THE CONSENSUS IMMUNOSCORE ADAPTED TO BIOPSIES IN PATIENTS WITH LOCALLY ADVANCED RECTAL CANCER: POTENTIAL CLINICAL SIGNIFICANCE FOR A "WATCH AND WAIT" STRATEGY

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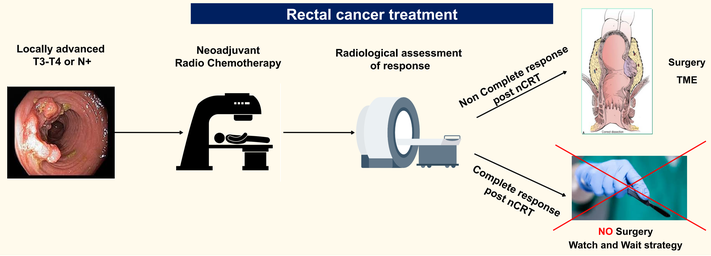
BACKGROUND

In colorectal cancers treated by surgery only as a first line treatment, the adaptive immune reaction composed of T lymphocytes (CD3+) with cytotoxic (CD8+) and memory (CD45RO+) phenotypes located in both the core of the tumor (CT) and the invasive margin (IM) have been repeatedly shown to highly predict recurrence and survival and to be superior to AJCC-UICC TNM classification[1]. In 2018, a consensus **Immunoscore** was developed and validated in an international colon cancer cohort of 3000 patients[2].

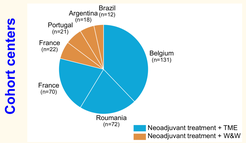
In **locally advanced rectal cancer** (LARC) characterized by a high risk of local recurrence, neoadjuvant chemoradiotherapy (nCRT) followed by radical surgery, namely protectomy with total mesorectal excision (TME), is recommended by international guidelines[3].

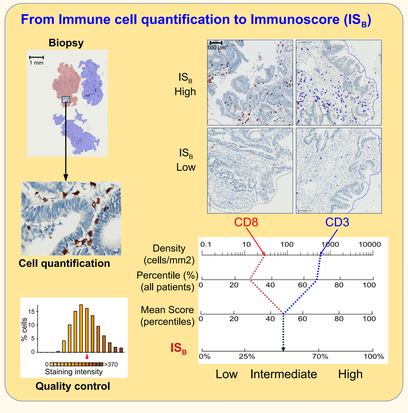
We investigated whether an adaptation of the Immunoscore to **pre-treatment rectal biopsies**, could predict the response to nCRT and delineate clinical responders that would benefit from a **"Watch and Wait"** (W&W) strategy[4] with acceptable outcomes.



METHODS

- Initial biopsies from **273 patients** with LARC treated by nCRT followed by total mesorectal excision, and **73** LARC treated by W&W strategy were immunostained for **CD3+** and cytotoxic **CD8+** T cells and quantified by **digital pathology** to determine the **Immunoscore-Biopsy** (IS_B).
- To calculate the IS_B : CD3 and CD8 densities are converted into **percentile** referring to the densities observed in all patients, then converted into a mean and categorized into IS_B Low, Intermediate and High
- Expression level of 44 immune related genes post-neoadjuvant treatment was investigated by **Nanostring** technology (n=62 patients).

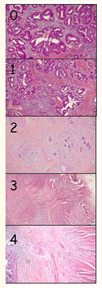




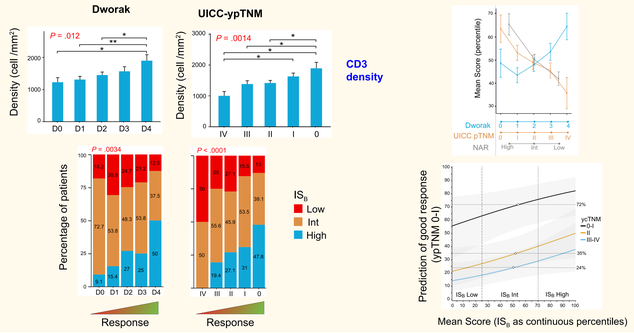
RESULTS

IS_B and response to neoadjuvant treatment

Dworak classification (Local regression)

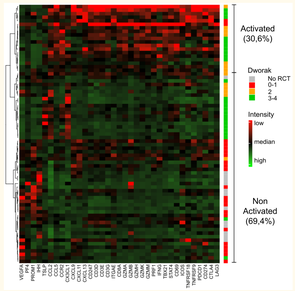


- IS_B was positively and significantly correlated with the response to nCRT, as evaluated by **Dworak classification**[5] (P=0.0034), **ypTNM** (P<0.0001) and neoadjuvant rectal (NAR) score (P< 0.0001).
- IS_B increased the accuracy of prediction of the very good pathological responders (ypTNM 0-I) compared to ycTNM classification alone.

