The Human Tumor Microenvironment

2

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Contents

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2.1 Introduction

For a long time, cancer therapy has had as its sole objective the direct elimination of tumor cells. In case of nonmetastatic disease, this is accomplished by surgery, which removes the primary tumor. Radiotherapy and conventional chemotherapies also aimed at targeting tumor cells preferentially. The high capacity of tumor cells to divide as compared to the normal cells makes them more sensitive to agents that physically, in the case of radiotherapy, or chemically, in the case of chemotherapy, attack DNA and lead to cell death. Targeted therapies targeting mutations in tumor cells such as BRAF have been developed as well. However, these approaches also destroy the nonmalignant cells and/or have systemic consequences. To increase specificity toward the tumor cells, cytotoxic agents have been coupled to antibodies that bind to the tumor cells in order to allow their specific targeting to the tumor and not to the normal cells. However, the entry of such constructs into tumors still remains a major issue.

The progresses that have been accomplished in the field of tumor immunology in these last 20 years have led to a drastic change in the representation of primary tumors and metastases and to cancer treatments. Tumors are not anymore represented as a simple accumulation of cells that have undergone oncogenic processes but as a complex and dynamic structure made of tumor cells and inflamed tissue. Tumors are infiltrated with blood vessels that bring nutriments and all kinds of leukocytes inside the tumor and at its periphery, in the so-called tumor stroma that also contains matrix proteins such as collagen fibers. The transformation of a normal cell into a clinically detectable tumor can last for decades such as in the case of breast or colon cancers. Thus, tumors are dynamic structures that derive from this long process of carcinogenesis occurring in an inflamed and reactive tissue microenvironment.

Importantly, the last 20 years of intense research in the tumor immunology field unraveled the proof of concept of the immunosurveillance theory that was brought by McFarlane and Lewis Thomas in the 1950s (reviewed in [1]). These two scientists anticipated that immunosurveillance is a physiological mechanism that protects against nascent tumors. The description of immune cells with effector and memory functions within primary tumors and their metastases and the discovery of the correlation between their density at the site of the primary tumor and patient's survival more than 10 years ago unambiguously demonstrated that the immune system is capable of recognizing and eliminating tumor cells. The immune system uses the same basic mechanisms to fight against cancer as those used to eliminate viruses such as the influenza virus. Both the innate and adaptive arms of the immune system cooperate to mount an antitumor response leading to the development of effector $CD4^+$ T cells that produce cytokines, of effector $CD8^+$ T cells that kill the tumor cells and produce cytokines, and of B cells that differentiate into plasma cells that produce antibodies. Most importantly, so-called memory lymphocytes develop in parallel. All these cell types accumulate into tumors, and the memory lymphocytes circulate for a long time, with the possibility of transforming into effector lymphocytes very rapidly. They protect locally against tumor cells and systemically against metastatic cells that may escape from the primary tumor and circulate before nidation in distant organs, where they proliferate and become metastatic. An immune response is raised directed against tumor antigens. More than 15 years ago, it was proposed that tumors grow until an equilibrium is reached between tumor cells and the immune system. Only tumors, in which the tumor cell growth potential overcomes the pressure exerted by the adaptive immune response, can subsequently grow and metastasize into distant tissues. Indeed, tumor cells develop a series of mechanisms to evade the immune defenses including the downregulation of tumor antigens or the production of molecules that suppress immune functions. Therefore, tumor cells have long standing interactions with the immune system, especially in the microenvironment in the primary tumor and later in the metastases.

Finally, studies on the tumor microenvironment brought another major issue regarding the mounting and the regulation of the antitumor defenses. Immune cells were found to form aggregates at the tumor sites, mimicking those

found in inflamed tissues that reflect local consequences of a chronic antigenic challenge. A large body of evidences suggests that these so-called tertiary lymphoid structures play an important role to mount, maintain, and control the local and systemic immune defenses.

This deep knowledge of the antitumor defenses and of the composition of the tumor microenvironment brought a new paradigm for cancer treatment. Instead of targeting the tumor cells by using radiotherapy or chemotherapy, drugs targeting the tumor microenvironment have been developed. This major step in cancer therapy has been accomplished these last years. Drugs aiming to alleviate the immune defenses by unlocking the effector functions of the T cells, such as anti-CTLA4 or anti-PD-1 antibodies, have been developed. Other drugs targeting the tumor vasculature such as antibodies against factors favoring the growth of cells lining the blood vessels (vascular endothelial growth factor, VEGF) or molecules inhibiting the signaling pathways in the endothelial cells downstream VEGF (sunitinib) have been approved by the FDA for some cancers. Indeed the tumor microenvironment offers an array of potential new targets that can be used alone or in combination with the classical approaches preferentially targeting the tumor cells such as chemotherapy or radiotherapy which may also in some cases increase immune reactions to the tumors.

In this chapter, we will first describe the tumor natural history, how tumor cells progressively grow in a tissue that becomes inflamed, and how the tissue both facilitate the development of tumors and participate to their elimination. We will then describe the different cell types that are found in the tumor microenvironment, their function, their location, and their organization in human tumors. The prognostic impact of the different cell types of the tumor microenvironment will then be compared, and the immunotherapy approaches targeting the tumor microenvironment will be described.

Regarded for a long time as a genetic and cellular disease, cancer is now considered as a tissular and systemic disease whose outcome depends largely on interactions with the host, especially within the tumor microenvironment. The tumor microenvironment can promote or inhibit tumor invasion and metastasis. It changes during the course of the disease, and the understanding of this dynamic interaction makes it possible to identify new therapeutic prognostic factors and new therapeutic targets at all stages of the disease.

2.2 Cancer's Natural History

More than 40 year ago, Peter Nowell proposed that genetic alterations—induced by diverse mutagenic stimuli—could be responsible for the transformation of normal cells toward neoplastic states [2]. According to his theory, these random mutations confer cells with autonomous proliferative capacity and immortality. This concept has barely changed, and today we know that genetic instability is the hallmark initiating event of cancer cells. In fact, tumor cells acquire a series of mutations over time, and it is believed that the stepwise accumulation of genetic abnormalities eventually generate their malignant transformation. In average, a tumor cells exhibit 120 non-synonymous mutations [3] that not only confer them autonomous and uncontrolled proliferative capacities but also several other characteristics that allow them to survive in the hostile human body environment.

In 2011, Hanahan and Weinberg proposed the main hallmarks or essential characteristics that a cancer cells exhibit and allow them to self-support the development of a tumor mass [4]. With genetic instability and increased proliferative capacity leading the list, it is currently recognized that tumor cells also need to actively interact with surrounding endothelial, stromal, and immune cells, to guarantee their own survival. Thus, human cancers often promote angiogenesis and inflammation and commonly develop mechanisms to evade the immune system. While the stepwise acquisition of new mutations allows the development of these pro-tumoral functions, the pressure of the hostile environment leads to the selection of the more malignant and aggressive cell clones [5].

