

International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study

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KEY POINTS

- Immunoscore® confirmed as a major risk factor, predicting risk of relapse in early colon cancer
- Immunoscore® exceeds all other clinic-pathological parameters in predicting the risk of recurrence and survival in colon cancer patients
- Immunoscore® quantification is reproducible, robust and quantitative
- First consensus immune biomarker validated worldwide, in the largest international prognostic biomarker study on colon cancer (n=2681 cases)

These results support the implementation of Immunoscore® as a new component for the classification of cancer, TNM-I (Immune).

Introduction

By measuring the density of CD3+ and CD8+ T lymphocyte populations in the center and at the periphery of the tumor, Immunoscore® had been previously shown to be a promising risk factor to guide treatment strategies for stages II and III colon cancer patients.

Following the first World Immunotherapy Council meeting in 2012, the decision was taken with the Society for Immunotherapy of Cancer (SITC), to initiate an international Immunoscore® consortium to validate the Immunoscore® assay in patients with stage I-III colon cancer.

The purpose of the multicenter study was notably to:

- validate the prognostic performance of the Immunoscore® in routine clinical settings.
- demonstrate the feasibility and reproducibility of Immunoscore®
- demonstrate the utility of Immunoscore® to predict stage II colon cancer patients with high-risk of recurrence

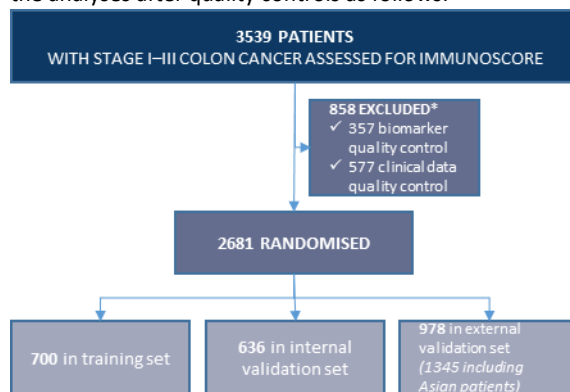
Methods

Patients

Biomarker data from 3539 AJCC/UICC-TNM stage I/II/III colon cancers patients were collected from 14 expert centers from 13 countries in North America, Europe, and Asia.

After stratification by center, T-stage, N-stage, and relapse, patients were randomly split into training and internal validation datasets (TS and IVS). An additional independent and external validation set (EVS) was also established.

Samples from primary tumors from 3539 patients were processed, and samples from 2681 patients were included in the analyses after quality controls as follows:



Procedures

Paraffin sections which contained the colon tumor and invasive margin from each patient were processed by immunohistochemistry, and the densities of CD3+ and cytotoxic CD8+ T cells in both regions were quantified by digital pathology.

The mean of four percentiles (two markers, two regions) was calculated and converted into an Immunoscore.

In a three-category Immunoscore analysis, a 0–25% density was scored as low, a density between 25% and 70% was scored as intermediate, and a density between 70% and 100% density was scored as high. In the two-category analysis, intermediate plus high categories were grouped as high Immunoscore.

Statistical analysis

Analyses were conducted according to a pre-specified statistical plan and in compliance with the STARD reporting guidelines.

Immunoscore cutoffs were defined in the Training set, and subsequently validated in the independent datasets.

Statisticians external to the consortium did the statistical analyses.

Outcomes

The primary endpoint was to evaluate: The prognostic value of Immunoscore® for TTR - time to recurrence (defined as time from surgery to disease recurrence).

Additional outcomes of interest were:

- DFS - disease-free survival time from surgery to first observation of disease recurrence or death due to any cause).
- OS - overall survival (time from surgery to death due to any cause).

Results

Overall, **2681 patients** were included in the analysis, among which 17% were stage I patients, 54% stage II, and 29% stage III.

In the training set, 155 (22%) patients had a low Immunoscore®, 357 (51%) patients had an intermediate Immunoscore®, and 188 (27%) patients had a high Immunoscore®.

A significant positive correlation was found between the densities of T cells in each tumor region and survival in the training set, with patients having a **high Immunoscore®** showing the **lowest risk of recurrence**.

