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REVIEW ARTICLE

Integrating histopathology, immune biomarkers, and molecular subgroups in solid cancer: the next step in precision oncology

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Abstract

For many years, the gold standard cancer grading and staging had focused on the characteristics of the cancer cells and often disregarded the non-tumoral cell compartments. The expansion of research on the tumor immune microenvironment, the successes and dissemination of immunotherapies to treat cancer, and the open access to large -omic databases have allowed the development of novel powerful immune-based prognostic and theranostic biomarkers. Although they often correlate with histopathologic characteristics and TNM staging, in many instances, they are independently associated with, and potentially superior predictors of, the patient's prognosis and response to immunotherapies. As pathologists in the era of precision medicine, we are uniquely positioned to participate in the integration of these histologic and molecular features of the tumor microenvironment to provide the best prognostic information to clinicians and patients. In this review, we summarize some of the most important immune-related prognostic biomarkers in solid cancer, how they integrate with traditional histopathologic (i.e., staging and grading) and novel molecular stratification systems, and their potential role as predictors to response to agents blocking the PD-1/PD-L1 axis.

Keywords Tumor immune microenvironment · Biomarkers · Molecular subgroups · Immune checkpoints

Introduction

Improving tumor subclassification systems (grading and staging) to predict the patient's clinical outcome and guide therapies has been one of the major endeavors of modern oncopathology. To date, the most widely used tumor staging system is the one proposed by the American Joint Committee on Cancer/Union Internationale Contre le Cancer (AJCC/UICC), which takes into consideration the size and local invasion of the tumor (T), the number of metastatic regional lymph nodes (N), and the presence of distant metastasis (M). The TNM has been validated in the vast majority of cancer types and has proven useful to predict patients' clinical outcome [1–4]. Also, several histopathologic characteristics of the tumor give clues regarding the malignant potential of cancer cells. For example, the presence of necrosis [5], an increased number of mitoses, higher nuclear grade or pleomorphism (variation in shape and size), the presence of lymphovascular invasion [6], and a sarcomatoid/rhabdoid architecture [7] point towards an aggressive tumor cell phenotype and correlate with poor clinical outcome.

For many years, these staging and grading systems had focused on the characteristics of the cancer cells and did not integrate other cell compartments present within the tumor microenvironment. However, in the last two decades, cumulative evidence has demonstrated that the non-cancer cell components of the tumor largely influence its growth rate and metastatic potential [8, 9]. Hence, the pathology field is currently experiencing a transition where new prognostic and theranostic biomarkers need to be integrated with more traditional staging and grading systems.

The two main non-cancer cell cellular components that shape the tumor's natural history are the infiltrating immune cells [8, 10] and the tumor-associated stromal cells [11]. In

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recent years, several groups, including ours, have extensively investigated the prognostic impact of different immune cell populations within cancer. Furthermore, a large amount of checkpoint blockade clinical trials have shown that the characteristics of the tumor immune microenvironment can predict the patient's response to therapy.

Novel cancer classification systems that integrate the biology of the tumor cells, the immune microenvironment, the associated-stromal compartments, and more traditional pathology grading systems are emerging in the literature [12–16]. In this review, we discuss the evidence linking the composition of the tumor immune microenvironment with patient's prognosis and response to checkpoint blockade in solid cancer, as well as more recent evidence integrating novel technologies with traditional pathology staging and grading systems to improve their predictive potential.

The tumor immune microenvironment

Innate immunity

The immune response can be divided into two main compartments: the innate and the adaptive [17]. By definition, the innate response comprises all the cellular and extracellular elements that provide the first line of immunity in an effective, but poorly specific manner, and generally lack memory responses. The cells and proteins of the innate immunity play a crucial role in the initiation and subsequent activation of the adaptive immune system. Cells within this compartment include polymorphonuclear cells (PMNCs), macrophages, and natural killer (NK) cells.

PMNCs and macrophages usually initiate the inflammatory response through the activation of pattern-recognition receptors that allow them to detect pathogen-associated molecular patterns (e.g., Toll-like receptors and nucleotide-binding oligomerization domain) on infectious organisms, damaged tissues, or tumor cells [18]. Neutrophils are the more abundant PMNC and are usually the first line of cellular host defense against pathogens. Although previously thought that these cells had a short half-life and low functional plasticity, it is now recognized that they can survive for up to 5 days in the circulation, may potentially live for weeks in tissues, and can exhibit a large spectrum of functional phenotypes [19, 20]. Furthermore, they play a key role in orchestrating the innate and adaptive immune responses by releasing cytokines and chemokines. The functional orientation of neutrophils within tumors can be polarized into an "N1" or "N2" phenotype depending on the cytokine milieu [21]. While N1-neutrophils express immunoactivating and antitumoral cytokines (e.g., TNF-α and IL-12), N2 express pro-angiogenic (e.g., VEGF) and immunosuppressive (e.g., TGF-β1) molecules [22].