The cornerstone of tumor cell emergence and development is then genetic mutations, which can

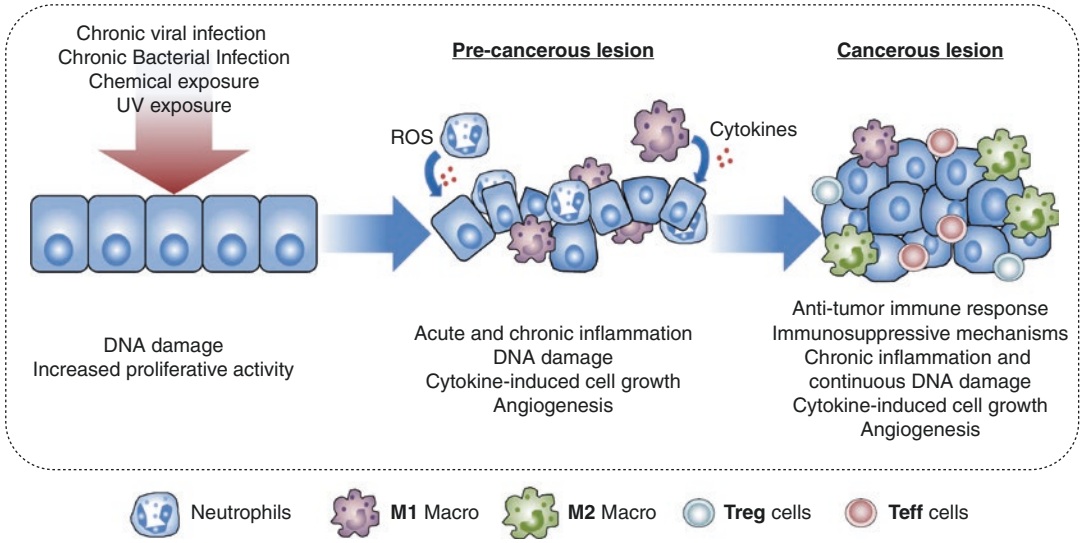

Fig. 2.1 Major immunopathological and genetic events occurring during carcinogenesis. Upon chronic inflammatory stimuli exposure, normal cells undergo transformation into precancerous cells. Local inflammation induces recruitment of myeloid-derived cells that fuel carcinogenesis via production of oxygen derivatives or cytokines. Later on, tumor growth and invasion into tissues are controlled by a balance between antitumor and immune escape mechanisms

be induced by diverse factors (Fig. 2.1). We are continuously exposed to mutagenic agent, such as UV light, pollution, or even viruses. Normal cells often possess efficient machineries that repair mutated DNA or intracellular cascades that promote cell death when the damages are irreparable [6]. Some hereditary diseases, such as xeroderma pigmentosum (associated with an extremely high risk of skin cancer at early ages due to defect in the DNA-repairing machinery), are examples of how important these proofreading systems are to prevent cancer development and how often we are exposed to mutagenic stimuli.

Inflammatory mediators are other well-known promoters of genetic alterations. In fact, many of the substances produced by the inflammatory immune cells (such as macrophages and neutrophils) can induce the direct damage of DNA in nonimmune cells. In the presence of noxious stimuli, chronic inflammation can both induce the development of driver tumorigenic mutations and promote the necessary genetic instability to allow other alterations to develop [7]. This process of cancer induced by chronic inflammation (Fig. 2.1) has been described in several pathologies, including gastric cancer in association with *Helicobacter pylori* infection, asbestos or cigarette smoke exposure and lung cancer, arsenic exposure and skin cancer, gastroesophageal reflux for cancer of the esophagus, inflammatory bowel disease for colorectal cancer, chronic pancreatitis for pancreatic cancer, and pelvic inflammatory disease for ovarian cancer [8].

Examples of inflammatory carcinogenic mediators include reactive oxygen species and matrix metalloproteinases, which can induce DNA damage and extracellular matrix disruption, respectively [9]. In addition, some cytokines can induce the growth of abnormal or preneoplastic cells, such as IL-1β for gastric carcinoma and IL-8 for melanoma. The preneoplastic potential of many other cytokines has also been described (e.g., IL-1β, IL-6, IL-23, and TNF-α).

In virus-related cancers, aside from the inflammation induced by the infection itself, the virus genetic material can integrate into the host genome and induce cell transformation by altering diverse oncogenic pathways [10]. Virus-associated cancers represent roughly 20% of all cancer types and include cervical cancer (induced by HPV), B cell

lymphoma (induced by EBV), Merkel cell carcinoma (induced by Merkel cell polyomavirus), hepatocellular carcinoma (induced by hepatitis B and C viruses), and some gastric cancer and H&N cancer (induced by EBV).

2.3 The Tumor Immune Microenvironment

As mentioned above, the tumor microenvironment is a very complex and dynamic ecosystem, where different cellular populations coexist. The major players include tumor, immune, and supporting cells (e.g., fibroblasts, stromal, and endothelial cells) [11]. Immune cells that circulate in the blood enter into tumors via transendothelial migration and are attracted by chemokines produced by tumor cells, fibroblasts, or inflammatory cells. Within the tumor mass, the immune cells locally proliferate, differentiate, exert their functions, and die, and some migrate back to the circulation. Within this population, one often can find cells related to acute inflammation (including neutrophils, basophils, and eosinophils), cells of the innate immune response (including macrophages, NK cells, and DC), and cells from the adaptive immune response (including cytotoxic $CD8^+$ T cells, Th1-/Th2-skewed T cells and B cells). We focused this subchapter in the last two populations.

2.3.1 Tumor-Associated Macrophages

Tumor-associated macrophages (TAM) represent an abundant population, and in many tumors they outnumber other immune cells [12]. Although the majority of TAM are found in the invasive margin of the tumor, we can often find also elevated densities within the tumor core [13]. TAMs exhibit an extremely plastic phenotype and function, and two main subtypes have been described: M1 TAM (induced by Toll-like receptor ligands [e.g., lipopolysaccharide and IFN-γ]) which preferentially express pro-inflammatory cytokines and inducible nitric oxide synthase and M2 TAM (induced by IL-4 or IL-13) which express arginase 1, CD206, CD163, IL-4R, TGF-β1, and PDGF [12]. Some works suggest that while M1 TAM potentiate the antitumoral Th1 response and antagonize the suppressive activities of regulatory immune cells, M2 promote angiogenesis, tumor growth, and metastasis [13].

