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Accessibility
Towards the introduction of the 'Immunoscore' in the classification of malignant tumours

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Abstract

The American Joint Committee on Cancer/Union Internationale Contre le Cancer (AJCC/UICC) TNM staging system provides the most reliable guidelines for the routine prognostication and treatment of colorectal carcinoma. This traditional tumour staging summarises data on tumour burden (T), the presence of cancer cells in draining and regional lymph nodes (N) and evidence for distant metastases (M). However, it is now recognised that the clinical outcome can vary significantly among patients within the same stage. The current classification provides limited prognostic information and does not predict response to therapy. Multiple ways to classify cancer and to distinguish different subtypes of colorectal cancer have been proposed, including morphology, cell origin, molecular pathways, mutation status and gene expression-based stratification. These parameters rely on tumour-cell characteristics. Extensive literature has investigated the host immune response against cancer and demonstrated the prognostic impact of the in situ immune cell infiltrate in tumours. A methodology named 'Immunoscore' has been defined to quantify the in situ immune infiltrate. In colorectal cancer, the Immunoscore may add to the significance of the current AJCC/UICC TNM classification, since it has been demonstrated to be a prognostic factor superior to the AJCC/UICC TNM classification. An international consortium has been initiated to validate and promote the Immunoscore in routine clinical settings. The results of this international consortium may result in the implementation of the Immunoscore as a new component for the classification of cancer, designated TNM-I (TNM-Immune).

Keywords: Immunoscore; colorectal cancer; colon carcinoma; tumour microenvironment; immune response; classification; prognostic markers; predictive markers; T cells

Introduction

Cancer is a complex and dynamic disease characterized by major hallmarks. They include sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism and evading immune destruction [1]. The most common system for classifying the extent of spread of cancer is the American Joint Committee on Cancer/Union Internationale Contre le Cancer (AJCC/UICC) TNM classification [2–4]. The tumour staging gives an estimation of the degree of tumour progression at the time of the surgical resection. The higher the degree of tumour progression, the greater the chance that the tumour will have undergone clonal evolution and acquired a set of unfavourable characteristics, such as the ability to invade lymphatic or blood vessels or to metastasize to distant sites. Furthermore, multiple tumour-cell parameters are an indication of the intrinsic biology of the tumour.

The TNM classification has been used for over 80 years and is valuable in estimating the outcome of patients for a variety of cancers [2–4]. There has been a continual refinement of the staging system. In 2009, the Union for International Cancer Control issued the seventh edition of the TNM classification guidelines. The pathologists generate data from a pathological snapshot. This static measurement integrating the tumour grade and stage is correlated with a dynamic process, such as the time to occurrence or death. The system is used in clinical trials to select patients who are eligible for inclusion, and in cancer registries to compare outcomes between different series, across different countries and over different time periods [5]. An accurate, stable internationally agreed staging system is essential to global progress in this disease. Its main aim should be to provide prognostic information and, based on this information, individual treatment decisions can then be made. In daily practice and in guidelines, the TNM category is directly linked to treatment strategies and, as such, changes in the TNM staging system have a considerable and direct impact on the cancer care that patients receive.

This TNM staging system has stood the test of time but provides incomplete prognostic information. Clinical outcome can significantly vary among patients within the same histological tumour stage [5]. In some patients, advanced-stage cancer can remain stable for years, and although rare, partial or full regression of metastatic tumours can occur spontaneously [6]. In contrast, relapse, rapid tumour progression and patient death is associated with approximately 25% of TNM III stage colorectal cancer (CRC) patients, despite complete surgical resection and no evidence of residual tumour burden or distant metastasis [6].

Unfortunately, the predictive accuracy of the traditional staging system relies on the assumption that disease progression is a tumour cell-autonomous process. The focus of this classification is solely on the
tumour cells and fails to incorporate the effects of the host immune response [7]. The phenotype of a tumour is not governed only by the epithelial component but also by the tumour environment, that is, other cells in contact with the tumour, the mesenchyme and the inflammatory infiltrate. These components determine the net inputs to the cell, which include ligands, cell–cell adhesion molecules, metabolites, oxygen and multiple soluble factors [1]. Relatively little information is available on the spatial organization of key proteins and cells, although new imaging techniques offer the potential for high-resolution measurements of the spatio-temporal dynamics of large numbers of proteins [8].

