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Immunologic Biomarkers and Biomarkers for Immunotherapies in Gastrointestinal Cancer

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Keywords

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Abstract

Gastrointestinal (GI) cancers contribute significantly to the worldwide cancer burden. Pathologic evaluation is indispensable for the estimation of prognosis and therapeutic strategy. At present, immunotherapies are evolving into efficient therapeutic approaches, which are accompanied by the need for biomarkers to predict therapy response. In colorectal cancers, the only predictive biomarker for Food and Drug Administration-approved immunotherapy is the mismatch repair status. Besides, pathogenic *polymerase epsilon* mutations, tumor mutational burden, neoantigen load, and features of the immune contexture could soon find their way into clinical routine. Furthermore, in colorectal cancer, the Immunoscore, which is defined by the amount of CD3+ and CD8+ T-cells in the tumor center as well as at the infiltrative margin, might supplement the TNM system in the future (as TNM-Immune). This immunologic biomarker was shown to be impressively prognostic and predictive in colorectal cancer. In conclusion, there is increasing evidence of immunologic as well as predictive biomarkers for immunotherapies in GI cancers. Nevertheless, future progress is necessary for the variety of current advances to be implemented in clinical care.

Introduction

It is estimated that about 14.1 million new malignant tumors occurred in 2012 worldwide. Approximately 6 million of them occurred in developed countries, and about one of five was of gastrointestinal (GI) origin (colorectal, gastric, liver, or pancreatic cancer) [1]. In developed countries, almost every primary cancer diagnosis is based on pathologic evaluation. Besides the diagnosis, the main task of pathologic evaluation is the prediction of the clinical course to facilitate an adequate therapeutic approach. Especially for personalized medicine, biomarkers are of increasing importance. The AJCC/UICC TNM classification and histomorphologic assessment are still the backbone of risk stratification, but during the last years, the pathologist's repertoire has been supplemented. In particular, genetic biomarkers have paved the way to targeted therapies [2]. Now, immunotherapies are evolving into established therapies. The first immunologic treatment attempts were already carried out during the end of the 19th century, and Smith postulated a role of the immune system in cancer just a few years later [3, 4]. Nevertheless, advances in recent history have placed immunotherapies and the tumor-immune system interaction in the current limelight of cancer research. In 2013, the

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journal *Science* selected cancer immunotherapy as "breakthrough of the year" [5]. The importance of this development was definitely documented by awarding the Nobel Prize 2018 to James P. Allison and Tasuku Honjo for their fundamental contributions in this field [6]. The different targets and strategies of immunotherapy have been described in excellent reviews by Stein et al. [7], Procaccio et al. [8], and Sanmamed and Chen [9] and are only briefly described herein. According to Sanmamed and Chen [9], immunotherapies can be divided into concepts that enhance the immune system and strategies that normalize or restore the immune response to cancer. Enhancers comprise interferons, interleukins, anti-CTLA4 antibodies, and the very recently introduced genetically engineered T-cells (e.g., CAR-T). These therapies are hampered by high rates of immune-related adverse events. Normalizing or restoring concepts aim to release the brake of the immune response to activate its natural function. This can be realized by application of antibodies against the programmed death receptor 1 (PD-1) or its ligand (PD-L1).

Now, 5 years after the proclaimed breakthrough of the year 2013, we will discuss in this article the role of immunologic biomarkers as well as biomarkers for immunotherapies in GI cancers. Because of its outstanding model role, we will mainly focus on colorectal cancer.

Overview of Biomarkers in Immuno-Oncology

Like other biomarkers in oncology, immunologic biomarkers can be either prognostic or predictive. In many circumstances they are both. The most investigated prognostic immunologic marker is the occurrence of infiltration of a tumor by immune cells. Tumor-infiltrating lymphocytes have gained the highest relevance in many cancer entities. High numbers of cytotoxic T-cells are effective in establishing immune surveillance that is countable by conventional histology and immunohistochemistry. Despite the currently high importance of tumor-infiltrating lymphocytes, other immune cells such as eosinophilic or neutrophilic granulocyte, macrophages, and dendritic cells could also be shown to be prognostic [10–13]. Recently, our group suggested lymph node size as a potential new and very easy evaluable marker for immune response, with enlarged lymph nodes indicating an enhanced response against a tumor [14, 15].

