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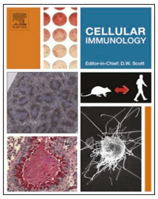

Review article

Role of tumor-infiltrating lymphocytes in patients with solid tumors: Can a drop dig a stone?

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ABSTRACT

In recent years, multiple strategies for eliciting anti-tumor immunity have been developed in different clinical studies. Currently, immunotherapy was clinically validated as effective treatment option for many tumors such as melanoma, non-small cell lung cancer (NSCLC) and renal cell carcinoma (RCC). Some surface receptors of immune cells, called immune checkpoint receptors, may inhibit activity of proinflammatory lymphocytes, following binding with specific ligands. Cancer cells exploit these mechanisms to inactivate tumor-infiltrating lymphocytes (TILs) to escape from immunosurveillance. Among the different tumor-infiltrating immune cell populations, including leucocytes, macrophages, dendritic cells and mast cells, TILs are considered a selected population of T-cells with a higher specific immunological reactivity against tumor cells than the non-infiltrating lymphocytes. In this review we will discuss the promising role of TILs as biomarkers reflecting the immune response to the tumor, describing their potential ability to predict the prognosis and clinical outcome of immunotherapy in some solid tumors.

1. Introduction

In recent years, multiple strategies for eliciting and enhancing anti-tumor immunity have been developed and evaluated in different clinical studies [1,2]. Currently, immunotherapy was clinically validated as an effective treatment option for many tumors such as melanoma, non-small cell lung cancer (NSCLC) and renal cell carcinoma (RCC) [3–5]. Some surface receptors of immune cells, called immune checkpoint receptors, may inhibit activity of killer and pro-inflammatory lymphocytes, following binding with specific ligands [6]. Cancer cells exploit these mechanisms to inactivate tumor-infiltrating lymphocytes (TILs) in order to escape from immunosurveillance and survive [7,8]. Three signals are required for T cell activation: i) the interaction between tumor-associated antigens exhibited by major histocompatibility complex (MHC) and T-cell receptor (TCR) [9]; ii) the interaction between receptors on T cells and co-stimulatory molecules on antigen-presenting cells (APCs) [9]; iii) the release of inflammatory cytokines such as IL-12 [10].

The binding of the Programmed Death 1 (PD-1) receptor, mainly expressed on the surface of T cells, to the PD-1 receptor ligands (PD-L1 and PD-L2), expressed on tumor cells, represses the T cell activation signal triggered by binding between MHC and TCR, by inducing T cell apoptosis, resulting in inhibition of their ability to target cancer cells. Therefore, an increase in expression levels of PD-1/PD-L1 may be detected in many tumors with poor prognosis [11]. Likewise, also the cytotoxic T lymphocyte-associated protein 4 (CTLA-4), has been shown to suppress T cell activation [12]. Monoclonal antibodies (mAbs) targeting mainly PD-1/PD-L1 and CTLA-4 were recently developed for cancer immunotherapy, showing a significant success in clinical practice [12–15]. The checkpoint blockade has been shown to act by activating directly cancer-specific T cells of the immune system in order to eradicate tumor. However, many others immune checkpoints are to date known and some of them could be involved in immune attack against cancer [6,16].

Among the different tumor-infiltrating immune cell populations, including leucocytes, macrophages, dendritic cells and mast cells, TILs

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are considered a selected population of T-cells with a higher specific immunological reactivity against tumor cells than the non-infiltrating lymphocytes [17,18]. The major component of tumor microenvironment is represented by TILs containing different fractions of CD3+ CD4+ (helper) and CD3+ CD8+ (cytotoxic) T cells [19]. At first, TILs were mainly used as potential effectors of adoptive T-cell therapy in metastatic melanoma through their isolation from tissue, *in vitro* clonal expansion and autologous administration. However, some limitations resulting from the restricted availability of TIL sources and significant toxicity induced by combination therapy with high-dose interleukin-2 (IL-2) led to the failure of this approach [20]. Subsequently, the presence of TILs in tumor tissues and their potential impact on the prognosis were studied by means of immunohistochemistry (IHC) analysis, suggesting a favorable prognostic role in melanoma. In recent years, similar results have been detected in other solid tumor, including lung cancer, RCC, breast and ovarian cancer, etc [21]. Currently, many studies are ongoing to assess the predictive value of TILs in several tumors subjected to different immunotherapeutic and chemotherapeutic treatments [22–24]. In fact, for some tumors, such as melanoma and ovarian cancer, TIL accumulation induces the CD8+ cell-mediated production and release of interferon gamma (IFN-γ) which, in turn, increases PD-L1 expression in tumor, leading to better immunotherapy response [25] (Fig. 1). In this review we will discuss the promising role of TILs as biomarkers reflecting the immune response to the tumor, describing their potential prognostic and predictive significance in some solid tumors.