The TNM classification was never surpassed in multivariate analysis by alternative methods such as immunohistochemistry for tumour biomarkers, flow-cytometry for DNA content, molecular signatures or genetic features. However, it was shown that the analysis of a specific type of intratumoural immune response was indeed surpassing the TNM classification in multivariate analysis [9,10]. Thus, tumour progression has now to be considered as the result of a balance between an invasive tumour process and a defence system whose major component is constituted by the host immune response.

Molecular subtypes of colorectal cancer

Several ways to classify cancer have been proposed (Figure 1). These rely on tumour cell characteristics, including morphology, molecular pathways, mutation status, cell origin and gene expression-based methods, and allow the distinction of multiple, often overlapping, subtypes.

A morphology-based classification allows the distinction between five major types of CRC. More than 90% of CRCs are adenocarcinomas originating from epithelial cells. A number of histological variants are described: mucinous, signet ring cell, medullary, micropapillary, serrated, cribriform comedo-type, adenosquamous, spindle cell and undifferentiated. These histopathological criteria have modest prognostic value.

A second class is based on molecular pathways. CRC is molecularly defined into three groups: chromosomal unstable (CIN), microsatellite unstable (MSI) and CpG island methylator phenotype (CIMP). Most cases arise through the CIN pathway, with imbalances in chromosome number and loss of heterozygosity. These cancers have accumulation of mutations in specific tumour suppressor genes and oncogenes that activate pathways critical for CRC initiation and progression. A high degree of MSI (MSI-high) is present in 15% of CRCs and represents a specific type of genomic instability characterized by frequent microsatellite length mutations. Frameshift mutations in microsatellite instability high (MSI-High) colorectal cancers are a potential source of targetable neo-antigens [11]. Most MSI-high cancers are caused by epigenetic silencing of a mismatch-repair gene (MLH1). This silencing typically occurs in tumours of the CpG island methylator phenotype (CIMP-high) [12,13]. MSI-high cancers overlap with those of CIMP-high cancers [13,14]. CIMP-high represents a specific type of epigenomic instability that is characterized by widespread promoter CpG island methylation and epigenetic gene silencing [15]. Nonetheless, different from the MSI subgroup, CIMP-high patients have similar characteristics to MSI, and have been associated with old age, female gender, proximal tumour location, poor tumour differentiation, BRAF mutation, wild-type TP53, high-level of global DNA methylation and stable chromosomes (CIN) [12,13]. MSI tumours have a more favourable prognosis and are less prone to lymph node or distant metastasis [16]. CIMP-high might be a prognostic marker independent of the presence of MSI and BRAF mutation [17].

A third method to classify CRCs is based on mutation analysis. Following the discoveries from Bert Vogelstein et al. that tumours result from the sequential accumulation of alterations in oncoproteins and tumour suppressor genes and the appearance of driver mutations, several mutations are now regularly tested in CRCs. These include the APC, Kras, TP53, BRAF, NRAS, PI3KCA and CTNNB1 genes. Somatic mutations in codons 12, 13 and 61 of KRAS, and more recently NRAS, predict innate resistance to mAbs targeting epidermal growth factor receptor [18–20]. The BRAF V600E mutation is an adverse prognostic factor in stage IV colorectal cancer but does not have clinical utility at present [21].