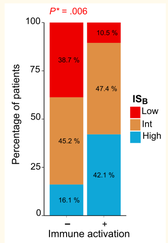


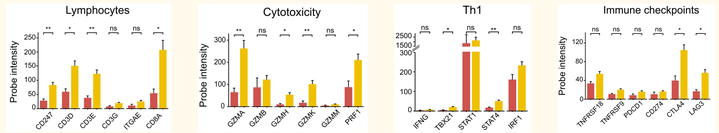
Immune gene expression post neoadjuvant treatment

- Unsupervised hierarchical clustering showed that 30.1% (n=19/62) of the **surgical specimen** presented signs of post-nCRT immune activation.
- The activation status was significantly correlated with the densities of CD3+ and CD8+ in biopsies before treatment.
- Good response was correlated with a higher expression of genes associated with lymphocyte infiltration, cytotoxicity, Th1 orientation and activation.



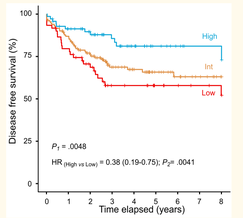
Initial IS_B vs transcriptional Immune activity post nRCT





Prognostic value of IS_B and Survival in LARC

DFS in LARC patients (n=273)



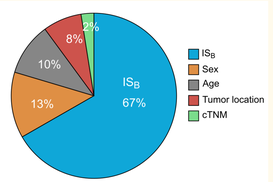
Multivariate analysis for DFS

	PHA test	HR (95% CI)	*P* value*
Age			
Under vs Over 65 y	0.935	0.73 (0.45-1.18)	0.20
Tumor Location			
Middle vs Inferior	0.16	0.99 (0.6-1.66)	0.98
Superior vs Inferior	0.36	0.6 (0.24-1.52)	0.28
Sex			
M vs F	0.073	0.68 (0.39-1.15)	0.15
cTNM stage			
III vs I-II	0.534	0.84 (0.49-1.43)	0.53
Immunoscore$_B$			
Intermediate vs low	0.668	0.58 (0.34-1)	0.051
High vs low	0.44	0.29 (0.14-0.62)	0.0013

c-index (+/- se): 0.63 (+/- 0.034)

NOTE.
*The significance of the Cox multivariate regression model was evaluated with the Wald p value
PHA=proportional hazards assumption, HR=Hazard ratio

Relative variable contribution to DFS



IS_B is:

- Positively associated with survival.
- Independent of other markers in multivariate analysis.
- More important than other initial clinical markers to predict survival.

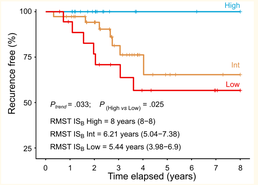
IS_B and Watch and Wait strategy

Clinical Characteristics for Watch and Wait patients

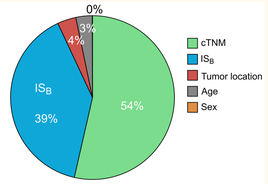
Characteristics	Overall No. (%)	IS_B Low No. (%)	IS_B Int No. (%)	IS_B High No. (%)	*P*
No. of patients	73 (100)	19 (26)	37 (50.7)	17 (23.3)	
Age					.28*
≤ 65 years	32 (43.8)	6 (31.6)	16 (43.2)	10 (58.8)	
> 65 years	41 (56.2)	13 (68.4)	21 (56.8)	7 (41.2)	
Gender					.32*
Male	40 (54.8)	10 (52.6)	23 (62.2)	7 (41.2)	
Female	33 (45.2)	9 (47.4)	14 (37.8)	10 (58.8)	
Tumor location					.04†
Inferior	43 (81.1)	15 (100)	20 (80)	8 (61.5)	
Middle	9 (17)	0 (0)	5 (20)	4 (30.8)	
Superior	1 (1.9)	0 (0)	0 (0)	1 (7.7)	
cTNM stage					.0021*
I	9 (17.3)	2 (14.3)	7 (28)	0 (0)	
II	10 (19.2)	6 (42.9)	2 (8)	2 (15.4)	
III	33 (63.5)	6 (42.9)	16 (64)	11 (84.6)	

NOTE.
* Chi square *P* value for independence between clinical variables and IS_B level
† Fisher's test *P* value for independence between clinical variables and IS_B level

TTR in W&W patients (n=73)



Relative variable contribution to TTR



- **No evidence of relapse** was noticed during the follow up in **IS_B High patients**
- Relative contribution of the IS_B to predict the occurrence of relapse is independent of other initial factors

CONCLUSIONS

- Altogether, IS_B could serve two purposes: prediction of the nCRT response and re-staging before surgery. This could facilitate a personalized multimodal treatment of rectal cancer particularly among patients with high IS_B tumors at baseline likely to exhibit excellent response and rare recurrence in the event of a complete clinical response.
- To be of clinical utility the IS_B has now to be validated in a large-scale prospective study, which is underway with the OPERA clinical trial (NCT02505750).

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