2. Role of TILs in melanoma

Melanoma is the most aggressive and serious form of skin cancer accounting for the sixth most common cause of cancer-related death [26]. To date, prevention and early diagnosis are the most effective strategies to decrease the incidence of this malignancy, since advanced stage melanomas are largely resistant to conventional therapies [27]. Melanoma is considered a multifactorial disease, which is caused both by genetic and environmental factors. The most important environmental risk factor for cutaneous melanoma is the sun exposure that may generate combined genotoxic and mitogenic effects arising from deleterious interactions between the melanocyte genome and ultraviolet (UV) radiations [28,29]. A key factor in the development of novel therapeutic strategies is the comprehension of the molecular mechanisms responsible for disease. Over the last decade, immunotherapy led to significant advances in metastatic melanoma, since the immune system responds to different types of antigens [30]. To date, the melanoma classification is based on the pathological characteristics of the primary tumor and spread of the disease at the time of diagnosis. In the tumor staging, the presence or absence of TILs is not often considered in melanoma, although several studies highlighted the prognostic value of this immune component [31]. Today, the advent of all new targeted therapies requires to the researcher a better elucidation of the prognostic function and significance of TILs, in both untreated melanoma and those treated with targeted agents [30]. In fact, the immune cell infiltration of tumor mass may affect the anticancer immune response, which is measured by analysis of the lymphocytic infiltrate at the base of the vertical growth of melanoma. Some studies showed that higher presence of immune infiltrate in tumors is associated with a better

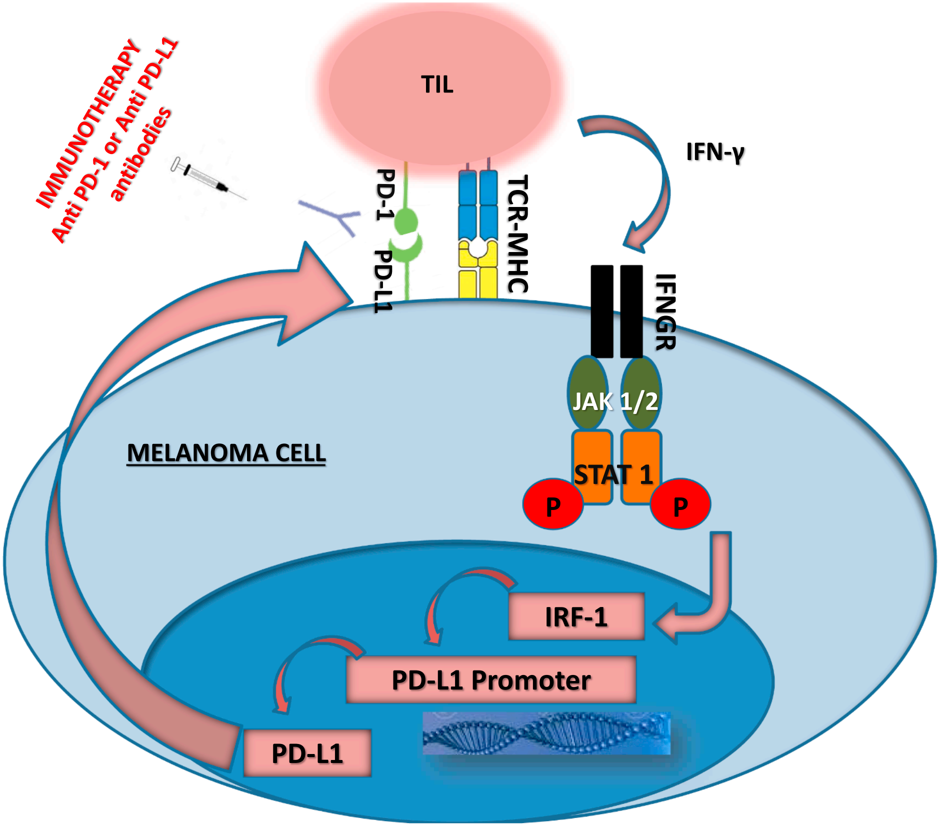

Fig. 1. The interferon gamma (IFN-γ) receptor pathway regulating PD-L1 expression in melanoma cell. TIL accumulation induces the CD8+ cell-mediated production and release of interferon gamma (IFN-γ) which, in turn, by binding its receptor (IFNGR) activates the Jak/STAT signaling increasing PD-L1 expression in tumor cell, leading to better immunotherapy response. Abbreviations: PD-1, Programmed Death 1 receptor; PD-L1, Programmed death ligand 1; IFN-γ, Interferon gamma; IFNGR, Interferon gamma receptor; IRF-1, Interferon regulatory factor 1; Jak 1/2, Janus Kinase ½;MHC, Major histocompatibility complex; STAT1, Signal transducer and activator of transcription 1; TCR, T-cell receptor; TIL, Tumor-infiltrating lymphocyte.