2.3.2 NK Cells

Natural killer cells are cytotoxic effector lymphocytes of the innate immune system whose primary function is to help control infections and tumors [14]. Two major mechanisms of recognition of tumor cells by this population have been described: they can recognize cells which have downregulated major histocompatibility complex class I expression (an immunotolerance phenomenon widely described in many cancer types), or they can bind to stress-induced ligands expressed on tumor cells (e.g., MICA or MICB, which bind to NKG2D expressed on the NK cell) [14].

2.3.3 Dendritic Cells

The main function of dendritic cells (DC) is to establish a bridge between the innate and adaptive immune response. Under physiological circumstances, DC engulf and process nonself-antigens, and when they are exposed to danger or activation signals, they become activated and travel to secondary lymphoid structures in lymph nodes where they prime naïve B or T cells [15]. The DC phenotype is rather plastic, and they can produce a wide range of pro-inflammatory or immunosuppressive cytokines, as well as expressing a large series of activating or inhibition receptors, depending of the environment where they are embedded. The secondary lymphoid organs are protected environments and often provide an ideal milieu to promote a DC phenotype that effectively activates the adaptive immune response [16].

In many cancer types, tumor cells produce molecules that induce pro-inflammatory or tolerogenic DC and block their maturation at different stages.

Often, intratumor DCs exhibit an immature and inhibitory phenotype [17]. Interestingly, in recent years, several works have described the presence of tertiary lymphoid structures (TLS) in the invasive margin of many cancer types [18], where in theory the DCs are protected from tumor-produced inhibitory substances and from where they can effectively prime the antitumor immune response [19].

2.3.4 Tertiary Lymphoid Structures

TLS are highly organized lymphoid aggregates that develop in inflammatory pathologies. In cancer, TLS often develop in the invasive margin of the tumors and/or in the stroma and resemble those arising in other chronic infectious or autoimmune diseases [19]. Figure 2.2A illustrates TLS found in clear cell renal cell cancer (ccRCC). Characteristically, TLS exhibit an organization similar to secondary lymphoid organs, including a T cell zone (Fig. 2.2Aa) and a B cell follicular zone (Fig. 2.2Ab), and are often surrounded by high endothelial venules [20]. B cells in TLS form germinal centers; they undergo active proliferative machinery and somatic hypermutation [19]. T cells have a CD62L$^+$/CD45RO$^+$ central memory or a naïve phenotype, and some can be found in contact with mature DC which expresses the DC-Lamp marker (Fig. 2.2Aa) or at the periphery of B cell follicles (Fig. 2.2Ac) [20]. Follicular dendritic cells are also detected

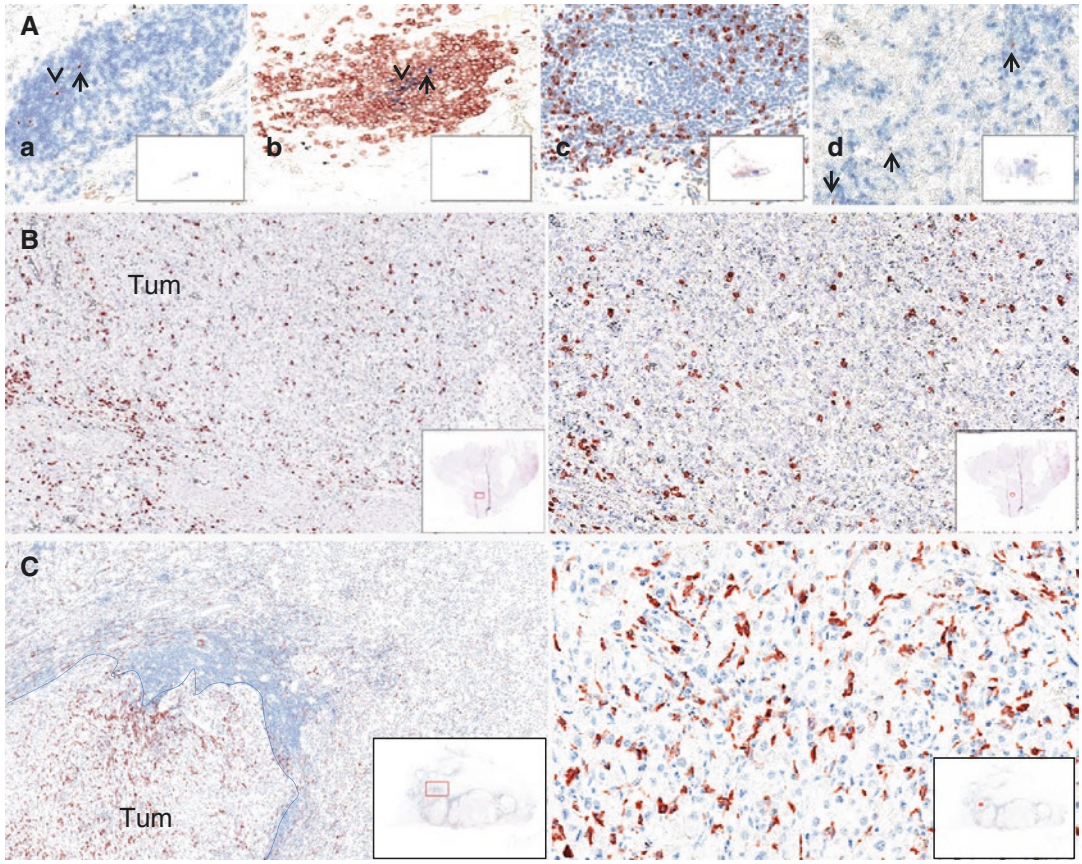

Fig. 2.2 The tumor microenvironment in human clear cell renal cell cancers as detected by IHC on paraffin sections. (**A**) Tertiary lymphoid structures: (*a*) DC-Lamp$^+$mature DC (*brown*) in the CD3$^+$T cell zone (*blue*); (*b*) CD20$^+$ B cells (*brown*) and CD21$^+$ follicular dendritic cells (*blue*) delineate the germinal center; (*c*) CD8$^+$ T cells (*brown*) are distributed around the germinal center; (*d*) non-TLS-DC-Lamp + DC (*brown*). (**B**) CD8 + T cells (*brown*) (*left* 5×, *right* 20×). (**C**) CD163+ macrophages (*red*) (*left* 5×, *right* 20×), Tum = tumor area

forming a network where immune complexes can form and be presented for selection of the high affinity B cells. Plasma cells that produce antibodies are located at the vicinity of TLS [21].

Primary tumors and metastases contain TLS at variable densities, depending on the tumor type and on the patient. As discussed below, it is assumed that TLS reflect the ongoing immune reaction within tumors. They allow the presentation of tumor antigens by mature dendritic cells to T cells leading to the differentiation of $CD4^+$ Th1 cells as reflected by the expression of the T-bet marker and the T-B cell cooperation for B cell differentiation into plasma cells. All of these events can thus occur locally, within the tumor bed. To what extent TLS bypass the need of secondary lymphoid organs to mount or control the antitumor immune reaction remains an open issue.