Recurrence at 5 years, was observed in 8%, 19% and 32% of the patients with a high, intermediate and low Immunoscore® respectively. The hazard ratio [HR] for high vs low Immunoscore® was 0.20, 95% CI 0.10-0.38 ($p < 0.0001$) (fig. 1).

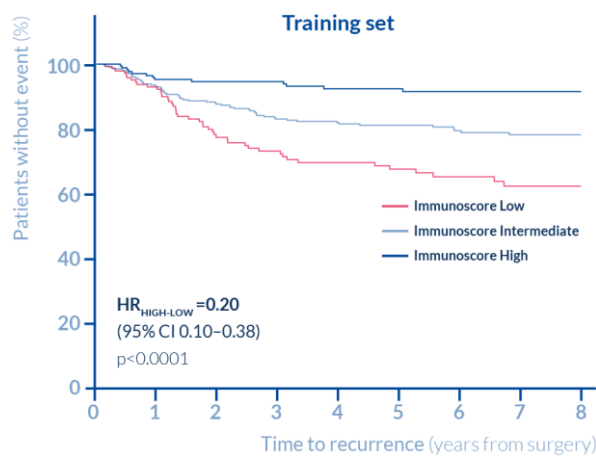


Figure 1

The findings were validated both in the internal and external validation sets (Fig. 2-3).

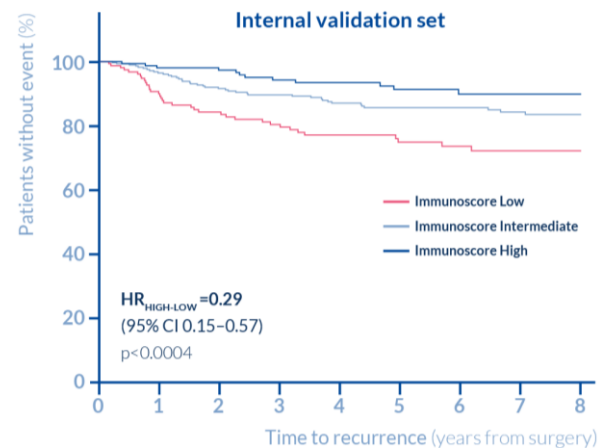


Figure 2

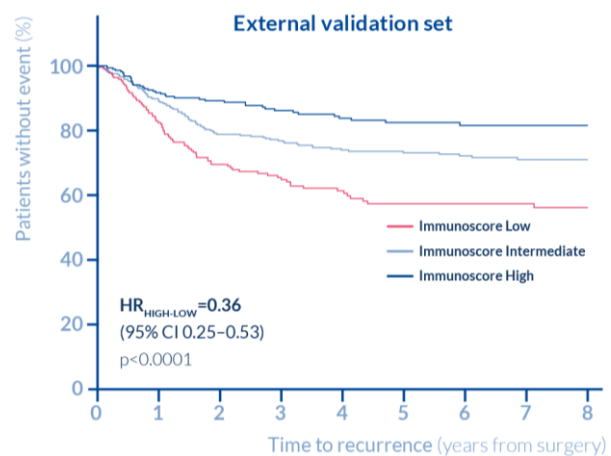


Figure 3

Patients with a high Immunoscore® had longest survival.

In the training set, overall survival at 5 years was observed for 82%, 77% and 62% of the patients with a high, intermediate and low Immunoscore® respectively (unadjusted HR for high vs low Immunoscore® 0.53, 95% CI 0.38-0.75; $p = 0.0004$).

These results were also validated in the internal and external validation sets (all $p < 0.05$).