prognosis, while others revealed an immunophenotypic and functional heterogeneity of TILs in melanoma [32]. Additionally, it has been shown that TIL function is compromised as a result of the accumulation of immunoregulatory cells and various tumor escape mechanisms. Because of this recent findings, it is necessary to collect further data on the composition and function of TILs in order to evaluate their prognostic significance.

For the first time in 1969, Clark et al. [33] described the lymphocytic infiltration of primary cutaneous melanoma. Later, Day et al. [34] and Tuthill et al. [35] reported that lymphocytic infiltration could have prognostic features. In fact, patients with high number of infiltrating lymphocytes in primary melanoma showed a better prognosis and a higher survival rate than patients with low or absent infiltration. Subsequently, Sambuco et al. [36] demonstrated that TILs had a prognostic significance only in the vertical growth phase (VGP), whereas, conversely, during radial growth (RGP), lymphocytic infiltration had no prognostic value. The study carried out by Clemente et al. [37] showed that TILs had a greater prognostic significance in T2-T4 primary melanoma patients, whereas in very T4-stage thick lesions the prognostic significance was lost. Several studies showed that the transfer of *ex vivo*-expanded TILs can cause substantial regression of metastatic melanoma [38].

The College of American Pathologist generally divides TILs into Brisk, non-Brisk and not identified. TILs "Brisk" are considered lymphocytes diffusely infiltrating the entire base of the invasive tumor, or showing diffuse permeation of the invasive tumor. Instead, TILs "non-Brisk" are considered lymphocytes infiltrating melanoma only focally or not along the entire base of the invasive tumor. TILs "not identified" are defined as absent lymphocytes, or lymphocytes present but not infiltrating tumor at all [39,40].

The knowledge of tumor-specific lymphocytes is based on the study of CD8+ cytotoxic T cells which circulate in peripheral blood or are present in the tumor. However, recently, it has emerged that other subgroups of lymphocytes may be equally important against melanoma, such as helper T cells, which can use other patterns and locate in other sites such as drenant lymphonodes [30]. Also CD4+ T lymphocytes seem to have a significant role in melanoma, because they showed the ability to eliminate melanoma cells in animal models. Another subset of CD4+ T cells present in the tumor environment is represented by CD4+ CD25+ regulatory T cells (Tregs) which are significantly increased in patients with malignant epithelial diseases. It has also been seen that Tregs exhaustion results in effective anti-tumor immunity in experimental models. Tregs TILs are mainly present in advanced melanomas, with increased concentration in metastatic lesions and deep VGP lesions. Recent data showed that a higher percentage of Tregs is associated with a significantly high risk of melanoma recurrence, so the accumulation of these cells may be associated with the progression of disease [41]. For natural killer (NK) cells, the role in melanoma immunoresponse has not yet been clarified. Although NK cells are only rarely present in the melanoma microenvironment, NK TILs has been observed only in patients with early-stage melanoma, but not in those with progressive disease. Therefore, several subgroups of lymphocytes contribute to the immune response to melanoma, so a better understanding of the specificity and immunobiology of different lymphocyte subgroups is needed in order to better predict the prognostic significance of TILs [30].

Several studies showed that blocking the interaction of CTLA-4 (also known as CD152) or PD-1 with their ligands, through the use of antibodies, has induced an effective immune response not only in melanoma, but also in RCC and NSCLC. Some clinical trials support the idea that TIL immunologic therapy, based on the persistence of the transferred tumor-specific T cell clones, is associated with tumor regression [38].

Recent studies demonstrated that anti-tumor response after TILs transfer, which determines a substantial regression of metastatic melanoma, is observed in more than 72% of patients in phase 2 clinical trials, and without side effects for most of the patients [17].

Not only the T cell repertoire reflects the ability of host immune response, but it is important for prognosis to evaluate the relationship between an increased accumulation of somatic mutations in tumor cells and inhibition of immune checkpoints. In fact, melanoma patients with high frequency of somatic mutations responded better to inhibition of checkpoints with anti-CTLA-4 and anti-PD-1 antibodies. In melanoma mice, loss of IFN-γ receptor 1 (IFNGR1) gene resulted in impaired response to anti-CTLA-4 therapy and reduced overall survival (OS) [42].