Since the indications of immune system-enhancing therapy regimens such as anti-CTLA4 antibody and engineered T-cell administration are not based on biomarkers, the topic of predictive biomarkers in immunotherapy can be restricted to anti-PD-1 and anti-PD-L1 therapies. There are several possible strategies to estimate the chance of a response to anti-PD/anti-PD-L drugs (Table 1). First, the number of cells expressing those receptors or ligands can be measured. Currently, this is the main method to predict the response of the several antibodies that have entered clinical routine. More precisely, the PD-L1 scored by immunohistochemistry is of interest, although PD-L2 would have been another possibility for the prediction. PD-L1 assessment is a big matter of debate, and based on the substances and entities, there exist different ways how it has to be evaluated (Table 2). Those differences concern the cells that are counted and the cutoffs in different therapy lines. In the US, the antibodies and the technical platforms are specified by the Food and Drug Administration (FDA).

A second way to predict the likelihood of a therapy response is to identify features of a tumor that cause a particularly strong immune response. It is currently widely accepted that the production of neoantigens by a tumor is associated with a strong immune response. Tumors with a high number of mutations are more likely to produce such neoantigens. Mismatch repair-deficient (dMMR) tumors are also known to induce a particularly intensive immune response very likely due to the production of neoantigens. Table 1 gives an overview of established, upcoming, and potential biomarkers.

Colorectal Cancer

The field of immuno-oncology has gained an extraordinary dynamic with the development of several substances targeting different structures that have entered daily routine. Moreover, there are many other promising drugs and combination therapies in the pipelines of the pharmaceutical companies. Generally, its principles are applicable in all cancer types, including carcinomas, hematologic neoplasia, primary tumors of the central nervous system, and sarcomas [16]. This dynamic is also recognizable in GI cancers. Because of its outstanding model role, we mainly focus on colorectal cancer. In addition to the histomorphologic classification of colon cancer, there are several ways to subtype colon cancers. For example, it is possible to distinguish four molecular subtypes: the DNA mismatch repair capability of the tumors (mismatch repair-proficient [pMMR] versus dMMR tumors), the CpG island methylation phenotype, or the level of chromosomal instability [17, 18]. The heterogeneity of colon cancer already indicates that personalized immunotherapies, with the need for predictive biomarkers, have to be implemented to offer an effective therapeutic approach.

Predictive Biomarkers for Immunotherapies

The first therapy approaches with PD-1 inhibitors were discouraging as no objective response was seen [19].

Table 1. Established, upcoming, and potential predictive biomarkers for checkpoint inhibitor therapies

Biomarker	Method	Routine use	Remarks	Relevance in GI cancers	Reference
PD-L1	IHC	established	several different cutoffs and analysis guidelines	ESC, GC, GEJC, AC, HCC	Hazama et al. [53]
Microsatellite instability	PCR	established	approval by the FDA, but not yet by the EMA	especially for CRC, but tumor agnostic in general	Le et al. [21]
Mismatch repair deficiency	IHC	established	approval by the FDA, but not yet by the EMA	especially for CRC, but tumor agnostic in general	Le et al. [21]
Tumor mutational burden	NGS	upcoming	CheckMate 026 trial revealed superiority over PD-L1 testing; also relevant in SCLC and urothelial cancer	not yet	Schumacher and Schreiber [29]; Yaghmour et al. [60]; Buchhalter et al. [61]
POLE	PCR	upcoming		unclear	van Gool et al. [62]
PD-L2	IHC	potential		unclear	Schmid et al. [63]; Yearley et al. [64]
PD-L1/2 amplification	FISH	potential		unclear	Inoue et al. [65]
T-cell repertoire	NGS, nCounter	potential		unclear	McGranahan et al. [66]; Newman et al. [67]; Wallden et al. [68]
Human papillomavirus	PCR	potential	virus-induced inflammation	unclear, perhaps AC	Hong et al. [69]
Epstein-Barr virus	IHC, ISH, PCR	potential	virus-induced inflammation	GC	Kelly [70]

AC, anal cancer; CRC, colorectal cancer; EMA, European Medicines Agency; ESCA, esophageal squamous cancer; FDA, Food and Drug Administration; FISH, fluorescence in situ hybridization; GC, gastric cancer; GEJC, gastroesophageal junction cancer; GI, gastrointestinal; HCC, hepatocellular carcinoma; IHC, immunohistochemistry; ISH, in situ hybridization; NGS, next-generation sequencing; PCR, polymerase chain reaction; PD-L1, programmed death ligand 1; PD-L2, programmed death ligand 2; *POLE*, *polymerase epsilon*; SCLC, small-cell lung cancer.