2.3.5 $CD4^+$ and $CD8^+$ T Cells

CD4+ T-helper cells are divided into different subtypes, including Th1, Th2, Th17, Tfh, and Treg; each subpopulation accomplishes specific roles in the antitumor immune response. Overall, a Th1-oriented response antagonizes the tumor growth and is often associated with good clinical outcome [22]. In fact, Th1-oriented cells potentiate in situ the antitumor function of cytotoxic T cells, through the production of several cytokines including IL-2 and IFN-γ. Tfh cells interact with B cells in TLS, helping antibody production.

The role of other subpopulations of tumor-infiltrating $CD4^+$ T cells (Th2, Th17, and Treg) is less well understood but is often associated with poor prognosis in different tumors [22]. Many studies suggest that Treg in cancer can dampen the antitumor immune response by two main mechanisms: (1) production of inhibitory cytokines (e.g., IL-10, TGF-β, and IL-35) and (2) suppression of DC development and maturation [23].

$CD8^+$ T cells exert a very import function in the antitumor immune response, as they are responsible of tumor cell recognition and elimination. Due to their genome instability, tumor cells often express mutant proteins at their surface. Many of these are neoantigens that can induce a tumor-specific immune response. The primed $CD8^+$ T cells are in charge of the tumor cells recognition and lysis, by mechanisms well described in the literature including the release of cytotoxic granules [24]. Interestingly, in the majority of tumors, infiltrating cytotoxic T cells express inhibitory receptors (e.g., PD-1, Tim-3, and Lag-3), whose function under physiological situations is to contract the immune response upon biding to their ligands. Many tumor cells in fact can take advantage of this inhibitory mechanism and in fact express a wide arrange of ligands (e.g., PD-L1, PD-L2) that help them escape for the T cell attack [25].

2.3.6 B Lymphocytes

In inflammatory settings other than cancer, B cells enhance T cell responses by producing antibodies and stimulatory cytokines and chemokines, serving as local antigen presenting cells and organizing the formation of TLS that sustain the immune response. In cancer, B cell can exert all of these functions and overall have an antitumor effect. In addition, recent evidence suggests they can also play an immunomodulatory role through the production of IL-10, among other cytokines [26].

2.3.7 Spatiotemporal Dynamics of the Tumor Immune Microenvironment

Chemokines ensure the local migration of these different cell types and cytokines allow their cooperation. In addition, many tumors are surrounded by a stroma containing an extracellular matrix composed of fibroblasts that form collagen fibers and produce enzymes—such as metalloproteases—that facilitate local invasion within tissues and ultimately the release of tumor cells that egress to the circulation and migrate in other tissues.

A direct consequence of these processes is that the tumor microenvironment is a tissue-dependent organized structure in which immune cells are common denominators. Figure 2.2B illustrates the presence of $CD8^+$ T cells in the

tumoral zone of clear cell renal cell cancer. A closer look into the organization of the immune microenvironment reveals that cells are not evenly distributed in the tumor area. Lymphocytes (T and B cells) are more abundant in the tissue border area called the invasive margin than in the center of the tumor [13]. They can be found dispersed or within aggregates, forming TLS in the invasive margin and/or in the stroma [18, 27]. Most of the T and B cells have a memory phenotype, $CD8^+$ T, $CD4^+$ Treg, Th1, Th2, Th17, and B cells being detected at variable densities, whereas naïve T cells and $CD4^+$ Tfh are exclusively present within TLS. NK cells are detected in the tumor stroma. Some T cells are found in close contact with tumor cells in the center of the tumor. Myeloid cells such as macrophages, myeloid-derived suppressor cells, mast cells, and neutrophils are present at high densities, both in the invasive margin and the center of the tumor. Figure 2.2C illustrates the high density of $CD163^+$ M2-oriented macrophages near the invasive margin of renal cell cancer. Immature dendritic cells are present at low densities, dispersed in the whole tumor area whereas mature dendritic cells are usually found within the TLS, in close contact with T cells (Fig. 2.2Aa). Importantly the immune composition of the tumor microenvironment evolves with the stages of tumor progression in a tumor-dependent manner. Thus, T cells are more numerous at the early stages of the disease in colorectal cancers and at their late stages in renal cell cancers [17, 28]. The density of B cells increases with tumor stage in colorectal cancers, as does that of the myeloid cells such as neutrophils, mast cells, immature dendritic cells, and macrophages. Thus, the tumor microenvironment is a complex structure, forming a tumor-dependent "immune landscape" that evolves during tumor progression.

2.4 The TME Dictates Clinical Outcome for the Patients

Quantification of immune infiltrates and its relationship with prognosis has been studied for more than 20 years. Following the observation that high T cell densities correlate with longer survival in ovarian cancer [29], the Galon, Pagès, and Fridman studies demonstrating for the first time in large cohorts of patients with colorectal cancers (CRC) the association between densities of memory T cells, early signs of metastasis, and patient's survival made a significant breakthrough in this field [28, 30]. Since then, important progresses in immunohistochemistry (IHC) with the multiplication of robust antibodies, the development of high through put technologies and of automated quantitative imaging has led to numerous studies on immune cell composition of the TME. This real enthusiasm was even more pronounced during the last 5 years with the emergence of checkpoint blockade therapy (CBT), which aims at reversing T cell exhaustion. Thus, T cell abundance in the TME and its link with outcomes and/or response to CBT is under intensive work by many teams worldwide.

2.4.1 T Cells

2.4.1.1 $CD8^+$ T Cells

T cell abundance within the TME has been extensively studied across the majority of tumor types. Our group published in 2012 a comprehensive review of the number of original articles linking immune cell populations infiltrating the tumor and prognosis [11]. We reported that high densities of $CD3^+$ T cells, $CD8^+$ cytotoxic T cells, and $CD45RO^+$ memory T cells were associated with a longer disease-free survival (DFS) and/or overall survival (OS) in most tumors (including melanoma, head and neck, breast, bladder, urothelial, ovarian, colorectal, and lung cancer) [1]. We noted at that time that clear cell renal cell carcinoma (ccRCC) was one of the rare exceptions to the rule. We updated these data last year and found similar results. In addition, we reported new tumor types such as GIST, biliary tract, thyroid, or oropharyngeal cancers where $CD8^+$ cell infiltration was associated with a good prognosis [22].

The poor prognostic value associated with $CD8^+$ T cells in ccRCC was confirmed by our group, both in kidney primary tumors [17] and in ccRCC lung metastases [31]. Besides ccRCC, studies in lung adenocarcinoma [32] and in HCC [33] also reported a poor prognostic value

associated with increased CD8+ T cell infiltration, in contradiction with other published studies. In prostatic adenocarcinoma as well, CD8+ T cell densities correlate with poor outcome [34], consistent with our own data [35].