Several studies showed that VEGFA expression increases in non-responders undergone to therapy, suggesting a therapeutic resistance mechanism [43]. In contrast, in resistant tumors there is a particular transcriptional signature, which has been associated with a greater expression of genes involved in the regulation of epithelial-mesenchymal transition, cell adhesion, remodeling of extracellular matrix, angiogenesis and wound healing.

The PTEN gene deletion, commonly present in melanoma, has a deleterious effect on antitumor immunity, as it leads to a "cold" tumor with high levels of immunosuppressive cytokines with rare and inactive T cells [44].

3. A link between "old" TNM system and "new" immune microenvironment in lung cancer

Lung cancer represents the second most frequent cause of death worldwide and is represented for 85% by NSCLC. Despite the progress in the treatment of lung cancer, the prognosis in the patients with this disease remains poor. Little is yet known about potential prognostic and predictive biomarkers for NSCLC. In the last century, the TNM system is been the only parameter used to give information about the prognosis of lung cancer patients [45,46]. However, this system is resulted insufficient to stratify patients with the same histopathological diagnosis of lung cancer [47]. The ability of neoplastic cells to interact with their immune microenvironment has acquired a great significance, during the past 15 years. The components of this microenvironment include a dynamic population of macrophages, mast cells, T cells, B cells, dendritic cells, NK cells, myeloid-derived suppressor cells, neutrophils, and macrophages which seems to represent a potential powerful prognostic instrument for lung cancer [48]. Almost all of this cell population is represented by T cells, especially cytotoxic T cells, memory T cells, and T helper 1 cells able to infiltrate the tumor tissue [49]. This dense cellular network of TILs can give supplementary information within each TNM stage, differentiating the clinical outcome of lung cancer patients. TILs are a heterogenity group of immunoinflammatory cells expressing various activation antigens [50]. Subsets of TILs include CD8+, CD3+, CD4+ and FOXP3+ cells. Also, CD4+ T lymphocytes, CD8+ cytotoxic T lymphocytes, $CD4^+$ T helper lymphocytes, CD45RO + memory T cells (Tm), and FOXP3+ Tregs are the principal cell types of T cells. All this cells may contribute in tumor eradication [51].

3.1. TILs: potential prognostic and/or predictive biomarkers in lung cancer

TILs may be localized within cancer cell nests (epithelial lymphocytes), in the central cancer stroma (stromal lymphocytes) and along the invasive margins (peritumoral lymphocytes). The dual role of TILs has been controversial, since they may act as suppressors or promoters of tumor growth moving into the different steps of tumor progression [52]. The scientific community, due to the urgency of new prognostic and/or predictive biomarkers, has shed light on TILs to evaluate their impact on a clinical setting of NSCLC [53]. Generally, their infiltration was associated with a greater outcome [49]. Most of the studies showed a higher number of tumor-infiltrating CD8+ lymphocytes than those present in corresponding peripheral blood samples. This phenomenon seems to be associated with tumor cell apoptosis in NSCLC [49,54]. Despite many uncertainties, it is important to stress the concept that the density, subset and localization of TIL make them useful sentinels of progression-free survival (PFS) and OS in patients with lung cancer [55,56]. Studies showed a prognostic role of CD8+ lymphocytes in function of their localization, but there are conflicting opinions about their prognostic role and that exerted by other more common lymphocytes. A recent meta-analysis reported that these cells can modify tumor epitelium (TN) and/or stroma (TS) in a way that reduces the tumor progression and metastasis [57]. High density of different subset of TILs was correlated with the survival of lung cancer patients. Geng et al. [58] showed that the value of $CD8^+$ T cells was better correlated with TS than TN. Moreover, patients with high levels of the same cells in both TS and TN showed better OS. In lung cancer patients, CD3+ cell infiltration was found in both TS and TN and high level of this infiltrating cell correlated with better OS. Conversely, high levels of tumor-infiltrating FOXP3+ T cells have shown a negative prognostic value. Yan and collaborators [59] confirmed that the presence of FOXP3+ TILs and IL-2 expression in tumor cells were associated with recurrence. The combination of different TIL subsets with opposite prognostic role would allow to predict the prognosis. These results seem to show the better positive prognostic role of TILs in TS than in TN and this evidence seems to be related to the pathway used by cell to escape the immune system. The lung cancer cells counterattack the immune system thanks to the deregulation of pathway Fas/FasL. Indeed, the level of expressed FasL is symptomatic of poor clinical outcome/prognosis and metastasis. The deregulation of the Fas/FasL pathway and, in particular, the Fas downregulation and FasL upregulation, allows to lung cancer cells to escape from cytotoxic T-cell effects. Lung cancer cells express constitutively FasL as well as also Fas, but at lower levels. This different level in the ratio of FasL/Fas in the tumor cells within intraephitelial compartment mediates the apoptosis process of TILs, triggered by high level of FasL [57,60]. Recently, Lizotte et al. [61] have identified by flow cytometric analysis two distinct immunological subsets in NSCLC: an immunologically "hot" cluster with abundant $CD8^+$ T cells expressing high levels of PD-1 and TIM-3 and a "cold" cluster with lower content of $CD8^+$ T cells and expression of inhibitory markers. Another recent study regarding the antigen characterization of peripheral blood T cells in patients with early stage NSCLC showed that a low CD4+/CD8+ ratio predicts a better prognosis compared to CD4+/PD-1+ cells [62].