Table 2. Overview of TPS, CPS, and ICS

Score	Definition	Cutoff	Category
TPS	$\text{TPS} = \frac{\text{number of PD-L1-pos. TC}}{\text{total number of TC}} \times 100\,[\%]$	>50% >25% and <50% >10% and <25% >5% and <10% >1% and <5% <1%	Cologne 5 Cologne 4 Cologne 3 Cologne 2 Cologne 1 Cologne 0
CPS	$\text{CPS} = \frac{\text{number of PD-L1-pos. TC} + \text{number of PD-L1-pos. IC}}{\text{total number of TC}} \times 100$	>10% (urothelial/renal cancer) >1% (GI/breast cancer) >1%	
ICS	$\text{ICS} = \frac{\text{area of PD-L1-pos. IC}}{\text{tumor area}} \times 100\,[\%]$	>10% >5% and <10% >1% and <5% <1%	IC 3 IC 2 IC 1 IC 0

CPS, combined proportion score; GI, gastrointestinal; IC, immune cells; ICS, immune cell score; PD-L1, programmed death ligand 1; pos., positive; TC, tumor cells; TPS, tumor proportion score.

Nevertheless, soon afterward studies elucidated that PD-1 inhibitor therapy was beneficial in metastatic dMMR colorectal cancer [20]. A phase II study with pembrolizumab, a PD-1 inhibitor, proved that the effectiveness of the immunotherapy depends on the mismatch repair status. None of the pMMR tumors responded to therapy, while dMMR tumors responded to a considerable extent [21]. Additionally, pembrolizumab was approved by the FDA in all solid dMMR tumors since it could be shown that deficient mismatch repair is predictive for treatment response, regardless of tumor origin [22]. Likewise nivolumab, another PD-1 inhibitor, is effective in metastatic dMMR colorectal cancer [23]. Biologically, dMMR tumors are predisposed for checkpoint inhibitor therapy as they are in particular dependent on checkpoint molecules [24]. Irrespective of that, the mentioned pembrolizumab studies also indicate that the number of somatic mutations and neoantigens play a central role in the effectiveness of PD-1 inhibitor therapy. High somatic mutation loads, a feature of dMMR tumors, were associated with prolonged progression-free survival [21]. Furthermore, T-cell clones that were reactive to mutant neopeptides were found in the tumor [22]. These findings are in alignment with other observations. In several indications, an increase in the tumor mutational burden has been associated with response to checkpoint inhibitor therapy [25–27]. Fabrizio et al. [28] recently identified in a cohort of 6,004 patients with colorectal carcinomas nearly 3% with pMMR tumors and high mutational burden. These patients could benefit from PD-1 inhibitor therapy, as do patients with dMMR tumors. Biologically, somatic mutations can generate neoantigens, which can be recognized by the host immune system [29]. Nevertheless, it has to be taken into account that the tumor-immune system interaction is complex. Galluzzi et al. [30] highlight that immunogenic cell death is dependent on antigenicity and adjuvanticity. According to that, the number of somatic mutations and neoantigens is just one of the two key factors.

Mutations in the *polymerase epsilon* (*POLE*) gene may serve as another potential predictive biomarker for PD-1 inhibitor therapy. About 1% of colorectal carcinomas harbor pathogenic somatic *POLE* mutations [31]. These *POLE*-mutated tumors also exhibit a hypermutated genome although they are pMMR tumors. Therefore, *POLE* mutations could function as another predictive biomarker for PD-1 inhibitor therapy. The first case of a clinical response of a metastasized, *POLE*-mutated colorectal carcinoma to pembrolizumab has already been published [32].

The predictive value of tumor-infiltrating lymphocytes regarding immunotherapy is not well established. One study described an association between tumor-infiltrating CD8+ T-cells and response to PD-1 inhibitory therapy in dMMR colorectal carcinomas [21]. However, this is in contrast with the observations in most other solid tumors [33].

Taken together, the mismatch repair status is an established predictive biomarker for treatment response for PD-1 inhibitor therapy in metastatic colorectal carcinoma. Tumor mutational burden as well as *POLE* mutations possess predictive value, but are not yet established as predictive biomarkers for PD-1 inhibitor therapy. Nevertheless, it can be assumed that in the near future, at least part of these biomarkers will find their way into routine clinical practice.