Macrophages are also actively implicated in the recognition and elimination of tumor cells. Similarly to neutrophils, macrophages can have at least two functional orientations in tumors depending on the cytokine milieu and the molecules triggering their activation. M1-macrophages are induced through Toll-like receptor activation or IFN-γ and preferentially express pro-inflammatory cytokines that potentiate the anti-tumor immune response. In contrast, M2-macrophages are induced by IL-4 or IL-13 and express immunomodulatory molecules (e.g., TGF-β1 and PDGF) implicated in fibroblast proliferation and extracellular matrix deposition [23].

NK cells are cytotoxic lymphocytes that, in contrast to $CD8^+$ T cells, do not need priming to exert anti-tumor activity. Our understanding of the function of NK cells in infectious processes and cancer has evolved significantly in recent years [24]. NK cells get activated when their receptors (e.g., NKG2D) binds to molecules expressed by cells in "distress," such as MICA or MICB on tumor cells. To avoid autoimmunity, NK cells also express inhibitory receptors that recognize major histocompatibility complex (MHC) class I molecules expressed in virtually all healthy cells. As tumor cells often downregulated MHC class I molecules, they are frequently targeted by NK cells [25].

Dendritic cells (DCs) are in charge of bridging the innate and adaptive immunity, as they process proteins expressed by the invading pathogens or tumor cells and present them as short peptides to T cells in the context of MHC molecules (aka priming) to initiate the adaptive immune response [26]. Through the production of cytokines, DCs play a key role in orienting the subsequent adaptive immune response. As neutrophils and macrophages, DC are highly plastic. While bona fide DC express all the cytokines that are necessary for T cell activation, pro-inflammatory tolerogenic DC can be immunosuppressive and promote a dysfunctional immune response. [27]

Classically, it has been thought that the T cell priming occurs exclusively in lymph nodes. Nevertheless, it is now well accepted that it can also occur within tertiary lymphoid structures (lymphoid aggregates present at the invasive margin of most tumors, TLS) [28]. These structures are rich in high-endothelial venules specialized in recruiting naïve T and B cells from the circulation and concentrate large numbers of activated DC.

The adaptive immunity

The adaptive immunity comprises all the cells that build an antigen-specific immune response. Based on their antigen specificity, this arm of the immune system demonstrates memory responses—enhanced reaction in response to repeated antigen challenge [29]. The cells in this compartment include T ($CD4^+$ and $CD8^+$) and B cells. $CD8^+$ T lymphocytes are extremely efficient cytotoxic cells that can destroy tumor cells

through the recognition of antigens expressed on their surface through HLA class I molecules. In contrast to NK cells, $CD8^+$ lymphocytes need to be primed before they can exert their cytotoxic functions [30].

$CD4^+$ T lymphocytes, also called helper T cells, exert multiple functions and are usually associated with the orchestration of all the cellular elements of the immune response. In fact, $CD4^+$ T cells can recruit PMNC, induce macrophage microbicidal activity, help B cells make antibodies, and activate DC to potentiate $CD8^+$ cell priming [31]. $CD4^+$ T cells can have multiple functional orientations, including Th1, Th2, Th17, and regulatory T cells (Treg). Classically, it has been described that Th1 cells potentiate the cellular immune response (IL-2 and TNF-a), Th2 the antibody-mediated immune response (IL-4, IL-5, and IL-13), and Th17 the mucosal immunity (IL-17, IL-21, and IL-22) [32]. Treg mainly mediate peripheral tolerance and help in the maintenance of the immune homeostasis. They exert this effect by suppressing the effector cell responses through different mechanisms, including the production of inhibitory cytokines (e.g., IL-10, TGF-β, and IL-35) and the suppression of DC development and maturation [33]. Finally, B cells are mainly not only specialized in the production of antibodies, but they also act as antigen-presenting cells (APC) and can prime T lymphocytes. The role of the humoral immune responses against cancer remains controversial. Nevertheless, it is well known that antibodies produced by B cells can opsonize tumor cells, activate the complement cascade, and contribute to NK cell-mediated tumor killing via antibody-dependent cell-mediated cytotoxicity [34].