The "Classical" Case of CRC

Colorectal cancer is the archetype of tumors where high CD8+ T cell densities are associated with good prognosis. Indeed a high infiltration of CD8+ T cells, particularly effector memory subtypes (TEM), is correlated with a low probability of metastatic spread and prolonged PFS and OS [28], suggesting T cells may control local invasion in primary tumors and confer a long-term systemic protection against metastasis. Moreover, IHC studies showed that compartmentalization of T cells in the center and the invasive margin of the tumors does matter. An immunoscore (IS) measures the density of CD3+ and CD8+ T cells in the center, and the invasive margin of the tumors has been developed by Jerome Galon's team and has been validated in a worldwide collaboration approximately 4000 CRC patients [36, 37]. Even if a high T cell density was more frequent in smaller tumors and MSI-positive tumors, the prognostic value of IS was independent from TNM stages and MSI status. Moreover IS was more accurate to predict the prognosis of patients with early stage CRC [37, 38].

The Discordant Case of ccRCC

We recently reported a clear negative association between CD8+ T cell infiltration and outcomes in ccRCC [17]. Within a cohort of 135 patients with available primary RCC tumors, we found that a high density of CD8+ cells, as assessed by IHC, was associated with a shorter disease-free survival and OS. These results were validated for OS in an independent cohort of 51 patients with (resected) lung metastases of ccRCC. The underlying mechanism for this poor prognosis value of CD8+ T cells is not fully understood. We showed that most of the intratumoral T cells have an exhausted phenotype, which may reflect impaired antigen presentation due to the presence of dysfunctional DCs with an immature phenotype (Fig. 2.2Ad). They express the DC-Lamp marker of mature DC but lack the high levels of MHC class II molecules and CD83 expressed by mature DC. They may be involved in the impairment of T cell antitumor response [17]. Consistently, in patients who have a higher density of DC within TLS, a high density of CD8+ was associated with good prognosis. Thus, antigen presentation by mature DC in the TLS seems to be a crucial event to drive antitumor response in ccRCC, in accordance with our previous observations in lung cancers [39]. Moreover, we showed by immunofluorescence (IF) that CD8+ T cells express immunoregulatory receptors such as PD-1 and/or LAG-3, suggesting a highly exhausted phenotype and both associated with poor outcomes [17].

2.4.1.2 CD4+-, Th2-, and Th17-Oriented T Cells

Consistent with CD8+ T cell infiltration, an increased in Th1-oriented CD4 T cell infiltration has been associated with favorable prognosis in almost all tumor types studied including breast cancer [40] or CRC [41].

Prognostic value of other T cell subsets (Th2, Th17) has been far less investigated first because of a low frequency in the majority of the tumors and second because of technical challenges to specifically identify these subsets.

2.4.1.3 Regulatory T Cells (Tregs)

The example of Tregs is eloquent. A high Treg density has been first associated with poor prognosis in ovarian cancer, which has been then confirmed in a variety of tumors such as in breast, lung, melanoma, or colorectal cancers (reviewed in [42]). Nevertheless, other studies reported longer survival associated with high densities of Tregs in colorectal, bladder, head and neck, or ovarian cancers. One of the reasons for these opposite results is the difficulty to identify the Treg population. Tregs are a heterogeneous population that should be ideally identified by a combination of markers (CD4+, CD25+, Foxp3+, T cells). The development of multicolor fluorescence imaging allows to increase the number of cell surface markers for their detection. Beyond the technical challenges, these results highlight that the prognostic impact of

immune cell populations depend on the tumor type and on the TME.

2.4.2 B Cells

The positive or negative role of B cells in antitumor immunity has been discussed for many years, mainly supported by mice studies. As compared to T cells, few clinical studies reported the prognostic role of intratumoral B cells. The majority of clinical studies have demonstrated that a high density of B cells within TME is associated with better prognosis including breast cancer [43], NSCLC [21], head and neck cancer [44], ovarian cancer [45], metastatic colorectal cancer [46], biliary tract cancer [47], and primary cutaneous melanoma [48]. Several nonexclusive mechanisms could explain the positive role of B cells in the antitumor immune response, some being antibody dependent by their capacity to trigger complement and antibody-dependent cell cytotoxicity (CDC and ADCC) or to form immune complexes able to activate DCs and others by acting as APC for CD4 [49] and $CD8^+$ T cell immune responses [50]. Indeed, it has been shown that B cells play a major role during initial priming and expansion of $CD4^+$ T cells [51], are able to cross-present antigens to $CD8^+$ T cells [52], and can promote cytotoxic T lymphocyte survival and proliferation [53].

On the opposite, few clinical studies reported a pro-tumoral role of B cells within the TME [54, 55]. B cells may play a pro-tumor function by the maintenance of a chronic inflammation [56], by the promotion of neoangiogenesis [57], and/or by the direct inhibition of cytotoxic T cell responses [55]. Moreover, a subpopulation of immunoregulatory B cells called "Bregs" has been described and has been shown to favor the differentiation and the recruitment of Tregs, thus amplifying the immunosuppressive environment [58].

Beyond the density of B cells, an increasing number of studies reported that the spatial localization of these cells have an impact on patient's outcome. In particular the density of B cell follicles characteristic of TLS is positively associated with outcomes. M.C. Dieu-Nojean and col. showed that an increase in B cell density within the TLS is associated with prolonged survival in NSCLC patients [21]. Similar results were reported in CRC [59] and oral squamous carcinoma [60].

2.4.3 Macrophages

Tumor-associated macrophages (TAM) are a major component of the TME, found both at the tumor core and the invasive margin. The prognostic value of TAM seems to be dependent of the tumor type. Increased density of TAMs is associated with a good prognosis in CRC [61], HCC [62], prostate [63], and cervical cancer [64]. At the opposite an increased TAM density is associated with poor prognosis in endometrial [65], gastric [66], urothelial [67], HCC [68], melanoma [69], breast [70], ovarian [71], bladder [67], NSCLC [72], and primary CRC tumors [13]. These discrepancies might be explained by the plasticity of these cells since we know that they can switch from a pro-tumoral function (M2) to an antitumoral function (M1) and vice versa [12]. M2 TAMs are associated with a shorter survival and M1 TAMs with a longer survival [22]. Unfortunately, there are no specific or consensual markers to define M1/M2 TAMs. Most of the studies used CD11c or NOS2 for M1 TAMs and CD163, CD204, or CD206 for M2 TAMs, but the use of these markers is still debated.