The fourth and fifth methods, assessing the cell of origin and gene expression, respectively, to classify CRCs are molecular based techniques. Using this approach, six groups of CRCs were recently defined, based on similarities with distinct cell types within the normal colon crypt and the response to classic chemotherapy and targeted therapies [22]. Other gene expression-based analysis described three groups of CRC patients [23], which correlated with two of the known molecularly defined groups, namely the MSI (renamed CCS2) and the CIN (CCS1) groups, whereas the third group corresponded partly with the CIMP group (CCS3). Further analysis of precursor lesions (serrated polyps) suggested that this latter group could be derived from the serrated pathway. Some partial overlap exists between both proposed gene expression-based classifications, especially with respect to the group that is defined by the MSI-high phenotype. Other gene signatures were also reported and, strikingly, most of the genes were unique to each signature. These signatures showed low prediction accuracy and moderate clinical usefulness [24].

Interestingly, both profiles emphasize the importance of ZEB1, a transcription factor regulating the epithelial–mesenchymal transition (EMT). However, EMT in CRC is generally not a feature of the whole
tumour but is seen at the invasive margin (IM). The stroma of human colorectal tumours was shown to contain TWIST1-positive cancer cells with mesenchymal phenotypes [25]. EMT is characterized by tumour cell budding, nuclear expression of β-catenin, loss of CDH1 (E-cadherin), gain of CDH2 (N-cadherin) and alteration of other epithelial cell adhesion molecules [26]. These features are often not molecularly defined but the result of interplay between the tumour and the microenvironment. Such local features lead to heterogeneity of the gene expression profile. Thus, gene expression classification may be prone to bias, due to different percentages of tumour cells in the sample, but also due to lack or uncontrolled presence of the IM in the sample used for RNA extraction. These issues were not addressed in either study [22,23]. Because of the limitation of gene expression signatures, and to facilitate implementation and testing in large patient series, both studies developed an immunohistochemistry approach based on gene expression data. Unfortunately, half of the patients turned out to be unclassifiable [22] and the absence of CDX2 positivity in all CCS3 samples (25% of CRCs) [23] does not correspond with extensive literature data (98% positivity in CRCs) [27].

The carcinogenic process that gives rise to an individual tumour is unique. It is postulated that tumours with similar characteristics share common pathogenic mechanisms and progression patterns. However, other major parameters have to be taken into consideration, in particular the tumour microenvironment. Importantly, neoplastic cells interact with host non-neoplastic cells (including immune cells) and extracellular matrix in the tumour microenvironment, and those components influence each other and modify an integral phenotype of any given tumour [28]. Many markers, signatures and methods have been described to evaluate the prognosis of cancer patients, yet very few such markers and laboratory assays translate into clinical practice or reach the statistical power of the TNM classification.

New ways to classify cancers using tumour microenvironment-related information

Modern classification of tumours is based on the recognition of disease entities that are characterized by morphological, phenotypical and genetic markers. Each classification system needs to be reliable, reproducible, clinically relevant and biologically meaningful. Many hurdles have to be taken into consideration. First, the inevitable presence of non-neoplastic cells, including immune cells, in ‘tumour areas’ means that DNA (or RNA) from the tumour area is not ‘pure’ DNA (or RNA) from neoplastic cells. These ‘contaminants’, often > 50% of non-tumour cells, may in fact have a profound biological meaning. Thus, the degree of immune cell infiltration may correlate with tumour molecular changes or may mask a correlation between
a tumour marker and the outcome, because ‘contami-
nating’ non-neoplastic cells can influence the results
of a tumour molecular assay. For sensitive mutation
detection, sequencing technologies allow the detection
of approximately 5% of mutant alleles. For quantita-
tive DNA methylation assays, a careful assessment of
a potential influence of contaminating normal cells is
necessary. This is even more problematic with gene
expression signatures, where a different degree of non-
neoplastic cells is observed among the assays.