Virtually, all these types of immune cells can infiltrate tumors, and they establish very complex interactions with the tumor and stromal cells (Fig. 1).

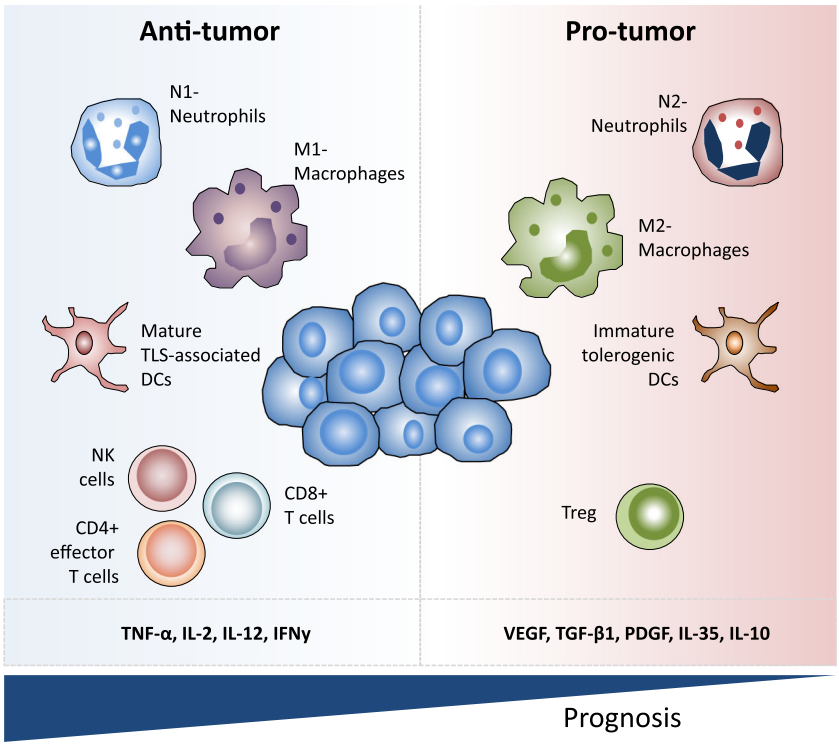

Fig. 1 The double-edged sword of the tumor immune microenvironment. The tumor-infiltrating immune cells are highly plastic and can exhibit a pro- or anti-tumoral functional orientation. Neutrophils can be attracted by chemokines secreted by the tumor, stromal, or other inflammatory cells within the tumor microenvironment (TME). In situ, these cells get activated by damage-associated molecular patterns derived from necrotic tumor cells and acquire an anti-tumoral or pro-tumoral function (N1 or N2, respectively). Similarly, monocytes are avidly recruited to the TME, and they play a significant role in potentiating and orchestrating the inflammatory immune response. While M1 macrophages usually promote tumor elimination through the activation of cells of the adaptive immunity, M2 macrophages are immunosuppressive. Intra-tumor NK cells can recognize and kill tumor cells expressing stress-related membrane receptors (e.g., MICA or MICB) that have downregulated HLA-class I molecules. Dendritic cells (DC) are in charge of priming the adaptive immune system. These cells process the tumor-related antigens and present them to the $CD8^+$ and $CD4^+$ T cells. Depending on the cytokine milieu, the DC can induce a dysfunctional or an effective T -cell activation and thus play a vital role in the orchestration of the adaptive anti-tumor immune response. Once activated by DCs, and in a process potentiated by Th1 $CD4^+$ lymphocytes, $CD8^+$ T cells can recognize antigens expressed in the membrane of tumor cells and release cytotoxic molecules to kill them. Interestingly, tumor cells can express immunosuppressive cytokines or membrane attached molecules (e.g., PD-L1) that inhibit the function of the infiltrating lymphocytes. Finally, regulatory $CD4^+$ T cells express several cytokines whose functions are to inhibit the cellular immune cell response and thus promote tumor cell growth indirectly. The composition of these immune cells within the tumor microenvironment strongly correlates with the patient's clinical outcome

Immune cells as prognostic biomarkers in solid cancer

The analysis of the immune component of the TME in large cohorts of cancer patients has established a clear correlation between the density and functional orientation of the infiltrating immune cells and patient's clinical outcome (reviewed in detail in [10, 12, 35]).