Tumors contain another heterogeneous subset of cells of myeloid origin, the myeloid-derived suppressor cells (MDSC). Such cells have an immature phenotype and exert profound immunosuppressive activities. Specific and robust tools are still needed for their identification in the human TME.

2.4.4 New Techniques to Estimate the Immune Cell Populations in Tumors

The most broadly used way to quantify tumor-infiltrating immune cells is to detect the protein expression of specific markers either by IHC or

IF. These techniques have been improved in the last decade, allowing to detect multiple proteins (multiplex IHC or IF) and to quantify cells automatically. Nevertheless, they remain expensive and difficult to standardize across laboratories, and available antibodies could lack sensitivity or specificity to accurately detect some of immune cell populations.

Efforts have been made to use transcriptome to estimate the composition of the TME. Nevertheless, variability in the signal has limited its applicability until recently. New methods such as CIBERSORT [13] or MCP-counter [73] aim at providing very precise quantitative information about the cell content of heterogeneous samples. Using MCP-counter, we estimated the abundance of immune cells, fibroblasts, and endothelial cell infiltrates, in transcriptomes of 25 different cancers (n = 19,000). The results showed the relative heterogeneity of the cellular composition of the tumor microenvironment in different cancers and confirmed that the inferred density of $CD8^+$ or cytotoxic T cells correlated with favorable prognosis in most cancer types [73] (Fig. 2.3).

Fig. 2.3 Estimation of the abundance of infiltrating immune and stromal cells and their prognostic significance across human solid tumors. *Left*, means of MCP-counter scores across malignant tissues (more than 19,000 tumors) in three transcriptomic platforms. *Right*, univariate prognostic values (overall survival) associated with MCP-counter scores in human solid tumors. *Green* represents significant favorable prognostic impact and *purple* significant poor prognostic impact. *Gray* represents no significant prognostic impact. Adapted from Becht E et al., Genome Biol. (2016) [73]

2.5 TME as Predictors of Response to Therapy

After decades of having targeted on tumor cells and their molecular alterations, new immuno-oncology (IO) agents such as CBT have shed a light on the crucial role of the TME. The currently approved CBT targets are CTLA-4 (ipilimumab) or the PD-1/PD-L1 axis (nivolumab, pembrolizumab, atezolizumab avelumab) [74]. These mAb block the negative signal received by T cells after their interactions with APCs or with tumor cells, thus being able to reverse T cell exhaustion.

As the main target of these agents are T cell infiltrating the tumor, efforts to predict CBT efficacy have been focusing on their characterization in terms of density, localization, phenotype and functionality, before and/or during treatment.

Other well-known and debatable candidates are still investigated as a "biomarker of efficacy" such as PD-L1 expression by IHC or the neoantigen/mutational burden, but are outside the scope of this chapter [75].

2.5.1 First Emerging Data from Checkpoint Blockade Treated Patients

2.5.1.1 Tumor-Infiltrating Lymphocytes

With the growing number of patients treated with anti-PD-1/PD-L1, translational data on the pharmacodynamics effect of these therapies on the TME are emerging. Tumeh et al. reported in patients with melanoma a higher density of CD8 TILs at baseline in responding patient to pembrolizumab (anti-PD-1) [76]. As with ipilimumab, serial biopsies on treatment showed an increased density of $CD8^+$ TILs in the responding group. In another exploratory study 53 melanoma patients who first received ipilimumab and then anti-PD-1 (pembrolizumab) at progression were serially biopsied before and on treatment. IHC analyses of the TME revealed that the increase of $CD8^+$ TIL density early on treatment was associated with response to ipilimumab, whereas baseline TIL density was not [77]. For the 46 patients who subsequently received anti-PD-1 after progression on ipilimumab, there was a statistically significant difference in the density of $CD8^+$, $CD3^+$, and $CD45RO^+$ T cells in pre-treatment samples of responders compared to nonresponders. In addition a very highly statistically significant difference in the expression of markers for T cell subsets—CD8, CD4, and CD3—and immunomodulatory molecules PD-1 and LAG3 was observed in early on-treatment tumor samples of responders versus nonresponders to therapy. Altogether these results highlight the unlocking effects of CBT on T cell response. In addition, the authors reported an increase in the ratio of $CD8^+$ TIL in the tumor center (TC) vs the IM in early on-treatment biopsies within responders compared to nonresponders suggesting an infiltration of the TILs from the IM to TC as a consequence to therapy [77]. Finally, IHC results were confirmed by gene expression analyses.

Another group performed the phenotypic analyses of TILs (flow cytometry) at baseline from 40 patients (discovery cohort and validation of 20 patients each) with metastatic melanoma treated with an anti-PD-1 [78]. CTLA4 expression by TILs was the only parameter significantly associated with a clinical response in multivariate analysis. The response rate (RR) and PFS were significantly correlated with the relative abundance of CTLA-4^{hi}PD-1^{hi} $CD8^+$ TILs.

In a multi-cohort phase I study of patients treated with atezolizumab (anti-PD-L1), both increased density of CD8 by IHC and high Teff signatures (genes regulated by interferon gamma (IFNg), including IFNg, CD8A, granzyme A, granzyme B, EOMES, and perforin) correlated with response in melanoma, but no association with clinical benefit was observed in RCC [79]. However, a higher ratio of Teff to Treg as revealed by gene expression was associated with atezolizumab response in RCC.

A translational study dedicated to investigate how VEGF blockade with bevacizumab could potentiate PD-L1 checkpoint inhibition with atezolizumab in mRCC was recently reported [80]. The authors showed that bevacizumab alone tends to increase the gene signatures associated with T-helper 1 (Th1) chemokines and CD8 T effectors, and the combination with atezolizumab further increases expression of these signatures. IHC showed similar results with an increase of $CD8^+$ density following bevacizumab, which was more pronounced with the combination. Interestingly the increased density of $CD8^+$ TILs seemed to reflect an increased trafficking into the tumor rather than

an in situ increased proliferation (unchanged ratio of Ki67+/Ki67- among CD8⁺ TIL) [80].

2.5.2 From the Molecular to the Immune Signatures

Escape to the immune surveillance has been proposed as an important mechanism of resistance to a number of systemic therapies including targeted therapies such as antiangiogenic agents [81]. Indeed, immune escape is one of the main mechanisms of resistance to VEGFR-TKI in ccRCC [82]. It was recently reported that metastatic ccRCC treated with sunitinib (VEGFR-TKI) could be classified into four distinct molecular groups (ccrcc1 to 4) using transcriptomic analysis [83]. The four groups had significantly distinct prognosis with ccrrcc1 and 4 having the poorest survival and response to sunitinib. Interestingly we found that immune cell infiltrates were different according to molecular groups [84]. For instance ccrcc4 tumors were the most highly infiltrated in T cells and had the highest expression of immunosuppressive markers such as PD-L1, PD-1, LAG-3, TIM-3, suggesting exhaustion of T cells within these tumors. Conversely, ccrcc1 tumors, which were also associated with poor prognosis, had the poorest T cell infiltration and a low expression of T CB markers. As the density of CD8⁺ infiltrating the tumor has been associated with CBT efficacy, we made the hypothesis that ccrcc4 could respond to PD-1/PD-L1 blockade alone. In contrast an anti-PD-1/PD-L1 alone might not be fully efficient in ccrcc1 due to the lack of CD8 T cells in the tumor. Another therapy able to attract T cells in tumors such as an angiogenesis inhibitor (VEGFR-TKI or anti-VEGF mAb) or CTLA4 blockade could sensitize tumors to anti-PD-1/PD-L1 therapy.

