A Diagnostic Biopsy-Adapted Immunoscore Predicts Response to Neoadjuvant Treatment and Selects Patients with Rectal Cancer Eligible for a Watch-and-Wait Strategy

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Purpose: No biomarker to personalize treatment in locally advanced rectal cancer (LARC) is currently available. We assessed in LARC whether a diagnostic biopsy-adapted immunoscore (ISb) could predict response to neoadjuvant treatment (nT) and better define patients eligible to an organ preservation strategy ("Watch-and-Wait").

Experimental Design: Biopsies from two independent cohorts (n1 = 131, n2 = 118) of patients with LARC treated with nT followed by radical surgery were immunostained for CD3+ and CD8+ T cells and quantified by digital pathology to determine ISb. The expression of immune-related genes post-nT was investigated (n = 64 patients). Results were correlated with response to nT and disease-free survival (DFS). The ISb prognostic performance was further assessed in a multicentric cohort (n = 73 patients) treated by Watch-and-Wait.

Introduction
Colorectal cancer is the third most common cancer in the world with an increasing incidence especially in younger adults (1). In locally advanced rectal cancer (LARC), neoadjuvant chemoradiotherapy (nCRT) followed by radical surgery is recommended by international guidelines (2, 3). Tumor recurrence and patient survival are strongly influenced by the quality of response to neoadjuvant treatment (nT); refs. 4-6). Recent advances in the management of patients with LARC have shown that it could be conceivable to avoid amputation of the rectum (preservation strategy; e.g., Watch-and-Wait) in patients with clinical and imaging features compatible with a complete response to nT (7, 8). These patients experience acceptable outcomes; however, about 25% of them develop early tumor regrowth (9). There are currently no molecular markers to predict responses to nCRT and guide treatment decisions (3), such as optimization or modification of nT in nonresponding patients, and better selection of patients eligible to preservative strategy.

Ionizing radiation has the capacity to prime/reinforce an adaptive T-cell–mediated immune response, which acts in the mechanisms of local tumor regression and of distant tumor inhibition and rejection.

Results: ISb positively correlated with the degree of histologic response (P < 0.001) and gene expression levels for Th1 orientation and cytotoxic immune response, post-nT (P = 0.006). ISb high identified patients at lower risk of relapse or death compared with ISb low [HR, 0.21; 95% confidence interval (CI), 0.06–0.78; P = 0.009]. Prognostic performance of ISb for DFS was confirmed in a validation cohort. ISb was an independent parameter, more informative than pre- (P < 0.001) and post-nT (P < 0.05) imaging to predict DFS. ISb combined with imaging post-nT discriminated very good responders that could benefit from organ preservation strategy. In the "Watch-and-Wait" cohort (n = 73), no relapse was observed in patients with ISb high (23.3%).

Conclusions: ISb predicts response to nT and survival in patients with LARC treated by surgery. Its usefulness in the selection of patients eligible for a Watch-and-Wait strategy is strongly suggested.
Translational Relevance

Biopsy-adapted immunoscore (ISb) performed on rectal cancer biopsy samples is an adaptation of the standardized IS performed on the surgical specimen in colon cancer. First, ISb provides a strong and independent prognostic factor for disease-free survival of patients with rectal cancer. Secondly, ISb predicts the response to neoadjuvant chemoradiotherapy (nCRT). Lastly, ISb combined with imaging post-nCRT discriminates the group of patients with a complete histologic response to nCRT (no residual tumor) that should benefit from less invasive therapeutic strategies (i.e., Watch-and-Wait or minimally invasive surgery), avoiding a disabling and useless rectal amputation surgery.

(i.e., the abscopal effect; refs. 10–12). This suggests that the quality and intensity of the natural immune reaction at tumor site before nT could influence the magnitude of response to nT and provide a predictive marker of response. Natural immune reaction at tumor site has been further associated with a favorable prognosis in various cancers (13), including colorectal cancer, treated by surgery alone (14, 15). Recent advances in digital pathology and image analysis have allowed a translation of immune assessment into a clinically relevant application (16). Using these technologies, the first standardized immunobased assay for colon cancer called “Immunoscore” (IS; i.e., the combination of CD3+ and CD8+ T-cell densities in the tumor and its invasive margin) has been developed. Its robustness and prognostic performance in stage I–III colon cancer have been consolidated through an international validation study (17). Thus, IS provides a reliable evaluation of the natural immune reaction at tumor site.