Second, a precise characterization of specific
tumour-infiltrating cells requires the use of an
immunohistochemical technique to detect in the tissue
the presence and localization of specific antigens
expressed by subsets of immune cells. The evaluation
of immune cells in haematoxylin and eosin (H&E)-
stained sections can be done at a low cost compared
to an immunohistochemical technique. However, an
evaluation of a specific subset of immune cells is
generally not possible with H&E-stained sections.
For example, lymphocytes with opposite functions,
such as CD4+ T cells with Th1 orientation versus
Th2 orientation versus immune cells with regulatory
functions (Treg cells), or NK cells, NKT cells, B
cells or cytotoxic CD8 T cells, are indistinguishable
without proper marker evaluation and require antibody
labelling by immunohistochemistry. Nowadays this is
simple, as specific antibodies with high affinity and
a high signal:noise ratio allow the detection of these
immune cell subpopulations with a single staining.

Third, it is known that immune cells are scattered
in the core of the tumour (CT) within the tumour
stroma and the tumour glands, in the invasive mar-
gin (IM) and in organized lymphoid follicles distant
from the tumour. A statistically significant correlation
between immune cell density in each tumour region
(CT and IM) and patient outcome has been shown in
colorectal cancer [9]. Further, the combined analysis of
tumour regions (CT plus IM) improved the accuracy of
prediction of survival for the different patient groups
compared with single-region analysis [9]. Given the
major clinical importance of distinct tumour regions,
it is appropriate to conduct immune cell infiltration
evaluation systematically in the two separate areas, the
core of the tumour and the invasive margin [9,10].
For routine practice, this requires immune cell evalua-
tion on whole-tissue sections, taking into account their
location.

Fourth, objective ways of counting immune cells
need to be achieved in order to remove the subjectivity
of field selection and imprecise semi-quantitative eval-
uation. Most of the tumour markers are generally more
complicated to quantify than immune cells, since only
a fraction of the tumour cells express the antigen and
the staining intensity has often been taken into consid-
eration. Standardized and reproducible measurement of
the intensity of staining, and hence quantitation of pro-
ten expression, is intrinsically difficult using immuno-
histochemistry. In contrast, immune cell types are
easier to quantify because well-characterized markers
exist, giving complete membrane staining for immune
cell subpopulations (eg CD3+ for T cells), allowing
counting cells as individual cells. Nowadays, digi-
tal pathology and image analysis software can detect
stained immune cells and determine their densities (n
cells/mm²) in histological sections. Validation studies
demonstrated the high concordance of these automated
systems in comparison to optical counts [9,29].
This is particularly important to facilitate routine pathology
and to speed up the process of quantification. In par-
cular, given the huge number of infiltrating immune
cells within a tumour (eg a mean of 75 000 CD3+
cells present on a 4 µm-thick section of tumour slide
from a CRC stage I/II patient), it would be unrealis-
tic to ask pathologists to count them all. In addition,
immune cells such as CD3+ lymphocytes are often
aggregated in complex cell clusters. Algorithms for
segmenting clusters of densely packed cells permit a
precise counting of cells. Given the major importance
of the determination of the density of immune cells
[9,30] to predict a patient’s outcome, it is now required
to take advantage of the digital pathology to determine
the exact count of stained cells and the surface of the
tissue analysed.

Fifth, the heterogeneity of a tumour applies to
tumour cells but to the microenvironment as well.
Upon evaluation of whole-tissue sections, a patholo-
gist often needs to choose specific fields to perform
detailed image analysis. The selection of the tissue
areas to study may depend on subjective interpretation,
and the evaluation needs to be validated by a sec-
ond independent observer blinded for the other results.
Inter-observer variability can reach very high levels
[31]. The use of computer-assisted image analysis pro-
vides important advantages, as all the fields are analy-
sed in the whole tumour section [32]. For example, the
determination of the mean density of stained cells in a
tumour region is supported by an objective computer-
based cell-counting method, leading to a good level of
reproducibility between users.