Tumor infiltration with cells from the innate immunity has been associated with various clinical outcomes. For example, increasing intra-tumor neutrophil densities have been linked with detrimental outcome in some cancer types, including renal cell carcinoma, urothelial cancer, head and neck cancer, or esophageal carcinoma [36–39], whereas it has been associated with better survival in colorectal and gastric cancer [40–42]. Similarly, several studies have found that the clinical outcome associated with increased numbers of tumor-associated macrophages (TAMs) is variable across tumor types [43]. For example, while high densities of this population are associated with favorable clinical outcome in colorectal carcinoma (CRC), gastric, non-small cell lung cancer (NSCLC), hepatocellular carcinoma (HCC), and prostate and cervical cancers, the opposite association has been observed in endometrial, esophageal, urothelial, oral, breast, ovarian, and bladder tumors (reviewed in [12]). The heterogeneous association of TAM with prognosis across tumor types is partially resolved when examining their functional status rather than simple representation. Thus, increased M1 TAM densities are associated with a favorable clinical outcome in NSCLC, ovarian, CRC, and gastric cancer, while M2 TAMs are linked to poor prognosis in mesothelioma, esophageal, gastric cancer, pancreatic, CRC, HCC, Hodgkin lymphoma, renal cancer (RCC), urothelial, breast, endometrial, ovarian, melanoma, and squamous cell carcinoma of the oral cavity (reviewed in [12]). Consistently with their efficient anti-tumor function, increasing densities of tumor-associated NK cells have been associated with favorable clinical outcome in CRC, gastric, vulvar, esophageal, HCC, NSCLC, and renal cancer (reviewed in [12]). As mentioned previously, DCs play an important role in the establishment of anti-tumor immunity and act as a bridge between the innate and adaptive immune cell responses. Overall, the augmented tumor infiltration with DC is associated with increased overall survival in many tumors types, including melanoma, HCC, gallbladder, oral, esophageal, gastric, NSCLC, CRC, bladder, breast, endometrial, and ovarian cancers (reviewed in [12]). The presence of TLS has also been associated with a positive prognosis in primary melanoma, NSCLC, RCC, colorectal, and breast cancer [12].

An overwhelming number of studies supports that tumor infiltration by the cells of adaptive immunity—in particular, cytotoxic $CD8^+$ and Th1 $CD4^+$ T cells—strongly correlates with good clinical outcome [10, 12]. More than 85 articles, with ~ 13,000 samples analyzed, have found that high infiltration by $CD8^+$ T lymphocytes is associated with favorable prognosis in multiple tumor types including lung, liver, stomach, CRC, breast, esophageal, bladder, melanoma, ovarian, and prostate cancers (reviewed in [10, 12]). However, there are exceptions to this rule, including diffuse large B cell lymphoma [44], Hodgkin lymphoma [45], and RCC [14, 46, 47], where high densities of tumor-infiltrating $CD8^+$ T and Th1 cells have been associated with poor prognosis. While the sheer volume of studies is not as immense as for T cells, there is evidence suggesting that tumor infiltration by B cells correlates with a favorable prognosis in NSCLC, primary cutaneous melanoma, breast cancer, and ovarian cancer [12].

TNM stage, histopathologic grading, and tumor-infiltrating lymphocytes

As expected from the strong correlation between the tumor immune microenvironment and patient's prognosis, numerous studies have established a close association between the number and type of tumor-infiltrating immune cells and other *classical* histopathologic prognostic biomarkers. However, despite this close correlation, the immune parameters have proven to be independent predictors of the patient's survival in multiple cancers. The association between the tumor immune microenvironment and histopathologic features has been extensively studied in several cancer, including breast cancer, melanoma, CRC, NSCLC, and renal cell cancer.

Studies analyzing large cohorts of breast cancer patients (~4000 in total) have established that higher tumor-infiltrating lymphocyte (TIL) grades or increasing $CD8^+$ cell densities are associated with higher histologic grades, higher proliferation indices, and no expression of ER, PR, and/or HER2. [48–55] Despite these associations, TIL grade has proven to be an independent prognostic factor for overall and disease-free survival (OS and DFS, respectively) in breast cancer [52, 56]. This is a good example where increasing numbers of TIL associate with higher tumor grade and stage but overall correlate with good prognosis.

In melanoma, the inclusion of semi-quantitative grading systems assessing the degree of TIL infiltration on H&E stained slides to the pathology reports (College of American Pathology, cancer protocol templates for melanoma [57]) has provided abundant evidence supporting a complex correlation between tumor grade, stage, immune microenvironment, and the patients clinical outcome. In fact, higher TIL grades have been associated with lower Breslow thickness, absent mitosis, but with higher growth phase (vertical and radial). Despite these correlations, TIL grade is an independent predictor of longer DFS in melanoma [58–61].