Preliminary studies in rectal cancer have suggested that the natural immune reaction of tumors could be evaluated on biopsies (18–20), the only sample material available before treatment. A derivation of the IS performed in initial biopsies (ISb) before nT has the benefit of evaluating the quality of the initial immune response in the tumor and its potential influence on both the degree of response to nT and clinical outcome.

The aim of this study was to determine whether the ISb correlates with response to nT, the in situ immune status after nT, and clinical outcome. We finally tested whether ISb could help to better select the subgroup of patients eligible to a preservative strategy, with acceptable outcome.

Materials and Methods

Patient population

Two retrospective consecutive cohorts of patients with LARC (n1 = 131, n2 = 118) with available biopsies, treated by nT and radical surgery by total mesorectal excision (TME), were analyzed. Cohort 1 was a monocentric cohort, and cohort 2 was multicentric (Supplementary Table S1 and Supplementary Fig. S1). Inclusion period ranged from 1999 to 2016. nT and surgery criteria were defined by each institution. Overall, 64.2% of patients were male, and the median age at diagnosis was 65 years [interquartile range (IQR), 53.3–74.1]. Patients were treated by nT [short (3.7%) or long (96.3%) course of radiation; 5-fluorouracil–based chemotherapy (82%); 18% did not receive chemotherapy]. According to the clinical TNM (cTNM) classification (UICC TNM 8th edition), rectal tumors were classified as cTNM I (1.2%), II (27.3%), and III (71.5%) based on baseline staging information provided by pelvic magnetic resonance and chest/abdominal computed tomography imaging. An additional cohort of patients (n = 73) with a complete/nearly complete response to nT (yCTNM 0–1), followed by a Watch-and-Wait strategy, was analyzed (Supplementary Table S2). A flow chart illustrating the studied population in each part of the investigations is provided in Supplementary Fig. S2. The median duration of follow-up for disease-free survival (DFS) of the cohort 1–2 was 45.4 months (IQR, 25.7–65.6). Duration of follow-up of each cohort for DFS, time to recurrence (TTR), and overall survival (OS) with the number of events is provided in Supplementary Table S3. The study was performed in accordance with the Declaration of Helsinki. Approval of all the centers’ Institutional Review Board committee was obtained for the study. Signed-informed consents from patients were obtained in each center.

Clinical outcomes

Patients were compared according to the degree of tumoral response to nT, using different tumor regression grade scoring systems: (i) the Dworak classification (21) defined as complete (Dworak 4), near complete (Dworak 3), moderate (Dworak 2), and no regression (Dworak 0); (ii) the neoadjuvant rectal (NAR) score classification (5), calculated using the equation [SpN-3(cT-pT) + 12]/29.61, and classified as low (<8), intermediate (8–16), and high (>16); (iii) the ypTNM stage, i.e., the postsurgical pathologic T and N evaluation; and (iv) downstaging of the tumor (4), defined as complete (ypT0N0), intermediate (ypT1–2N0), or weak/absent (ypT3–4 or N+). For patients who underwent surgery, the events were local, systemic recurrences and death from the date of surgery for DFS, recurrences for TTR, and death from any cause for OS. All patients who were managed with the Watch-and-Wait strategy were considered to have clinical complete response (yCTNM0) and were offered a strict surveillance protocol.

Immunohistochecmistry

Initial biopsies of all patients performed for diagnosis purpose were retrieved from all centers. Two formalin-fixed paraffin-embedded (FFPE) tumor tissue sections of 4 μm were processed for IHC with antibodies against CD3+ (2GV6, 0.4 μg/mL; Ventana) and CD8+ (CB11/44B, 3 μg/mL; Dako) according to the previously described protocol (17) revealed with the Ultraview Universal DAB IHC Detection Kit (Ventana) and counterstained with Mayer’s hematoxylin.