Immunologic Biomarker: Immunoscore

In 2006, the first landmark study was published by Galon et al. [34]. The group characterized immunologically a large cohort of human colorectal cancers by in situ immunohistochemistry as well as gene expression profiling. To summarize, it can be noted that the immunologic data outperformed the histopathologic prognostic factors for patient survival. Furthermore, similar results could be shown for the predictive value of CD8+ T-cells according to recurrence and patient survival [35]. The superiority might be explained by an inverse correlation of the immune infiltrate density (a positive prognostic factor) with the T classification, but constantly low immune infiltrate density in patients with relapsing early-stage tumors [35, 36]. Additionally, the predictive and prognostic value for tumor recurrence and survival could be shown for early-stage colorectal cancer as well as for rectal cancer [35, 37, 38]. In rectal cancer tumor biopsies, a high infiltration of CD3+ and CD8+ T-cells was associated with downstaging after chemoradiotherapy [38]. Additionally, the immune contexture was shown to be predictive for response to radiochemotherapy [39, 40]. Noteworthy, the prognostic value of the Immunoscore is independent of the microsatellite status [41, 42].

To sum up, these studies underscore the impact of the immune contexture, which is defined as type, functional orientation, density, and location of adaptive immune cells within distinct tumor regions [36, 43]. Based on these findings the Immunoscore was developed. It consists of a standardized immunohistochemical evaluation of T-cells (CD3+ and CD8+ cells) in the tumor center as well as the infiltrative margin [36]. A worldwide taskforce was already founded 2012 to evaluate and validate the Immunoscore [44]. The Immunoscore could be a new approach to classify cancer, designated as TNM-Immune [45–47]. In Mai 2018 the results of the international validation study of the consensus Immunoscore for the classification of colon cancer were published [48]. Tissue samples from 2,681 patients were included in the analyses (training set: 700 patients; internal validation set: 636 patients; external validation set: 1,345 patients).

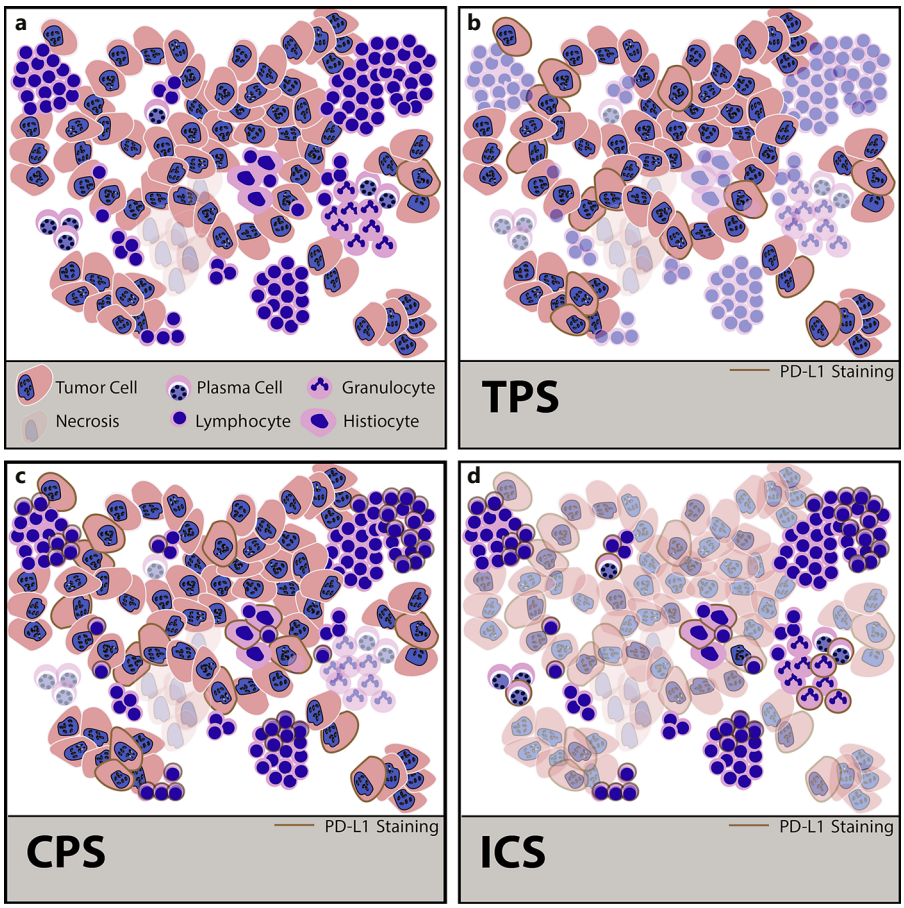

Fig. 1. a Schematic illustration of an epithelial cancer with a strong immune response consisting of plasma cells, lymphocytes, neutrophilic granulocytes, and histiocytes. **b** Tumor proportion score (TPS). Only the PD-L1-positive (membranous expression) tumor cells are counted. Immune cells stay unscored. **c** Combined proportion score (CPS). PD-L1-positive (membranous expression) tumor cells plus lymphocytes and histiocytes (membranous and/or cytoplasmic expression) are scored. The score has no dimension, the maximum value is 100 by definition. **d** Immune cell score (ICS). Only immune cells, including plasma cells and granulocytes, that cover the tumor area are counted. The score dimension of this score is percentage of area.