Clinical performance of tumor-related parameters and Immunoscore®

The performance of Immunoscore to predict survival was compared to that of existing tumor risk parameters such as grade of differentiation, MSI status, mucinous colloid type, sidedness, venous emboli, lymphatic invasion, perineural invasion and VELIPI. Of all clinical parameters, the relative contribution to the risk showed that Immunoscore® (47%) was better than TNM staging (28%), grade of differentiation (15%), VELIPI (8%), sex (<3%), mucinous, and MSI status. Thus, Immunoscore® was stronger than all these clinical parameters, showing the highest contribution to predict survival (fig. 4)

Immunoscore® was stronger than clinical parameters to predict survival

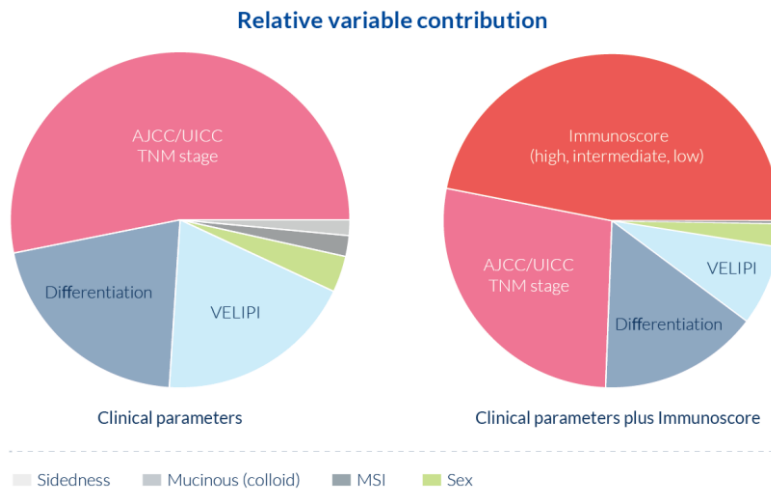


Figure 4

Furthermore, Cox multivariate regression analysis for OS stratified by center, for all available clinical parameters revealed that Immunoscore® and TNM stage were significant risk parameters, whereas all other existing tumor risk parameters were no longer significant (Tab. 1).

	Hazard ratio (95% CI)	Wald p value
Gender Female vs Male	0.89 (0.72-1.11)	0.3083
AJCC/UICC TNM stage II vs I	1.65 (1.11-2.46)	0.0127
AJCC/UICC TNM stage III vs I	2.10 (1.38-3.18)	0.0005
MSI Status MSI vs MSS	0.97 (0.71-1.32)	0.8416
VELIPI YES vs NO	1.26 (0.99-1.61)	0.0580
Differentiation moderate vs Well	0.91 (0.67-1.25)	0.5664
Differentiation poor-undif vs Well	1.41 (0.93-2.13)	0.1035
Mucinous (Colloide) Type YES vs NO	1.01 (0.78-1.32)	0.9095
Sidedness distal vs proximal	0.97 (0.77-1.22)	0.7784
Immunoscore Intermediate vs Low	0.68 (0.53-0.87)	0.0019
Immunoscore High vs Low	0.47 (0.33-0.65)	<0.0001

Table 1

Immunoscore® significantly predicted survival in patients with Stage II colon cancer

In patients with stage II tumors (n=1434), a high Immunoscore® was associated with lowest risk of recurrence and highest DFS and OS (p<0.05) (Fig. 5).

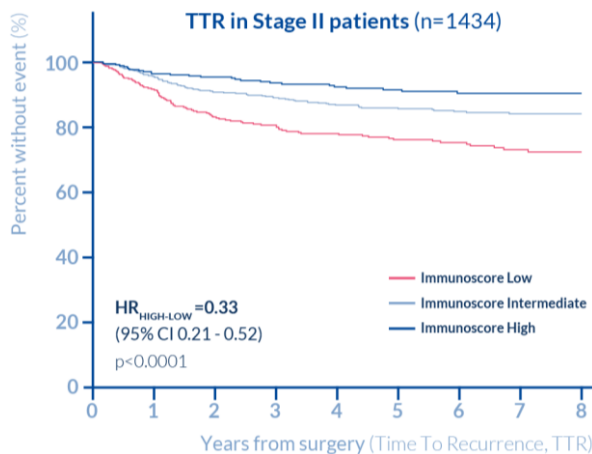


Figure 5

Recurrence at 5 years was observed in 8% 14% and 23% of the patients with high, intermediate and low Immunoscore® respectively (unadjusted HR for high vs low Immunoscore® 0.33, 95% CI 0.21–0.52; p<0.0001; C index=0.60).