ISb determination

Digital images of stained tissue sections were obtained with a 20× magnification and a resolution of 0.45 μm/pixel (Nanoworries HT). Delimitation of the tumor component excluding normal tissue and low/high-grade dysplasia-associated lesions was performed by an experienced pathologist (C. Lagorce). The mean densities of CD3+ and CD8+ T cells in the tumor region were determined with a dedicated IS module of the Developer XD image analysis software (Definiens; Fig. 1A). The mean and distribution of the staining intensities were monitored providing an internal staining quality control. A final quality check was performed to remove nonspecific staining detected by the software. Determination of ISb was directly derived from the methodology used to determine the Immunoscore® (HalioDx, France) in the international validation cohort of IS in colon cancers which have shown a strong interobserver reproducibility (17). CD3+ and CD8+ T-cell densities in the tumoral region of each patient were compared with that obtained for the whole cohort of patient and converted accordingly into percentile. Then, the mean of the two percentiles (CD3 and CD8) was translated into one of the three ISb categories (Fig. 1B): ISb low (0%–25%), ISb intermediate (25%–70%),...
Figure 1.
A, Left: Representative image of tumor region (pink) with normal tissue or dysplasia (blue) excluded from the analysis. Left-mid: The tumor region divided into tiles for the analysis. Right-mid: Histogram of positive cells staining intensities detected by the software in a case of adequate immunostaining intensity. Red triangle defines mean case staining intensity of 220. Right: CD3⁺ T-cell (red) detection in a tile (magnification, ×200).
B, Chart illustrating the ISB calculation method. Densities of CD3⁺ and CD8⁺ T cells in the tumor region are converted into percentile values. The mean percentile of two markers is calculated to generate IS percentile value, where ISB low, ISB intermediate, and ISB high subgroups are reflected by 0%–25%, >25%–70%, and >70%–100% percentile, respectively.
C, Representative images of positive CD3⁺ and CD8⁺ T cells (cells/mm²) infiltrating the rectal tumor in patients with ISB low and ISB high.
D–E, DFS according to ISB low (red), ISB intermediate (orange), and ISB high (blue) in patients who met the biomarker and clinical data quality control evaluation in two independent cohorts (n₁ = 124 and n₂ = 114). P₁ = 0.012
P₁(high vs. low) = 0.009
HR(high vs. low) = 0.21 (95% CI, 0.06–0.78)
P₂ = 0.021
P₂(high vs. low) = 0.019
HR(high vs. low) = 0.25 (95% CI, 0.06–0.78)
and ISB high (>70%–100%). The ISB determination was performed blinded to the study endpoint.

RNA extraction and transcriptomic analysis by NanoString technology

Total RNA from 20-μm FFPE tumor tissue sections from all patients for which both biopsies and the corresponding surgical specimen post-nT was available (cohorts 1 and 2; n = 62) and from patients with colorectal cancer not treated with nT (n = 13) was isolated using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion Thermo Fisher). Distribution of tumor extension T and N stages among patients with or without nT did not display any statistical difference. The quality and quantity of the isolated RNA were measured using Agilent RNA 6000 Nano kit (Agilent Technologies) and NanoDrop 2000 (Thermo Fisher Scientific), and 100–400 ng RNA of each sample was processed using an in-house panel of 44 immune-related genes (Nanostring Technologies; Supplementary Table S4). Reporter-capture probe pairs were hybridized, and the probe/target complexes were immobilized and counted on the nCounter analyzer. Background subtraction was applied to raw data, and normalization based on the geometric mean of positive control and internal housekeeping genes (GUSB, SP2) was performed using the nSolver Analysis software, version 2.5.