Sixth, specific markers need to be selected.
They should be robust, well-established and have
high predictive value. A growing body of litera-
ture [9,10,30,33–36] supports the hypothesis
that cancer development is influenced by the host
immune system. This may offer powerful prog-
nostic information and facilitate clinical decision
making regarding the need for systemic therapy
[7]. Accumulat-ing evidence suggests that CD3+
[9,10,30,34,37–43], CD8+ [9,10,34,38,43–52] and
CD45RO+ [9,10,34,40,47,49,53,54] cells have roles
in antitumour immune responses. Numerous data col-
lected from large cohorts of human CRCs (with sample
sizes n = 415, 599 and 602, respectively) demonstrated
that the number, type and location of tumour immune
infiltrates in primary tumours are prognostic for
disease-free survival (DFS) and overall survival
(OS) [9,10,30]. Altogether, these immune parameters
are designated the ‘immune contexture’ [33,35,55].
Notably, several large studies of CRCs (with sample
sizes $n=843$ and $n=768$, respectively) have shown that tumour lymphocytic reaction and T cell sub-
populations (CD8, CD45RO, FOXP3) are significant prognostic biomarkers, even after adjusting for stage,
lymph node count and well-established prognostic tumour molecular biomarkers, including microsatel-
lite instability (MSI), $BRAF$ mutation and LINE-1 hypomethylation [54,56]. A possible association
exists between MSI status and immune cell infiltrates [38]. MSI tumours often contain intra-epithelial T
cells in response to the expression of neo-antigens on the cell surface [42], in particular those that do
not undergo non-sense medicated decay [11]. This probably contributes to the better prognosis of patients
with MSI tumours. Furthermore, CRCs from a large cohort ($n=1197$) and external validation ($n=209$)
confirmed the prognostic value associated with CD3$^+$, CD8$^+$ and CD45RO$^+$ (PTPRC) T cell infiltration in
CRCs [43].

A recent meta-analysis [55] summarized the impact of immune cells, including B cells, NK cells, MDSC,
macrophages and all subsets of T cells on clinical outcome from more than 120 published articles. Import-
antly, the beneficial impact of the immune infiltrate with cytotoxic and memory T cell phenotype has
been demonstrated in cancers from diverse anatomical sites; including colorectal cancer but also malig-
nant melanoma, lung, gastric, oesophageal, head and neck, breast, bladder, urothelial, ovarian, cervical, pro-
static and pancreatic cancer, hepatocellular carcinoma, medulloblastoma and Merkel cell carcinoma [55]. It is
interesting to note that the implications of this immune phenotype apply not only to various organs of cancer
origin, but also to various cancer cell types, ie adeno-
carcinoma, squamous cell carcinoma, large cell cancer and melanoma. Thus, general characteristics emerge in
which cytotoxic T cells, memory T cells and $T_{H1}$ cells are associated with prolonged survival [35,57,58]. In
contrast, the prognostic impact of other immune cells, such as B cells, NK cells, MDSCs, macrophages and a
subset of $T$-helper populations ($T_{H2}$, $T_{H17}$, $T_{REG}$ cells), differ depending on the type of cancer and on the
on the cancer stage [55]. Altogether, the publications indicate that a precise analysis of the immune component of the
tumour microenvironment by computer-based analysis may be essential to managing patients better. Thus,
precise analysis of the tumour microenvironment by pathologists may be essential for future clinical implement-
ation and better patient management. An expertise in this new emerging field is now warranted to translate it into the clinical practice.

‘Immunoscore’ as a new approach for the classification of cancer

A potential clinical translation of these observations is the establishment of a scoring system des-
ignated ‘Immunoscore’ (Figure 2), derived from the

![Figure 2. (Top) Immunoscore definition and methodology. (Bottom) Immunohistochemistry of a colorectal tumour stained for CD3$^+$ T cells (brown).](image)

![Figure 3. Correspondence between the immune contexture and the Immunoscore.](image)
OS, including at early-stage colorectal cancers; and second, it has biological meaning (adaptive immune response to tumours) and provides a tool or a target for novel therapeutic approaches, including immunotherapy (as recently illustrated in clinical trials boosting T cell responses with anti-CTLA4, anti-PDCD1 (PD-1) and anti-CD274 (PD-L1) [59–62]. Current immunohistochemical technologies allow the application of such analyses in routine diagnostic pathology. Thus, considering the probable universal character of the immune control of tumours, it is essential for patients to take into account the immune parameter as a prognostic factor and to introduce the Immunoscore as a component of cancer classification [6,7,35,63,64].