CRC is another cancer type where the association between TIL, tumor stage, and patient's prognosis has been studied in

detail. Most studies evaluating the density of TIL in CRC have adopted a standardized method of digital pathology quantification of IHC stains called Immunoscore. This method is based on the quantification (cells/mm^2) of two lymphocyte populations (CD3$^+$ and CD8$^+$) within the central region and the invasive margin of the tumor and provides a scoring system ranging from Immunoscore 0 (I0) to Immunoscore 4 (I4) [62, 63]. Centralized classification of thousands of CRC (> 3500 cases) has shown a strong association between higher T cell densities and longer DFS and OS [16, 64]. Furthermore, this association seems to be independent of the T and N stages and MMR status. The Immunoscore represents an example of how elements of the immune microenvironment can be quantified in a standardized way and incorporated into a prognostic index. Other studies in CRC, not using Immunoscore, support the association between increasing CD8$^+$ T cell densities with lower T stage tumors and poor histologic differentiation [65, 66].

As mentioned previously, RCC is one of the few tumors where increased densities of CD8$^+$ T cells are associated with poor clinical outcome. Our group has previously documented that the nuclear grade (Fuhrman grade) of the tumors, as well as the presence of necrosis, is associated with CD8$^+$ TIL densities and the expression of T cell inhibitory receptors (i.e., PD-1, Tim-3, and Lag-3) [46, 47]. Interestingly, we have also observed that within a given tumor, the density of TIL is higher in areas with increased nuclear grade (unpublished data; Fig. 2). This finding suggests that more aggressive/poorly differentiated tumor cells more avidly recruit TIL from the circulation due to either the expression of pro-inflammatory factors or changes in their immunogenicity.

Integrated tumor classification: molecular subgroups

With the development of high throughput technologies—whole exome sequencing, transcriptomics, and proteomics—multiple novel methods of tumor subclassifications are being developed and validated (methods reviewed in [67]).

One of these novel classification schemes termed "Molecular subgroups" refers to subcategorizing the tumors according to their gene expression profile. In general terms, this is achieved through techniques that allow quantifying the levels of messenger RNA of thousands of genes in fresh frozen or FFPE tumor specimens, a method known as transcriptomic classification. This data is integrated through unsupervised clustering methods whose main goal is to find