Statistical analysis and data visualization

Statistical analyses and data visualizations were performed using the R software version 3.5.1 with the add-on survival, survminer, ggpubr, ggplot2, rms, and coin packages. The associations between ISB and clinical characteristics were assessed through the $\chi^2$ or Fisher tests of independence. Association level between CD3$^+$ and CD8$^+$ cell densities was measured by Pearson’s correlation coefficient $r$ and related $P$ value. Survival univariate analyses were performed using the log-rank test and the Cox proportional hazards model. Survival curves were estimated by the Kaplan–Meier method. The log-rank test for trend from the survminer package was performed to detect ordered differences in survival curves. Multivariate survival analyses were performed with Cox proportional hazards model to test the simultaneous influence of all covariates. The proportional hazards assumption for each covariate was tested using the cox.zph function. The relative importance of each parameter to survival risk was assessed by the $\chi^2$ from Harrell’s rms R package. The association between ISB and nT ordinal response level was assessed using a unilaterial linear-by-linear association test. The associations between nT response levels, CD3$^+$ T-cell densities, CD8$^+$ T-cell densities, and gene intensities were assessed by Kendall’s correlation test, $T$ test, and Mann–Whitney $U$ test. The Wilcoxon test adjusted to control FDR by using the Benjamini and Hochberg procedure was used to test treatment response level in transcriptional analysis. The ycTNM staging and ISB were included in the TTR (Supplementary Fig. S3A). The CD3$^+$/CD8$^+$ T-cell ratio was highly variable among patients, with a coefficient of determination ($r^2$) between both markers of 0.58 (Supplementary Fig. S3B). ISB was derived from the CD3$^+$ and CD8$^+$ T-cell densities (Fig. 1B). CD3 and CD8 densities in the tumor were converted into percentiles referring to the densities observed in all patients. ISB mean percentile of CD3 and CD8 was calculated for each biopsy (ISB mean score). No statistical difference for the mean score was observed between the two cohorts [cohort 1: 51.9 ± 29.1, cohort 2: 48.8 ± 23.4 (mean ± SD); $P = 0.36$]. After converting the mean score into ISB scoring system, overall 22.7%, 52.5%, and 24.8% of patients had ISB low, intermediate, and high, respectively. Of note, ISB intermediate category was more represented in cohort 2 (61.9%), as compared with cohort 1 (43.5%). Representative tumor biopsies from patients with ISB low and ISB high are shown in Fig. 1C.

ISB-associated prognostic value

Distribution analysis of ISB did not display any association with age, sex, or tumor location (Supplementary Table S1). The magnitude and reproducibility of the ISB prognostic performance were tested in two independent cohorts. In cohort 1 (n = 131), a significant difference in DFS between patients stratified by ISB was observed [P test for trend (P trend) = 0.012; HR(High vs. Low) = 0.21; 95% confidence interval (CI), 0.06–0.78 and illustrated by Kaplan–Meier curves, Fig. 1D]. Patients with ISB high were at low risk of relapse, with the 5-year DFS of 91.1% (95% CI, 82.0–100) versus 65.8% (95% CI, 49.8–86.9) in patients with ISB low. These results were confirmed in second independent cohort [n = 118; P = 0.021; HR(High vs. Low) = 0.25; 95% CI, 0.07–0.86; Fig. 1E]. Identical results were obtained when removing the 3 patients with UICC-TNM stage I tumors (Supplementary Fig. S4). In pooled analysis (n = 249), a significant difference between patient’s groups stratified by ISB was evidenced by univariate analysis (Supplementary Table S5) illustrated by Kaplan–Meier curves for TTR (P < 0.001), DFS (P < 0.005), and OS (P = 0.04; Supplementary Fig. S5).