Little is known about the potential predictive role of TILs in lung cancer. Blockade of immune checkpoints by targeting inhibitory receptors on T cells is a promising therapeutic strategy in human cancer. It is important also to know if the T cells express immune checkpoints. CD4+ and CD8+ T cells are capable of expressing multiple inhibitory receptors at the same time (PD-1, TIM-3, LAG-3, CTLA-4). Clinical trials of anti-PD-L1-directed therapy showed that PD-L1 overexpression in cell membranes of NSCLC patients could represent a valid biomarker of response and prognosis [63,64]. Also, the response to immunotherapy in advanced NSCLC patients can be predicted through the study of the tumor microenvironment [65]. Also, a tumoral context dense of CD8+ and FOXP3+ Treg TILs can be considered as predictive marker of response to platinum-based neoadjuvant chemotherapy in advanced NSCLC patients [66,67], as well as high levels of the transforming growth factor β (TGF- β) in TILs are predictive of poor post-operative survival [59]. Furthermore, there is still much to be studied to evaluate possible epigenetic modifications that can drive a more conscious therapeutic choice for lung cancer patients.

4. Role of TILs in renal cell carcinoma (RCC)

The recent revolution in the treatment of metastatic renal cell carcinoma (mRCC) resulted in significant improvements in median OS of cancer patients. The identification of novel pathways, new data from several early clinical trials and promising immunotherapy agents is finally translate to real benefit in clinical practice. Now more than ever before, the information from tumor microenvironment (TME) represents an excellent opportunity for kidney cancer patients to access new drugs and for clinicians to find prognostic and predictive "immune biomarkers".

Already in the past, before the "TKI-era", the mainstay of treatment for mRCC was systemic immunotherapy, however limited to cytokines such as IL-2 and interferon α (IFN-α), characterized by poor efficacy and severe dose-limiting toxicities [68]. The subsequent identification of angiogenesis-related molecular alterations involved in RCC pathogenesis allowed to design several and efficacy multitargeted tyrosine kinase inhibitors (TKIs) with a greater number of therapeutic opportunities [69].

Finally, the recent advances in immuno-oncology, promising frontier for many malignancies, provide substantial benefit in OS for renal cancer patients. The inhibition of immune checkpoints expressed on T cells (PD-1, CTLA4) with specific inhibitors (Nivolumab, Atezolizumab, Ipilimumab) has been demonstrated to be clinically effective in clinical trials performed on metastatic disease [70].

RCC lesions are often infiltrated by high levels of tumor-infiltrating lymphocytes. The parallel clinical observations of spontaneous tumor regression in some RCC patients has suggested a critical role for immune system in development and regulation of the neoplastic disease [71].

Despite numerous evidences in solid tumors suggest that increased TILs are associated with good prognosis [72], the role of TILs in RCC is controversial. Some studies showed that high density of CD8+ TILs is associated with poor clinical outcome [73–75].

Several elements and new hallmarks of cancer could possibly explain this negative correlation. The increase in TILs was found to be associated with higher tumor grade [74]. Furthermore, RCC with high density of CD8+ TILs showed increased expression of co-stimulatory checkpoints such as PD-L1. The expression of inhibitor ligands in the TME leads to the PD1+ TIL inhibition [75], with negative immunomodulatory effects.

A recent study concerning tumor-infiltrating immunophenotypes on tissue from localized renal cell carcinoma (ccRCC) identified a subgroup of ccRCC TILs defined as "immune-regulated". These lymphocytes which present CD8+ PD-1+ Tim-3+ Lag-3+ immunoprofiles are associated with high PD-L1 expression in immune infiltrate, and are peculiar of patients with aggressive histological features and high risk of relapse after primary surgery [76].