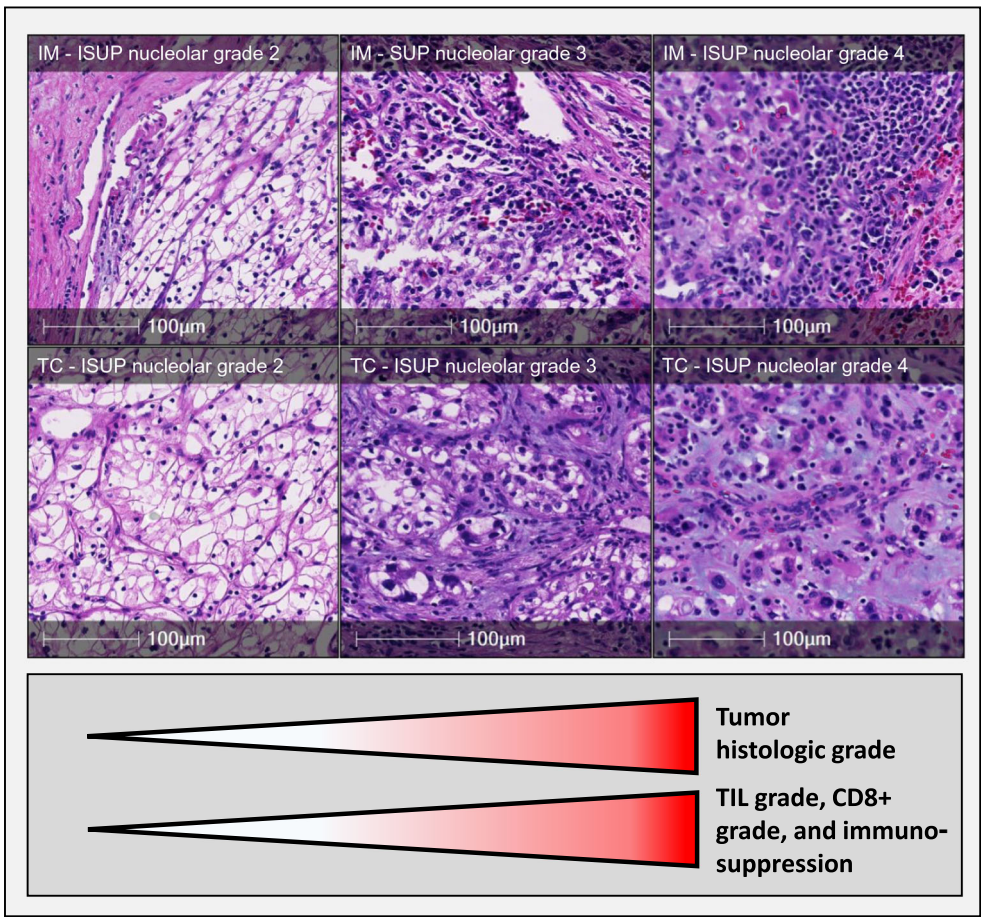

Fig. 2 Histopathology heterogeneity and immune cell infiltration in ccRCC. Photomicrographs of different representative areas of one clear cell RCC (H&E stain). As shown in these images, the inflammatory infiltrates increased with the nucleolar International Society of Urological Pathology (ISUP) grade. This is one representative case. IM invasive margin, TC tumor center

tumor subgroups with similar gene expression profiles. Several studies have also included DNA sequencing analysis, methylation studies, and micro-RNA into these classification algorithms. Interestingly, although these molecular subgroups are defined based on the gene expression profile only, they share in common molecular pathways (e.g., Wnt, MYC, and RAS expression), genetic mutations (e.g., KRAS mutation or MSI tumors), and clinicopathologic characteristics. This method has been widely used in numerous tumor types, including CRC [68] and RCC [14].

CRC is one of the cancer types where molecular subgroups have been more widely studied. In general, four consensus molecular subgroups have been defined in this tumor, as follows: microsatellite instable-enriched subgroup (CMS1), canonical subgroup (CMS2), metabolic (CMS3) subgroup, and mesenchymal subgroup (CMS4). CMS1 (~ 14%) is characterized by defective DNA mismatch repair machinery (microsatellite-instability, MSI, high), causing an increased rate of mutagenesis as compared to microsatellite-stable tumors. This results in an increased infiltration with immune cells, overexpression proliferation-related genes, and overall good prognosis. CMS2 (~ 37%) predominantly display epithelial genes including WNT and MYC signaling activation and are associated with intermediate patient's prognosis. CMS3 (~ 13%) is enriched in KRAS-activating mutations that induce multiple metabolism signatures, and intermediate prognosis. Finally, CMS4 (~ 23%) shows overexpression of epithelial-mesenchymal transition genes, complement-associated inflammation, matrix remodeling, stromal invasion, and angiogenesis, in addition to poor prognosis. Interestingly, these subgroups correlated with different histopathologic parameters. For example, CMS1 are predominantly right side lesions with advanced histologic grades. CMS2 are predominantly well-differentiated tumors. And CMS4 are usually stages III and IV at the time of diagnosis, exhibit prominent desmoplastic reaction, fibroblasts proliferation, and angiogenesis [13, 15].

With similar methods, clear cell RCC has been subdivided into four molecular subgroups [14]. ccrcc1 is characterized by upregulation of MYC targets and a stem cell phenotype; ccrcc2 is characterized by a silent phenotype, with no specific molecular pathway activation; ccrcc3 molecularly resembles no-tumoral kidney tissue; and ccrcc4 is characterized by upregulation of MYC targets and prominent inflammation. Interestingly, ccrcc4 subtype demonstrated specific pathologic characteristics, including sarcomatoid differentiation, more advanced nuclear grades, and is associated with worse prognosis.

Recently, multiple efforts have been undertaken to establish *universal* molecular classification across all cancer types (pan-cancer molecular subgroups). Through unsupervised clustering methods integrating data from 5 platforms (aneuploidy, DNA hypermethylation, mRNA, microRNA, and proteins) in 10,000 tumors, Hoadley et al. [69] recently described 28 pan-cancer subgroups. Approximately two thirds of them were enriched in tumors deriving from a single organ, for example, pan-kidney, pan-gastrointestinal, and pan-gynecological, suggesting that—from a molecular point of view—tumors derived from the same organ system share similar molecular signatures. These clusters could be further subdivided into subgroups, based on specific genetic mutations. For example, pan-GI could be subdivided into Epstein barr-associated tumors or MSI high and pan-squamous cell carcinoma into human papilloma virus (HPV)-related and HPV-unrelated clusters. The other third of the clusters were dominated by specific mutational patterns (i.e., chr9 deletion, BRCA-HER2 amp, and ERBB2-amplified tumors). Finally, they also described a subgroups of tumors across cancer types with a mesenchymal-signature. Due to the novelty of these findings, their clinical relevance has been not assessed in detail.