ISB and response to neoadjuvant treatment

We investigated whether the prognostic value associated to ISB was at least partly a consequence of a relationship between ISB and the quality of the nT response. The quality of response to nT was assessed 6 to 8 weeks after nT by imaging (ycTNM) and microscopic examination of the resected tumor, by the Dworak classification, a tumor regression grading system, ypTNM, downstaging, and the NAR score. In our cohorts (n = 249 patients), high CD3$^+$ and CD8$^+$ T-cell densities were significantly associated with a good response to nT evaluated by both Dworak classification and ypTNM staging (all P < 0.005; Fig. 2A). The mean of CD3$^+$ and CD8$^+$ percentiles (ISB mean score) were correlated with the NAR score, Dworak classification, and ypTNM staging (Fig. 2B). The ISB level and distribution were positively correlated with tumor response to nT (Fig. 2C). ISB high patients were not found in the nonresponder Dworak 0 group, and 52.9% of patients with undetectable tumor cells (i.e., the Dworak 4 group) were ISB high (P = 0.0006). The same correlation was observed with the ypTNM, tumor downstaging, and NAR (Fig. 2C). Good responders to nT were 6 times more frequent in the ISB high group than in the ISB low group according to the NAR scoring system (Supplementary Fig. S6). Immune consequences of nT were then investigated on...
post-nT tumor samples (Dworak 0–4; \( n = 62 \)) by analyzing 44 immune-related genes (Supplementary Table S4). Gene expression levels were highly variable among patients (Supplementary Fig. S7). Unsupervised hierarchical clustering showed that 30.6% (\( n = 19 \)) of patients presented with signs of local immune activation after nT (Fig. 3A). The immune activation status after nT was positively correlated with the densities of CD3\(^{+}\) and CD8\(^{+}\) T cells (i.e., ISB) before treatment (Fig. 3B; \( P = 0.006 \)). Nonresponder tumors (Dworak 0–1) presented a similarly low level of immune-related genes expression compared with tumors not treated by nT (Fig. 3A). Patients with a partial/complete response to nT had a significantly higher expression of genes associated with adaptive immunity (CD3D, CD3E, CD3Z, and CD8A), Th1 orientation (TBX21/Tbet and STAT4), activation (CD69), cytotoxicity (GZMA, GZMH, GZMK, and PRF1), immune checkpoints (CTLA-4 and LAG3), and chemokines (CCL2, CCL5, and CX3CL1), as compared with nonresponders to nT (Fig. 3C). This suggests a link between the quality of the natural adaptive cytotoxic immune response (ISB), the presence of a post-nT immune activation, and the degree of response to nT. Gene expression data analysis through a PCA visualization further reinforced the putative link existing between the response to nT and the immune environment by showing distinct patterns of gene expression depending on the degree of response to nT (Fig. 3D). The combination of the second and third dimensions was the most accurate to discriminate responders/nonresponder patients.

ISB, a biomarker to optimize patient care

We investigated whether the ISB could provide valuable prognostic information when combined with clinical and pathological criteria available: (i) before nT [i.e., initial imaging, cTNM (UICC TNM 8th edition)], (ii) after nT (i.e., imaging post-nT, ycTNM), and (iii) after surgery (pathologic examination, ypTNM). In Cox multivariate analysis, ISB was a stronger predictive marker of DFS than other clinicopathologic parameters including cTNM (ISB high vs. ISB low: HR, 0.2; \( P < 0.001 \)) and ycTNM (ISB high vs. ISB low: HR, 0.25; \( P = 0.039 \)). ISB further remained a significant independent parameter associated with DFS when combined to ypTNM (Table 1). The important contribution of ISB for the prediction of the relapse and/or death compared with other clinical parameters is illustrated in Fig. 4A. It is known that the accuracy of the complete response post-nT defined by imaging is imperfect. Only 25% to 50% of clinical complete responders have no residual tumor (i.e., complete histologic response; refs. 22–24). ISB combined to imaging post-nT (ycTNM) increased the accuracy of prediction of histologic good responders (ypTNM 0–1) as compared with ycTNM alone (Fig. 4B). Three out of 32 patients with good response to nT (ycTNM = 0–1, \( n = 32 \)) experienced distant relapses, and no local relapse was observed. Importantly, no relapse was observed in ISB high patients (Fig. 4C). Thus, ISB could help to select patients who could achieve a very favorable outcome and be eligible to a Watch-and-Wait strategy.