The Immunoscore was shown to be very powerful, for instance, in CRC patients with clinically localized colorectal cancer, with no detectable tumour spreading to lymph nodes or distant organs. These patients are usually treated only by a surgical removal of the tumour; however, approximately 25% of these patients will have recurrence of their disease, indicating that occult metastases were already present at the time of curative surgery. No tumour-associated marker predicts the recurrence of this subgroup of patients that could have a benefit for an adjuvant therapy. The Immunoscore (I) approach (with an enumeration of CD8+ and CD45RO+ cells in the CT and the IM) was applied to two large independent cohorts (n = 602). Only 4.8% of patients with a high I4 relapsed after 5 years and 86.2% were still alive. In contrast, 72% of patients with a low score (I0 and I1) experience tumour recurrence and only 27.5% were alive at 5 years. These I0 and I1 patients could potentially have benefited from adjuvant therapy if the Immunoscore had been incorporated into the tumour staging [34].

The Immunoscore classification, demonstrating the prevalence of immune infiltrates, was shown to have a prognostic significance superior to that of the AJCC/UICC TNM classification system. For all patients with CRC stages I/II/III, multivariate Cox analysis revealed that the immune criteria remained highly significantly associated with prognosis (DFS, DSS, OS). In contrast, the histopathological staging system (T stage, N stage and tumour differentiation) was no longer significant [10]. Tumour invasion was shown to be statistically dependent on the host immune reaction. Indeed, the immune pattern remained the only significant criterion over the classical AJCC/UICC TNM classification for DFS and OS, and led to an editorial entitled ‘TNM staging in colorectal cancer: T is for T cell and M is for memory’ accompanying the publication by Mlecnik et al [10,65]. It has thus been suggested that the prevalence of tumour immune infiltrates, more than the tumour status, could be a key indicator for recurrence, metastasis and therefore clinical outcome (Figure 4). These results suggest that once human cancer becomes clinically detectable, the adaptive immune response plays a critical role in preventing tumour recurrence. The ability of effector-memory T cells to recall previously encountered antigens leads to a protective response. Following primary exposure to antigen, memory T cells disseminate and are maintained for long periods of time [66]. The trafficking properties and the long-lasting antitumour capacity of memory T cells could result in long-term immunity in human cancer. Over the past few years, the area of immune regulation at the level of the tumour microenvironment has gained a forefront position in cancer research, in CRC [9,10,30,34,55], melanoma [67] and all other cancer types [7]. The Immunoscore, initially described several years ago [9] as a prognostic factor [10,34], could also play a role as a marker to predict the response to biotherapies targeting the immune checkpoints [63,64].

The inherent complexity of immunohistochemistry, in conjunction with protocol variability, contributes to the variability of the results obtained. A standardized consensus method is required. Large-scale assay harmonization efforts have already been witnessed, conducted for commonly used immunological assays of peripheral blood immune cell populations [68,69]. It is therefore essential to pursue assay uniformity to reduce these limitations. A clinical validation of the Immunoscore with standardized procedures is necessary to reach clinical applicability for individual patients.

We performed multiple Immunoscore quality controls to test the accuracy and repeatability of the method. We first observed that the automated cell-counting method achieved a very good level of correlation with the optical counting for CD3 and CD8 immunostainings and an excellent reproducibility of