A similar effort to develop a pan-cancer immune classifier was recently undertaken by Thorsson et al. [70], who analyzed 10,000 tumors and described six immune clusters (C1-C6). C4 and C6 subtypes conferred the worst prognosis and displayed a macrophage dominated, low lymphocytic gene signatures. In contrast, C2 and C3 were dominated by type I immune response (e.g., cytotoxic T cells and Th1 $CD4^+$ T cells) and had the most favorable prognosis. Interestingly, among these groups, C2 showed a less favorable survival despite having an IFN-γ dominant high lymphocytic infiltrate, a $CD8^+$ T cell-associated signature, and high M1-macrophage content, suggesting a robust anti-tumor immune response. This could be related to the fact that IFN-y can upregulate different immunosuppressive mechanisms within tumors, such as PD-L1 expression.

The tumor immune contexture as a theranostic biomarker

Manipulating the immune response to control and eliminate cancer cells has been tested for at least 20 years. In the late 1990s, the treatment with recombinant IL-2 was associated with response in up to 10% of patients with melanoma or renal cell carcinoma. The discovery of inhibitory receptors expressed on the T cells leads to the development of blocking monoclonal antibodies that boosted the immune response (checkpoint blockade). The first antibody of this class was ipilimumab, which targeted an inhibitory molecule (CTLA-4) preferentially expressed on $CD4^+$ T cells. By boosting the activity of these cells, ipilimumab helps to expand the number of $CD8^+$ T cell clones infiltrating the tumors. Due to a moderate improvement in the overall survival of patients with metastatic melanoma, ipilimumab was approved by the FDA as monotherapy for advanced melanoma in 2011.

Another inhibitory receptor that has become widely known in recent years is PD-1. This molecule is expressed on $CD8^+$

and CD4$^+$ T cells, B cells, and NK cells (Giraldo et al. 2018), and the interaction with its ligands (i.e., PD-L1 and PD-L2) induces a strong inhibition of cytokine production, proliferation, and cytotoxic granule formation [71–73]. Several clinical trials evaluating the activity of PD-1/PD-L1 blocking agents in different tumor types have resulted in remarkable outcomes [74]. The durable objective response rate (ORR) following these therapies ranges from 32–42% in melanoma to 14–21% in RCC. The vast majority of recent FDA approvals of checkpoint blockade agents have been molecules targeting PD- or its ligand, PD-L1.

Although remarkably successful, it has become a challenge to identify with patients will respond to PD-1/PD-L1 blocking therapy. Several biomarkers to predict clinical response to have been evaluated. Both the pre-therapy abundance of tumor-infiltrating lymphocytes (TIL) and the expression of PD-L1 by the tumor or immune cells have proven to be reliable markers to predict the tumor's sensitivity to anti-PD-1/PD-L1 agents [75].

TIL densities and response to anti-PD-1/PD-L1 therapies

Some clinical trials have evaluated the correlation between TIL densities and clinical response to anti-PD-1/PD-L1 therapies. Chen and colleagues [76] studied an extensive set of immunohistochemical immune markers in a cohort of patients with metastatic melanoma, initially treated with anti-CTLA-4 followed by anti-PD-1 at progression ($n = 46$). They reported higher densities of CD3$^+$, CD8$^+$, and CD45RO+ (memory) TIL in the pre-treatment biopsies from patients who responded to anti-PD-1 (nivolumab) as compared to non-responders. A similar association between CD8$^+$ TIL numbers and response to treatment has been reported in patients with melanoma and colorectal cancer treated with pembrolizumab (human IgG4 anti-PD-1 monoclonal antibody) [77, 78] or atezolizumab (human IgG1 anti-PD-L1 monoclonal antibody) [79]. Interestingly, two independent studies have reported that the degree of CD8$^+$ TIL infiltration is not associated with response to anti-PD-1 in metastatic RCC [79, 80].

