) and, in a close future, immune modulation. IS_B could serve as a companion biomarker for clinical trials assessing the benefits of adjusting the monitoring and/or the therapeutic strategy in patient groups with different prognoses.

From a fundamental perspective, high quality of in situ immune infiltration before treatment could promote an immediate response to nCRT and decrease recurrences by inducing immune surveillance. Interestingly, we have now observed signs of additional B lymphocyte activation in the draining lymph nodes of patients with cCR. Although hypothetical and speculative, these findings raise the possibility that the preserved lymph nodes in the W&W strategy may not only be considered as a risk factor for local recurrence, but also as a relevant immune site. If further confirmed by a large-scale study, this would provide an unexpected additional argument in favor of the organ-preservative strategy.

Our work has some limitations. First, IS was determined on biopsies with heterogeneity pitfalls. However, we previously observed that two fields of 1 mm² were sufficient to obtain a reproducible prediction of recurrence.[13] Herein, a mean of 14 mm² of tumor area was investigated per patient. Second, heterogeneity of treatment and follow-up modalities was observed among different centers across countries. Such an approach, however, illustrates the prognostic performance of IS_B in real-life clinical practice. No patient treated by TNT was included. IS_B should also be tested in this promising strategy. Third, the proficient or deficient mismatch repair gene expression (pMMR or dMMR) status was not available. Approximately 5% of rectal adenocarcinomas are dMMR. These patients will most likely be IS_B High, given the high frequency of neoantigens expressed by such tumors. A recent study evidenced an impressive cCR rate of 100% for all of 12 patients with dMMR LARC after PD-1 blockade therapy.[18] It would be interesting to evaluate whether pMMR IS_B High patients could equally benefit from this immunotherapy. Notably, the NICHE study[31] showed 100% (32/32) and 30% (9/30) pathologic responses in dMMR and pMMR early-stage colon cancers, respectively, treated by neoadjuvant PD-1 and CTLA-4 blockade. Preexisting CD8+PD-1+ T lymphocyte density predicted response to immune checkpoint inhibitor therapy.

In summary, the data from this large international cohort of rectal cancer patients with cCR managed nonoperatively validate the prognostic value of IS_B and could pave the way for prospective therapeutic trials guided by IS_B to adjust monitoring and/or therapy of W&W patients.

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DATA SHARING STATEMENT

The study protocol is included in the Data Supplement. Clinical data collected for the study and IS_B determined for each patient are provided in the Data Supplement. The information on sex and age is available upon request.

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International Validation of the Immunoscore Biopsy in Patients With Rectal Cancer Managed by a Watch-and-Wait Strategy

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