Figure 4. Schematic representation of cancer classification based on tumour-microenvironment-related parameters (Immunoscore), based on tumour extension and invasion (T, N, M stages), and based on five alternative tumour-related methods.
the count of stained cells. In addition, the variability between users for the determination with the software of the immune cell densities in tumour regions was 5.2% and 2.5% for CD3 and CD8, respectively (unpublished data). All the tests indicated a reliable assessment of the Immunoscore. To be used globally in a routine manner, evaluation of a novel marker should have the following characteristics: do-able in routine, feasible, simple, inexpensive, rapid, robust, reproducible, quantitative, standardized, pathology-based and powerful. The Immunoscore has a potential to fulfill these key aspects (Figure 5). Importantly, for patients with rectal cancer treated by chemoradiotherapy (pCRT) before surgery, preoperative chemoradiation therapy induces histological reactions precluding the realization of an Immunoscore, since the delineation of the analysed tumour regions (CT and IM) is often no longer practicable. Also, assessment of anti-tumour immunity by the Immunoscore is inappropriate in biopsies, since the limitation of the material precludes a precise delimitation of the tumour and the invasive margin. However, immune cell quantification remains an interesting possibility in tumour biopsies.

To assess the Immunoscore in clinical practice and measure its prognostic value, we are conducting a prospective multicentre study (French PHRC programme) for 600 patients from seven hospitals bearing a colorectal cancer treated by primary surgery. In an effort to promote the utilization of the Immunoscore in routine clinical settings, we initiated a worldwide Immunoscore consortium, with the support of the Société pour l’Immunothérapie du Cancer (SITC) [64]. Several thousands of tumours from patients will be Immunoscore-tested by the 23 centres from the worldwide consortium. The worldwide Immunoscore consortium, composed of international expert pathologists and immunologists, identified a strategy for the organization of worldwide retrospective study for the validation of the Immunoscore in colon cancer. Evidence-based selection of specific markers for the Immunoscore was discussed. Because of background staining and loss of antigenicity in stored sections (CD45RO) and granular staining (GZMB), it was decided to employ the two easiest membrane stains, CD3 and CD8. Thus, the combination of two markers (CD3+ and CD8+) in two regions (CT and IM) was agreed for validation in standard clinical practice. Precise quantification is currently performed on whole slide sections (Figure 2), following the recommended initial guidelines.

The purpose of the ongoing Immunoscore worldwide consortium is to validate the following points: first, to demonstrate the feasibility and reproducibility of the Immunoscore; second, to validate the major prognostic power of the Immunoscore in routine for patients with colon cancer stages II/III; and third, to demonstrate the utility of the Immunoscore to predict stage II colon cancer patients with high risk of recurrence. Twenty-three international pathology expert centres are now participating in the Immunoscore enterprise. These participants represent 23 centres from 17 countries, including Asia, Europe, North America, Australia and the Middle East (Australia, Austria, Canada, China, Czech Republic, France, Germany, India, Italy, Japan, The Netherlands, Qatar, Spain, Sweden, Switzerland, the UK and the USA). It is hoped that this initiative will result in the implementation of the Immunoscore as a new component for the classification of cancer, TNM-I (Immune). The Immunoscore should better define the prognosis of cancer patients, better identify patients at high risk of tumour recurrence [70], help to predict and stratify patients who will benefit from therapies [35] and, ultimately, help save the lives of patients with cancer.

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| Good biomarker | Immunoscore characteristics |
|----------------|----------------------------|
| Routine        |  Technique to be performed by pathologist using bright field and precise cell evaluation |
| Feasible       |  Established pathology techniques, using 2 regular whole slide FFPE sections |
| Simple         |  Automated immunochemistry |
| Inexpensive    |  Two simple stainings less costly than complicated molecular techniques |
| Rapid          |  Autostainers, scanner, digital pathology reduce the time to perform an Immunoscore |
| Robust         |  Two strong membrane stainings, with no background, allowing the enumeration of individual cells |
| Reproducible   |  Inter-observers variability is removed by the use of digital pathology, taking into account cell location and counts |
| Quantitative   |  Precise cell density (cell number / surface area) |
| Standardized   |  Standardized operating procedure should be performed to ensure reproducibility and worldwide comparisons |
| Pathology-based|  Necessity of pathologist expertise to validate cell type, cell location, and cell counts perform by digital pathology |
| Powerful       |  The Immunoscore has a prognostic value highly significant even in Cox multivariate including TNM classification

Figure 5. Characteristics of a good biomarker and of the Immunoscore.
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