PD-L1 expression and response to anti-PD-1/PD-L1 therapies

The first clinical trial using nivolumab in patients with cancer (melanoma, NSCLC, RCC, prostate cancer and colorectal cancer) suggested that the expression of PD-L1 in pre-treatment specimens was associated with response to treatment (ORR 36% in patients with PD-L1+ tumors vs. 0% in patients with PD-L1− tumors) [81]. Another clinical trial evaluating the activity of atezolizumab in different solid malignancies (NSCLC, melanoma, RCC, head and neck, breast, colorectal, among other) also described a significant association between the expression of PD-L1 by tumor cells and ORR (45% vs. 16% in PD-L1+ and PD-L1− tumors, respectively) [82]. Subsequently, the majority of clinical trials assessing the response to PD-1–PD-L1 blockade have evaluated the protein expression of PD-L1 by the tumor or infiltrating immune cells and established its association with clinical outcome. We reviewed the clinical trials published to date that have studied the association between the activity of agents blocking the PD-1–PD-L1 axis and the protein expression of PD-L1 (Fig. 3, unpublished data). We included patients who received anti-PD-1 or anti-PD-L1 blocking agents in the first line setting, as well as higher order therapy. Overall, the ORR to anti-PD-1 therapy in patients with PD-L1+ tumors has been approximately 38%, as compared to 20% in those harboring PD-L1− cancers. Similarly, the ORR to anti-PD-L1 therapy in patients with PD-L1+ tumors is 29% versus 11% in patient's whose tumors are negative for PD-L1.

Although the general pattern is consistent, the absolute response rates and strength of the association between the expression of PD-L1 and response to PD-1/PD-L1 blockade vary across tumors. In NSCLC, clinical trials with nivolumab [83–87], pembrolizumab, [88, 89] atezolizumab [82, 90], and avelumab [91] have shown that 30% and 19% of the patients with PD-L1+ and PD-L1− tumors, respectively, respond to these agents. Similarly, in urothelial cancer clinical trials using nivolumab [92], atezolizumab [93–95], durvalumab [96, 97], and avelumab [98] have shown response in 27.5% of patients with PD-L1+ tumors and 11% in patients with PD-L1−. In renal cell carcinoma, treatment with nivolumab [99], or atezolizumab [79], has yielded responses in 24% of patients with PD-L1+ tumors and 16% in the one with PD-L1− ones. In melanoma, clinical trials have only evaluated the efficacy of nivolumab [100–104] and have reported an ORR in 53% of the patients with tumors expressing PD-L1 and 34% in those without expression. Other clinical trials have found similar

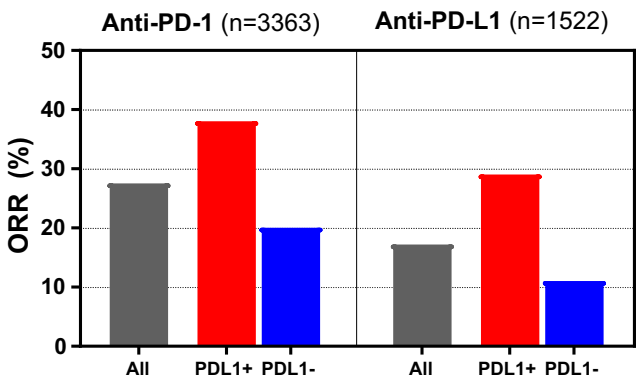

Fig. 3 ORR to anti-PD-1 or PD-L1 therapy in cancer according to the expression of PD-L1 by tumor cells. The ORR to PD-1/PD-L1 blocking agents (nivolumab, pembrolizumab, atezolizumab, or durvalumab) in 28 clinical trials [79, 81–108], including $n = 3363$ patients, stratified by PD-L1 status (cutoff for defining positivity varies from 1 to 50%). Last update, May 2018

results for head and neck squamous cell carcinoma [105] and gastric cancer [106]. Interestingly, in Merkel cell carcinoma (MCC), one study evaluating the efficacy of pembrolizumab [107] reported a trend towards higher ORR in patients bearing PD-L1− tumors (45%) as compared to PD-L1+ (33%). In contrast, one clinical trial using avelumab [108] in MCC showed higher ORR in patients with PD-L1+ tumors (34%) as compared to PD-L1− (19%).

Conclusions

Pathology has been the cornerstone of the tumor grading and classification systems, whose ultimate goal is to predict the patient's clinical outcome and to determine the need for neo-adjuvant or adjuvant treatments. Until recently, these systems mostly relied on the analysis of the tumor cell morphology and cancer's local extension. With the tremendous expansion of our understanding of the cancer biology in the last 15 years, it has become evident that the characteristics of the tumor cells (i.e., genetic or epigenetic traits), immune cells (i.e., densities and phenotype), and stromal cells (i.e., abundance and phenotype) needed to be integrated with the tumor histomorphologic features in order to predict with precision the patient's clinical outcome. As pathologists in the era of precision medicine, we are uniquely positioned to perform this integration and should continue to strive to advance of histologic and molecular understanding of the tumor microenvironment to provide the best prognostic information to clinicians and patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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