We therefore hypothesize that combination of molecular and immune signatures might be a better predictor of CBT efficacy than each signature alone. Figure 2.4 shows an example of an integrated view

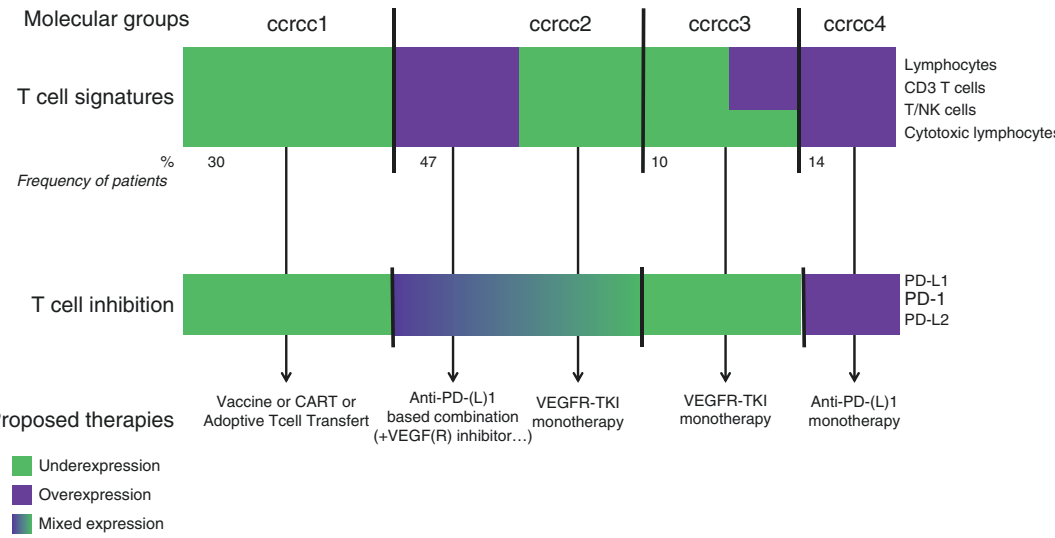

Fig. 2.4 Integrative view of biomarker-driven treatment: example of ccRCC. Using a 35-gene classifier, molecular grouping according to Beuselinck et al. [83] identified four groups of patients (ccrcc1 to 4) with distinct response to sunitinib, ccrcc3 having the best response to sunitinib. The ccrcc molecular groups have different gene expression immune profiles: immune-desert (enriched in ccrcc1), immune-competent (enriched in ccrcc3), immune-high (enriched in ccrcc4), and mixed (enriched in ccrcc2) tumors. CD8⁺ T cell infiltration evaluated by immunohistochemistry confirmed these four phenotypes [83]. T cell inhibition signatures based on the gene expression of immunoregulatory checkpoints and their ligands refine the four immunophenotypes and provide additional information to drive patient and treatment selection. ccrcc1 tumors are immune-desert and patients may benefit from a T cell attractant-based therapy such as vaccine or CAR-T cell or adoptive T cell transfer; ccrcc4 tumors are immune-high with a high density of T cells and high expression of immunoregulatory checkpoints; ccrcc4 patients may benefit from anti-PD-(L)1 alone. ccrcc3 tumors are immune-competent with a high infiltration of T cells but low expression of immunoregulatory checkpoints; VEGFR-TKI alone provides excellent results in this ccrcc3 group of patients [83]. ccrcc2 tumors are mixed in terms of T cell infiltration as well as expression of immunoregulatory checkpoints; ccrcc2 patients may be treated according to T cell infiltration and expression of immunoregulatory checkpoints

of how to combine multiple biomarkers to drive patient selection in ccRCC.

To confirm these hypotheses, we launched in March 2017 the first biomarker-driven trial to date in ccRCC called BIONIKK (BIOmarker-driven trial with Nivolumab and Ipilimumab or VEGFR tKi in naïve metastatic Kidney cancer, NCT02960906) [85]. This trial randomizes mRCC patients to receive a first line of systemic therapy with nivolumab (anti-PD-1), ipilimumab (anti-CTLA4), the combination, or a TKI according to their molecular subgroup. The primary endpoint is the objective response rate according to therapy and molecular groups. Immune infiltrates and their correlation with outcome and molecular groups will be evaluated using IHC and gene expression analyses (MCP-counter).

Conclusion

The findings of complex interactions between tumor cells and the host has led to define the concept of the immune contexture which include organization, location, density, and functional orientation of immune cells in the TME. This immune contexture helps to understand pathophysiological mechanisms that support the clinical impact of various cells of the immune response [86].

The growing approval rate of CBT targeting the PD-1/PD-L1 axis through many tumor types stimulates research teams worldwide to go deeper in the comprehension of the immune contexture to better optimize the efficacy of these agents. In addition, the high number of IO agents currently evaluated in clinical trials provides a huge competition between companies which in turn force them to understand the importance of selecting patients and to make financial efforts to support translational studies.

Many efforts are currently done to find a way to select patients who will have a durable benefit from CBT. Characterization of the tumor-infiltrating immune cells may provide one of the most promising biomarkers of efficacy. Nevertheless, some technical challenges explain why such promising biomarkers are not reproducible or difficult to assess. One of these challenges is inherent to the technique of IHC or IF. Even if major advances have been made on this field, we have to deal with high intratumor heterogeneity and lack of specific markers and to interpret a static evaluation of a dynamic process. The first two points could be partially resolved by the progress in transcriptomic analyses and particularly in the immune signatures that were recently developed such as in MCP-counter. It provides a high accuracy in defining the proportion of immune cells, is reproducible, is less dependent to tumor heterogeneity, and finally allows to compare between tumor types.

Characterization of the immune TME together with the deep characterization of malignant cells using next-generation sequencing (NGS), RNA sequencing, as well as multiplex IF will allow to treat patients with the most appropriate precision medicine and to closely monitor the dynamic changes during CBT.