TIL content might also be predictive of clinical outcome and response to medical treatment. The initial study by Siddiqui et al. suggested the negative impact on patient survival for FOXP3- Tregs rather than FOXP3+ [77].

A predictive role seems to be provided by the co-expression of specific inhibitory receptors on CD8+ T cells. The recent study of Granier et al. suggests that the presence of a specific intratumoral CD8+ T cell subset, co-expressing PD-1 and Tim-3, confers a higher risk of relapse and a poorer OS [78] (Fig. 2).

These and future studies characterizing the tumor immune microenvironment explain how the TILs can represent valid prognostic and predictive biomarkers that may be useful in routine clinical practice to identify tumor types potentially responsive to TME-modulating agents.

5. Role of TILs in breast and ovarian cancers

Breast cancer is characterized by genomic instability and a large number of gene alterations are involved in the malignant transformation [79–81]. Now, the majority of these is used in the clinic practice as essential molecular biomarkers. At the center of recent studies there is the presence of some neoantigens considered target for a local immune response [82,83]. Several independent studies have shown the presence of lymphocytes in the microenvironment of breast cancer, investigating very large cohorts of patients and highlighting a crucial role played by TILs in this tumor type. In particular, the presence of TILs has been associated with a better prognosis especially in triple negative (TNBC) and human epithelial growth factor receptor 2 positive (HER2+) breast

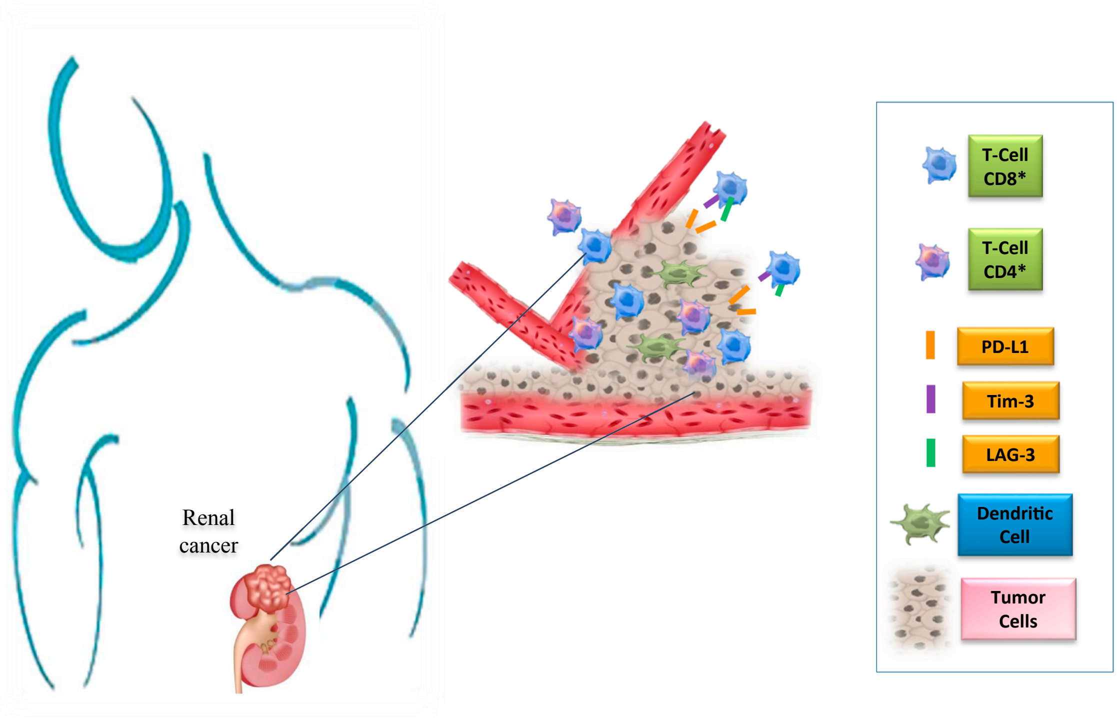

Fig. 2. Predictive and prognostic role of TILs in patients with RCC. The presence of a specific intra-tumoral CD8+ T cell subsets, co-expressing PD-1 and Tim-3, confers a higher risk of relapse and a poorer overall survival.

cancers.