The results confirm a high level of reproducibility as well as the prognostic relevance of the Immunoscore. The hazard ratio for recurrence was only 0.2 for patients with a high versus low Immunoscore in the training set (95% CI 0.10–0.38; $p < 0.0001$). Eight percent of patients with a high Immunoscore had a recurrence at 5 years, in contrast to 19% with a low Immunoscore. These findings could also be confirmed in the two validation sets. Moreover, the Immunoscore had the highest relative contribution to the risk of all clinical parameters and existing prognostic factors. In a subpopulation of patients with UICC stage II cancer, the Immunoscore was also able to perform a risk stratification for recurrence at 5 years (hazard ratio for high versus low Immunoscore 0.33, 95% CI 0.21–0.52; $p < 0.0001$). In conclusion, the authors state that the results support the implementation of the consensus Immunoscore as a new component of a TNM-Immune classification of cancer [48]. Indeed, the results are promising and support the introduction in the evaluation of colon cancer. As the results were just recently published, it remains to be seen whether the Immunoscore becomes implemented in general recommendations and guidelines.

Immunologic Biomarker: Lymph Node Size

Recently, our group suggested lymph node size as a surrogate marker for the immune response in colon cancer. Lymph node enlargement is associated with an increased number of intratumoral lymphocytes and with favorable outcome [14, 49, 50]. Moreover, it correlates with the number of detected lymph nodes. Several findings indicate that that prognostic effect of lymph node count is not a stage migration effect, also called Will Rogers phenomenon. Instead, its true explanation is likely that the status of the immune response against the tumor is expressed in lymph node size [51].

Other GI Cancer Types

Because of its high incidence and prevalence, colorectal cancer is of importance and attracts major attention. However, other GI cancers occur less frequently but show more aggressive behavior than colorectal cancers, with 5-year survival rates between 9 and 31 months [52]. This causes an urgent need for improved therapy regimens.

It is beyond this review that focuses on biomarkers to discuss all these new substances and ongoing studies. In

the following, we will only give a rough overview. Concerning the details we refer to recently published excellent reviews that summarize the clinical aspects of immuno-oncology in GI cancers [7–9, 53].

Again, checkpoint inhibitors are the only immunotherapy concept that currently reaches praxis. Since the effectivity of pembrolizumab inhibitors has been proven in 12 entities other than colon cancer, the FDA approved pembrolizumab tumor agnostically for all microsatellite-unstable or dMMR-positive cases [21].

Beyond their important role in microsatellite-unstable/dMMR tumors, checkpoint inhibitors gain importance in GI cancers independent from mismatch repair status. Nivolumab received FDA approval in hepatocellular carcinoma [54] and pembrolizumab in gastric and gastroesophageal junction cancer [55]. Additionally, nivolumab is approved in Japan in gastric and gastroesophageal junction cancer [56]. A phase II trial has been conducted testing nivolumab in esophageal squamous cancer [57]. Several other phase III studies are ongoing to evaluate checkpoint inhibitors in earlier treatment lines. One phase II study investigated nivolumab in metastasized anal cancer and showed a response in 24% of patients. The response was dependent on PD-L1 expression [58]. Disappointing results came from investigations which aimed to introduce immuno-oncological approaches to the treatment of pancreatic cancer [59].

In contrast to colorectal cancer, immunohistochemical PD-L1 expression plays a major role in predicting the response of checkpoint inhibitors. The assessment of PD-L1 is complicated by several different guidelines depending on the tumor entities and the different inhibitors. The three currently used assessment regimens are explained in Table 2 and Figure 1. These assessment rules relate to the cell types that must be evaluated or excluded and the cutoff values. In opposite to the European Medicines Agency, the FDA also specifies the immunohistochemical antibodies and the staining platforms.

Conclusion

Immunotherapeutic approaches can be seen as the currently most promising new concepts in cancer. This is underscored by the Nobel Prize 2018 that went to researchers in this field. In GI cancers only checkpoint inhibitors have yet reached daily routine. The mismatch repair/microsatellite instability status is currently the only relevant predictive marker in colorectal cancer and has been identified as tumor agnostically predictive. In other GI cancers, however, the immunohistochemically determined PD-L1 status is the main predictor for these drugs. There are many more biomarkers under investigation, some of which may become relevant. They most likely ones are *POLE* and tumor mutational burden.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

All authors declare that no conflict of interests exists.

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