Figure 2. A, Bar charts represent means ± SEMs of CD3\(^{+}\) and CD8\(^{+}\) T-cell densities in the tumor biopsies before nT according to the subsequent response evaluated by the Dworak score and ypTNM classification (\( * \), \( P < 0.05 \) and \( ** \), \( P < 0.01 \)). \( P \) obtained for the Kendall’s correlation test. B, Variation in mean percentile of CD3\(^{+}\) and CD8\(^{+}\) T-cell densities depending on the degree of tumor regression, as evaluated by the Dworak score, pTNM classification, and NAR score. C, The frequency of ISB low (red), ISB intermediate (orange), and ISB high (blue) according to tumor regression. Significant differences for the ISB distribution are observed with a unilateral linear-by-linear association test.
ISB in patients managed with Watch-and-Wait strategy

In a series of patients treated by the Watch-and-Wait strategy \( (n = 73) \), we retrieved the initial diagnostic biopsies to evaluate the ISB and the associated clinical outcome. Overall, 23\%, 51\%, and 26\% were classified as ISB high, ISB intermediate, and ISB low, respectively. Time to relapse was significantly different among patients stratified for ISB \( [P_{\text{High vs. Low}} = 0.025; \text{Fig. 5A}] \). No evidence of relapse was noticed during the follow-up period in ISB high patients.
Under the Cox proportional hazards regression model, the 5-year probability of survival without recurrence ranged from 46% to 89% according to ISB mean score (Fig. 5B). In Cox multivariable analysis, ISB was related to the patient’s TTR, independent of age, tumor location, and the cTNM classification (UICC TNM 8th edition; \( P = 0.04 \); Supplementary Table S6). The relative contribution of ISB to the prediction of occurrence of disease relapse is illustrated in Fig. 5C.

### Table 1. Multivariate Cox models for DFS according to ISB combined with available clinical parameters.

<table>
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<tr>
<th>Characteristics</th>
<th>Before neoadjuvant treatment</th>
<th>After neoadjuvant treatment</th>
<th>After surgery</th>
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<tr>
<td></td>
<td>PHA test</td>
<td>HR (95% CI)</td>
<td>P value</td>
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<td>Age</td>
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<td>Under vs. over 65 years</td>
<td>0.922</td>
<td>1.38 (0.85–2.24)</td>
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<td>Tumor location</td>
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<tr>
<td>Middle vs. inferior</td>
<td>0.433</td>
<td>1.1 (0.67–1.8)</td>
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<tr>
<td>Superior vs. inferior</td>
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<td>0.66 (0.26–1.69)</td>
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<td>Sex</td>
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<td>Male vs. female</td>
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<td>1.54 (0.9–2.63)</td>
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<td>Immunoscore (ISB)</td>
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<td>Intermediate vs. low</td>
<td>0.476</td>
<td>0.65 (0.38–1.1)</td>
<td>0.111</td>
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<td>High vs. low</td>
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<td>0.2 (0.08–0.49)</td>
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<tr>
<td>III vs. I–II</td>
<td>0.59</td>
<td>1.18 (0.68–2.04)</td>
<td>0.56</td>
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<td>ycTNM stage</td>
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Note: Significant P values (\(< 0.05\)) with the Wald test are written in bold.

Abbreviation: PHA, proportional hazards assumption.

*The significance of the multivariate Cox regression model was evaluated with the Wald test.

Under the Cox proportional hazards regression model, the 5-year probability of survival without recurrence ranged from 46% to 89% according to ISB mean score (Fig. 5B). In Cox multivariable analysis, ISB was related to the patient’s TTR, independent of age, tumor location, and the cTNM classification (UICC TNM 8th edition; \( P = 0.04 \); Supplementary Table S6). The relative contribution of ISB to the prediction of occurrence of disease relapse is illustrated in Fig. 5C.
Discussion

This work highlights the links between: (i) the quality of natural intratumor immunity evaluated by the ISB, (ii) the intensity of in situ immune reaction post-nT, (iii) the extent of the tumor regression post-nT, and (iv) the clinical impact in terms of prevention of tumor recurrence and survival. From a clinical point of view, ISB provides a reliable estimate of both the quality of response after nT and of the risk of recurrence and death in patients with LARC. ISB combined with imaging could further identify patients with a complete clinical response who can benefit from a close surveillance strategy post-nT, thus avoiding a disabling and useless rectal amputation surgery. ISB can be performed on routine diagnosis biopsies without any additional medical procedure. The rigorous and standardized quantification of immune cell infiltrates was achieved as for the IS colon study (17).