In 2005, Dieci et al. [84] analyzed the prognostic role of TILs in 781 patients out of 816 cases enrolled by two multicentric randomized trials comparing anthracyclines versus no chemotherapy in breast cancer. The result of this study was very strong, showing a 10-years OS rate of 89% for high TILs vs 68% for low TILs in TNBC patients, and 78% for high vs 57% for low TILs in HER2+ population. However, this study demonstrated only the prognostic value of TILs, but not their predictive role. Indeed, it has been shown that TILs are not predictive for the efficacy of anthracycline treatment.

Subsequently, in 2016 Loi et al., in association with the International Working Group on Immuno-oncology Biomarkers, published a work aimed to standardize the investigation approach in order to detect TILs in breast cancers reporting "the reproducible evaluation of TIL method" for clinical practice [85]. After this study many others confirmed the prognostic value of TILs in BC, but very few data were available about their predictive potential.

The study by Issa-Nummer Y et al. [86] showed that an increased immunological infiltrate was predictor of response to antracycline/taxane-based neoadjuvant chemotherapy (NACT). This research group has designed a substudy of Gepar Quinto Trials (NCT 00567554) including 93 centers investigating different chemotherapy regimens with similar immunological effects [87] and evaluated the tumor lymphocytic infiltrate, confirming that patients with a predominant presence of lymphocytes exhibited a complete pathological response of 36%. Subsequent studies confirmed this point of view revealing that high levels of TILs were associated with known clinico-pathological factors in breast cancer patients, including young age, viral diseases (HBV, HCV), HER2+ overexpression. Furthermore, high TIL concentration has been detected in 20% of luminal A cancers, 28% of luminal B cancer type, 89% of non-luminal cancer type, 8% of BRCA1/2 mutated patients, regardless of different mutation carriers. Conversely, the presence of TILs did not significantly vary in relationship with tumor grade, size and number of metastasis [88]. Recently, another study revealed that patients with high density of CD8+ TILs had a longer disease-free survival (DFS), and CD8+/PD-L1 was a positive prognostic factor associated with longer OS in patients with luminal/HER-EBC [89].

Recently, different studies reported the presence of PD-L1 also in breast cancer suggesting its role as a new therapeutic target mostly in HER+ tumors, in which are found the major levels of PDL-1 expression [90–92].

Indeed, the expression of PD-L1 on activated T cells maintained an immunosuppressive tumor microenvironment, offering the possibility to use monoclonal antibodies targeting PD-L1 and its receptor PD1 which are able to reactivate the antitumor immune response [93]. Even if limited by a lack of standardization in testing method, the IHC analysis represents the current standard approach to detect PD-L1 protein on tumor tissue samples. However, as already demonstrated in other tumor types such as melanoma and lung cancer, a potential combination of PD-L1 mRNA levels with TILs abundance could enhance our ability to identify those patients who are most likely to respond to immunotherapy [94]. Only in this view we could consider TILs as potential predictive biomarkers of response to the immune checkpoint blockade in breast cancer.

Ovarian cancer (OC) is a heterogeneous disease, classified according to tumor grade, histotypes, and clinical stage. However, the clinical indications for all patients is chemotherapy and/or surgery. Currently, patients with BRCA-mutated ovarian cancer are candidate to receive new targeted drugs known as poly ADP ribose polymerase (PARP) inhibitors, but they are only the 5–10% of all OC population. Several studies attempted to stratify the molecular and clinico-pathological features of OC in order to offer new winning treatment strategies according to the different cancer subtypes [95,96].

Many result are coming by various works investigating the role of immune cell response in OC patients. Although OC may not be considered an immunogenic disease like other types of solid tumors, such as melanoma or lung cancer, however some evidences revealed an immune system activity in epithelial OC (EOC) [97]. Among the first works, Zhang et al. have associated the presence of intra-tumoral T cells with increased expression of interferon-γ, interleukin-2, and lymphocyte. Their attractive role for chemokines inside the tumors and absence of intratumoral T cells was associated with increased levels of vascular

Table 1
Studies regarding the potential prognostic role of TILs in ovarian cancer.