The beneficial effect of a high Immunoscore® persisted in the presence of signs of tumor emboli (i.e. in patients with venous emboli, lymphatic invasion, or perineural invasion). Similar results were found for the adjusted model for Immunoscore®, age, sex, T stage, N stage, and MSI status and when stratified by city centers for **TTR (p<0.0001)**, **DFS (p<0.0001)** and **OS (p=0.002)**.

Immunoscore® and MSI status

1579 patient samples were included in the analysis of MSI status. When stratified into 3 Immunoscore® categories, MSI tumors were associated with a high Immunoscore® in 138 (45%) of 304 cases, whereas a high Immunoscore® was observed in 273 (21%) of 1275 patients with MSS tumors. When stratified into 2 Immunoscore® categories, patients with high Immunoscore® had prolonged DFS, TTR and OS, **irrespective of their microsatellite status** (Fig. 6).

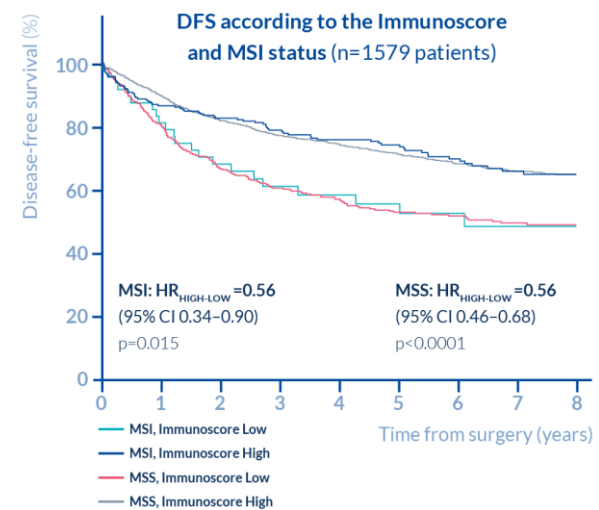


Figure 6

Additionally, patients with weakly infiltrated MSI tumors did not have a survival advantage as compared with patients with MSS tumors.

Similar results were found when analyzing Immunoscore® and MSI in patients with stage II tumors (Fig. 7).

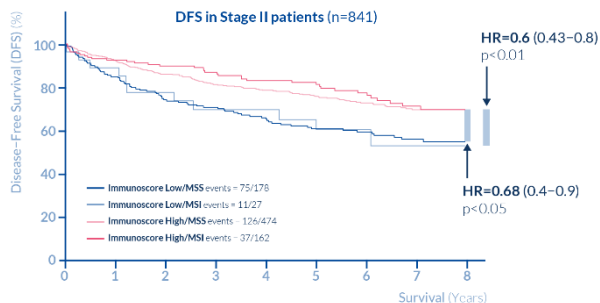


Figure 7

Stage II patients with high Immunoscore® had prolonged DFS, TTR and OS irrespective of their microsatellite status.

In multivariate Cox model combining MSI status with the Immunoscore®, MSI remained a significant factor for TTR but was not significant for DFS and OS and was dependent on Immunoscore®. The beneficial effect of the MSI status was therefore related to its capacity to induce a strong immunity (i.e., high Immunoscore).

Immunoscore® was also significant for TTR, DFS and OS (all p<0.0001) within the subgroup of patients with stage II cancer, MSS, and who were not receiving chemotherapy.

Immunoscore® showed a high level of reproducibility

Immunoscore® assay was shown to be highly reproducible between observers and centers (r=0.97 for tumor; r=0.97 for invasive margin; p<0.0001).

Furthermore, patient survival was predicted more accurately with Immunoscore® than by visual assessment of tumor-infiltrating T cell.

Overall, Immunoscore® quantification was standardized, reproducible, robust, and quantitative.

Conclusions:

This large international SITC-led validation study confirms the strong prognostic value of Immunoscore® assay in early stage colon cancer.

Patients with high Immunoscore® have prolonged DFS regardless of their microsatellite status, in Stages I-III, and in Stage II sub-set.

These results validate the reliability of Immunoscore® in identifying patients with a high risk of recurrence, independently of the TNM staging system.

Prognostication by the TNM staging was improved by Immunoscore®, supporting its implementation as a new component of a TNM-Immune classification of cancer.

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