In the current study, ISB was positively and significantly correlated with tumor response to nT. This observation is consistent with our previous preliminary result (18) and with studies using an optical semiquantitative evaluation of immune cell infiltrates (19, 20, 25). In the ISB low group (22.7% of the cohort), only 5% of patients experienced complete response (low NAR score), suggesting that an optimization or modification of nT such as adjunctive therapies (26), immunotherapy (27), or drug repositioning may provide greater benefits for these patients in order to achieve a better response. We evidenced an association between signs of in situ cytotoxic adaptive immune response and inflammatory interferon type I–associated molecules production post-nT and the response to treatment. Type I IFNs play a key role in antitumoral immunity by promoting the maturation and presentation capacity of dendritic cells and their migration to lymph nodes (28). This immune state was influenced by the quality and the intensity of the natural immune response pre-existing before nT. ISB high could not only favor nT-dependent tumor cell death, but also promote the presence of resident immune components that could be essential to avoid local recurrences in organ preservative strategies such as Watch-and-Wait. Of note, a few ISB high patients did not achieve a good response, highlighting that treatment resistance is also guided by independent tumor-intrinsic factors (29) or...
the presence of a suppressive microenvironment (30). Neoadjuvant treatment with development of clinical complete response post-nT has raised the possibility of organ-preserving strategies, as radical resection of the rectum results in functional outcomes, immediate morbidity, and even mortality rates (31). However, imaging after nCRT (ypTNM) has low accuracy in predicting pathologic complete response due to over- or under-staging (32). Importantly, no relapse was observed in good responders with ISb high patients. In addition, ISb increased the accuracy prediction for very good responders (ypTNM 0–1) evaluated by imaging and identified a subgroup of patients treated with an organ-preserving strategy (Watch-and-Wait) with a very favorable outcome. No biomarker is currently available to help select candidates of organ preservation including patients with ISb high and complete clinical response to nT, but also those with a delayed complete clinical response (i.e., "nearly complete responders") that are presently classified as incomplete responders (33).

This study has some limitations. The immune densities associated with predefined cut points (i.e., 25th and 70th percentiles) are closely linked to the clinical characteristics of the studied cohort. The densities used as cut point are relevant to patients with LARC treated by nT before surgery. In addition, assessment of ISb was performed on initial biopsies; this implies analysis only of a small fraction of the tumor (10%–15% of the surface of cut from a tumor block available after TME) and no analysis of the invasive margin absent on biopsies. In order to evaluate the correspondence of ISb and IS in resected tumor, we analyzed 33 colon cancer biopsies and their associated resected specimen and the multiple types of patient care, the strong and constant prognosis value associated with ISb highlights the robustness of the test and its generalizability. Prognostic parameters such as mismatch repair, KRAS, and BRAF status not available in our study were not included in multivariate analysis with IS scoring system. Despite this limited surface analysis and the absence of invasive margin, the prognostic value of the ISb was retained suggesting the accuracy of the immune evaluation on initial diagnostic biopsy when the surgical piece is unavailable or is impossible to analyze due to architectural changes secondary to the nT. In addition, performing IS on postoperative specimen would not allow an assessment of its predictive value of the response to nT. Furthermore, due to the deep histologic modifications after nT (no clear delimitation of the tumor and its invasive margin), an IS on post-nT specimen is not feasible. The study was performed on patients who came from different countries and received standard-of-care treatment in real-life clinical practice. Despite the size of the specimen and the multiple types of patient care, the strong and constant prognosis value associated with ISb highlights the robustness of the test and its generalizability. Prognostic parameters such as mismatch repair, KRAS, and BRAF status not available in our study were not included in multivariate analysis with IS scoring system. However, MSI+ cases are rare in rectal cancer (<5%; ref. 34), and we recently evidenced that IS was an independent prognostic parameter for survival when associated with MSI, KRAS, and BRAF status in colon cancers (35). Most of the rectal cancers included in this study were adenocarcinomas. A subanalysis by histologic subtypes could not be performed due to the large multicentric character of the cohorts studied, with heterogeneous level of histopathologic description and the obvious small effect of mucinous carcinomas, signet ring cell carcinoma, or tumor budding to address their relative prognostic impact with enough power. This study emphasizes the importance of the initial diagnostic biopsies, often done in private practices, and not easily available in some cases. Patients with rectal cancer would benefit from a close partnership between private pathology practices, clinics, and teaching hospitals in order to initially assess their immune status (ISb). This material could become in the near future essential and be part of the personal medical file of patients with rectal cancer as it is the sole material available before any nT. ISb may facilitate a personalized multimodal treatment of rectal cancer particularly in patients with ISb high tumors at baseline and with signs of tumor regression by imaging. These patients should benefit the most from the conservative strategy and in turn preserve their quality of life.