Study	Highlights
Stumpf M et al., 2009	CD3+ T cells and CD8+ T cells are associated with increased survival
Webb JR et al., 2014	CD103+ TILs activated CD8+ T cells
Mantia-Smaldone G et al., 2014	CD4+ depletion combined with PLD[‡] significantly prolonged OS ($p = 0.0204$) in BRCA1-tumor-bearing mice
Wouters MC et al., 2016	CD8+ TILs interact with treatment to affect prognosis
Schalper KA et al., 2016	*In situ* tumor PD-L1 mRNA expression is associated with increased TILs
Wang Q et al., 2017	Higher number of intraepithelial CD3+ or CD8+ TILs is an independent prognostic factor for longer OS
James FR et al., 2017	TILs in EOC improve clinical outcome

[‡] PLD, Pegylated Liposomal Doxorubicin.

endothelial growth factor (VEGF), thus favouring the tumor growth and metastatic potential [98]. As suggested by Clark et al., the presence of TILs is often detected in EOC, likely because CD8+ TILs are more frequently associated with the tumor epithelium than stroma. The evidence that T cell infiltration in epithelial cancers is considered a marker of better prognosis is today widely confirmed [99–101]. A recent meta-analysis including ten studies regarding 1850 OC patients confirmed that CD8+ TILs are associated with a significantly better outcome, playing an important role as prognostic biomarker [102]. This work has further evaluated the expression of the integrin CD103 induced by CD8+ TILs, that is considered a specific marker of TIL signaling revealing their presence in tumor tissue. Finally, this study explained that a lack of intraepithelial TILs is associated with a worse survival and how TIL presence, instead, may restrict tumor growth facilitating surgery. Studies regarding the potential prognostic role of TILs in OC are reported in Table 1.

A remarkable evidence showed that patients with BRCA-mutated or homologous recombination (HR)-deficient EOC benefited more than those BRCA wild-type by the treatment with immune checkpoint inhibitors, likely because lesions rich of mutations stimulated the recruitment of TILs overexpressing immune checkpoints targets such as PD-1 or PD-L1 [103].

Strickland et al. [104] evaluated the PD-1 and PD-L1 expression comparing two cohorts of patients, BRCA-mutated patients or with other HR alterations versus patients with no HR alteration. They observed that density of both intraepithelial and peritumoral lymphocytes was significantly higher in BRCA1/2-mutated patients and those with other HR alterations compared to tumors which did not harbor HR alterations. The PD-L1 expression on tumor-infiltrating immune cells was also different between HR-proficient and BRCA1/2-mutated tumors, whereas no differences were observed in tumor cells. The authors also evaluated the number of CD3+ TILs, showing a significant correlations with the number of PD-1+ lymphocytes, suggesting a potential role as “surrogate biomarkers” [104].

Nowadays, the scientific interest is directed to better focusing the relationships of all these biomarkers with responses to the immunotherapies and several studies are currently including ovarian cancer patients.

6. Potential role of TILs in Gastrointestinal stromal tumors (GISTs)

Gastrointestinal stromal tumors (GISTs) are rare cancers, considered as a paradigm of molecular biology in solid tumors. The receptor tyrosine kinase KIT is an established oncogenic driver of tumor growth in GISTs. In the last years, several studies were performed to achieve information about GIST biology and investigate novel potential biomarkers with diagnostic, prognostic and predictive value. Recent evidences also identified a considerable potential for KIT to influence tumor growth indirectly based on its expression and function in cell types of the innate immune system [105].

The blockade of CTLA-4 and PD-1, known critical immune checkpoint molecules that negatively regulate T cell activation, with specific antibodies, is emerging as an effective and promising treatment option against several solid tumors [6]. Unlike other tumors, the significance of immune microenvironment, including tumor-infiltrating lymphocytes, and potential role of immune checkpoints in GISTs are unknown.

Recently, analysis of tissue microarrays from pathology samples showed the association between CD8+ or CD3+ TIL counts, PD-L1 immunohistochemical expression with tumor size, mitoses, and GIST outcomes. TILs expressing high CD8+ or CD3+ expression levels were associated with decreased PD-L1/IDO-positive GIST size [106].

Further studies, beyond the known mutations involving KIT and PDGFRA genes, are requested to investigate the microenvironment in GISTs, and prognostic and predictive function of TILs. These information could represent a promising tool to select clusters of patients who may benefit from specific immunologic treatments.

7. Conclusions

Since the efficacy of immunotherapeutic agents greatly varies across patients and among different tumor types, the identification of predictive biomarkers able to monitor the immunotherapy response represents the next step for further advances towards precision immune-oncology approaches [107]. In particular, it is essential to define the immune environment of the tumor focusing the attention on molecules modulating the immunological response produced by cancer cells. The finding that complexity of the T cell population in tumor could predict a good response to checkpoint blockade therapy highlights the importance of understanding which antigens can elicit an effective antitumor immune response. For this purpose, the main objective of clinical research will be to further evaluate the potential prognostic and predictive role of TILs in tumors in order to define, before initiation of treatment, clusters of patients who may benefit from treatments targeting immunomodulatory molecules in a patient-specific manner.

Conflict of interest

The authors declare no conflict of interest.

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