In conclusion, our results indicate that ISb could be used: (i) to predict tumor response after nT, (ii) to restore local disease after nT, and (iii) to predict clinical outcome. This method may facilitate a personalized multimodal treatment of rectal cancer particularly in patients with ISb high tumors at baseline and with signs of tumor regression by imaging. These patients should benefit the most from the conservative strategy and in turn preserve their quality of life. ISb is yet to be validated on larger Watch-and-Wait cohorts both retrospectively and prospectively. Such validations are planned in international validation studies using the International Watch-and-Wait database and in the OPERA ongoing clinical trial (NCT02505750).

Disclosure of Potential Conflicts of Interest

B. Milecnik reports patents WO2014023706A1 and WO2017194556A1 on methods and kits for screening patients with cancer, licensed and with royalties paid from HaloDX. F. Bibeau reports personal fees from Amgen; grants and personal fees from BMS; personal fees from MSD, Merck, and Bayer; and grants and personal fees from AstraZeneca outside the submitted work. D. Tougeron reports personal fees from Sanofi (board), MSD (board); personal fees and other from AMGEN (board, travel for congress); Servier (board, travel for congress); personal fees from Roche (board), RMS (board), AstraZeneca (board), and Bayer (board) outside the submitted work. R.O. Perez reports personal fees from Johnson & Johnson and personal fees from Roche outside the submitted work. J.-P. Gerard reports grants from Centre Antoine Lacassagne outside the submitted work. J. Galon reports personal fees from HaloDX (HaloDX co-founder) and personal fees from INSERM (licensure) during the conduct of the study; grants from Immcheck Therapeutics, AstraZeneca, MedImmune, IObiotech, Janssen, Perkin Elmer, Ulivive; personal fees from BMS (consulting), 10Biotech (consulting), Northwest Biotherapeutics (consulting), Gilead (consulting), Amgen (consulting), Catalyn (consulting), and Sanofi (consulting) outside the submitted work; in addition, the author has a patent for biomarkers and methods for predicting survival pending, licensed, and with royalties paid from HaloDX & INSERM. F. Pagès reports grants and personal fees from BMS; grants from HaloDX; personal fees from Gilead, Merck, Roche, and Janssen outside the submitted work; in addition, the author has a patent for WO2007045996 on an in vitro method for the prognosis of progression of a cancer and of the outcome, and a patent for WO2013/186374 on the quantification of immune cells in tumor tissues, both owned by HaloDX and both applications by INSERM. No potential conflicts of interest were disclosed by the other authors.

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G. Zeißen: Conceptualization, supervision, data curation, writing-original draft, writing-review and editing.
F. Pages: Conceptualization, data curation, supervision, funding acquisition, investigation, methodology, writing original draft, writing review and editing.

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References

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