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References

1. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004;21(2):137–48.
2. Nowell PC. The clonal evolution of tumor cell populations. Science. 1976;194(4260):23–8.
3. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science. 2013;339(6127):1546–58.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
5. Greaves M, Maley CC. Clonal evolution in cancer. Nature. 2012;481(7381):306–13.
6. Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol. 2008;9(4):297–308.
7. Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. Nat Rev Cancer. 2013;13(11):759–71.
8. Giraldo NA, Becht E, Vano Y, Sautès-Fridman C, Fridman WH. The immune response in cancer: from immunology to pathology to immunotherapy. Virchows Arch Int J Pathol. 2015;467(2):127–35.

9. Crusz SM, Balkwill FR. Inflammation and cancer: advances and new agents. Nat Rev Clin Oncol. 2015;12(10):584–96.
10. Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer. 2010;10(12):878–89.
11. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012;12(4):298–306.
12. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol. 2010;11(10):889–96.
13. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity. 2013;39(4):782–95.
14. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol. 2008;9(5):503–10.
15. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer. 2012;12(4):265–77.
16. Gardner A, Ruffell B. Dendritic cells and cancer immunity. Trends Immunol. 2016;37(12):855–65.
17. Giraldo NA, Becht E, Pagès F, Skliris G, Verkarre V, Vano Y, et al. Orchestration and prognostic significance of immune checkpoints in the microenvironment of primary and metastatic renal cell cancer. Clin Cancer Res. 2015;21(13):3031–40.
18. Sautès-Fridman C, Lawand M, Giraldo NA, Kaplon H, Germain C, Fridman WH, et al. Tertiary lymphoid structures in cancers: prognostic value, regulation, and manipulation for therapeutic intervention. Front Immunol. 2016;7:407.
19. Dieu-Nosjean M-C, Goc J, Giraldo NA, Sautès-Fridman C, Fridman WH. Tertiary lymphoid structures in cancer and beyond. Trends Immunol. 2014;35(11):571–80.
20. de Chaisemartin L, Goc J, Damotte D, Validire P, Magdeleinat P, Alifano M, et al. Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid structures in human lung cancer. Cancer Res. 2011;71(20):6391–9.
21. Germain C, Gnjatic S, Tamzalit F, Knockaert S, Remark R, Goc J, et al. Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. Am J Respir Crit Care Med. 2014;189(7):832–44.
22. Becht E, Giraldo NA, Germain C, de Reyniès A, Laurent-Puig P, Zucman-Rossi J, et al. Immune contexture, immunoscore, and malignant cell molecular subgroups for prognostic and theranostic classifications of cancers. Adv Immunol. 2016;130:95–190.
23. Vignali DAA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol. 2008;8(7):523–32.
24. Zhang N, Bevan MJ. CD8(+) T cells: foot soldiers of the immune system. Immunity. 2011;35(2):161–8.
25. Speiser DE, Ho P-C, Verdeil G. Regulatory circuits of T cell function in cancer. Nat Rev Immunol. 2016;16(10):599–611.
26. Balkwill F, Montfort A, Capasso M. B regulatory cells in cancer. Trends Immunol. 2013;34(4):169–73.
27. Dieu-Nosjean M-C, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol. 2008;26(27):4410–7.
28. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313(5795):1960–4.
29. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. N Engl J Med. 2003;348(3):203–13.
30. Pagès F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med. 2005;353(25):2654–66.
31. Remark R, Alifano M, Cremer I, Lupo A, Dieu-Nosjean M-C, Riquet M, et al. Characteristics and clinical impacts of the immune environments in colorectal and renal cell carcinoma lung metastases: influence of tumor origin. Clin Cancer Res. 2013;19(15):4079–91.
32. Tian C, Lu S, Fan Q, Zhang W, Jiao S, Zhao X, et al. Prognostic significance of tumor-infiltrating $CD8^+$ or $CD3^+$ T lymphocytes and interleukin-2 expression in radically resected non-small cell lung cancer. Chin Med J. 2015;128(1):105–10.
33. Che Y-Q, Feng L, Rong W-Q, Shen D, Wang Q, Yang L, et al. Correlation analysis of peripheral blood T cell subgroups, immunoglobulin and prognosis of early hepatocellular carcinoma after hepatectomy. Int J Clin Exp Med. 2014;7(11):4282–90.
34. Ness N, Andersen S, Valkov A, Nordby Y, Donnem T, Al-Saad S, et al. Infiltration of CD8+ lymphocytes is an independent prognostic factor of biochemical failure-free survival in prostate cancer. Prostate. 2014;74(14):1452–61.
35. Petitprez F, Fossati N, Vano Y, Freschi M, Becht E, Lucianò R, et al. European Urology Focus, 2017, ISSN 2405-4569, http://dx.doi.org/10.1016/j.euf.2017.05.013. (http://www.sciencedirect.com/science/article/pii/S2405456917301517)
36. Mlecnik B, Bindea G, Angell HK, Maby P, Angelova M, Tougeron D, et al. Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity. 2016;44(3):698–711.
37. Galon J, Mlecnik B, Marliot F, Ou F-S, Bifulco CB, Lugli A, et al. Validation of the Immunoscore (IM) as a prognostic marker in stage I/II/III colon cancer: Results of a worldwide consortium-based analysis of 1,336 patients. J Clin Oncol. 2016 34:15_suppl, 3500–3500.
38. Kirilovsky A, Marliot F, El Sissy C, Haicheur N, Galon J, Pagès F. Rational bases for the use of the immunoscore in routine clinical settings as a prog-

nostic and predictive biomarker in cancer patients. Int Immunol. 2016;28(8):373–82.
39. Dieu-Nosjean M-C, Giraldo NA, Kaplon H, Germain C, Fridman WH, Sautès-Fridman C. Tertiary lymphoid structures, drivers of the anti-tumor responses in human cancers. Immunol Rev. 2016;271(1): 260–75.
40. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A, et al. CD4+ follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest. 2013;123(7):2873–92.
41. Boissière-Michot F, Lazennec G, Frugier H, Jarlier M, Roca L, Duffour J, et al. Characterization of an adaptive immune response in microsatellite-instable colorectal cancer. Oncoimmunology. 2014;3:e29256.
42. Fridman WH, Remark R, Goc J, Giraldo NA, Becht E, Hammond SA, et al. The immune microenvironment: a major player in human cancers. Int Arch Allergy Immunol. 2014;164(1):13–26.
43. Mahmoud SMA, Lee AHS, Paish EC, Macmillan RD, Ellis IO, Green AR. The prognostic significance of B lymphocytes in invasive carcinoma of the breast. Breast Cancer Res Treat. 2012;132(2):545–53.
44. van Herpen CML, van der Voort R, van der Laak JAWM, Klasen IS, de Graaf AO, van Kempen LCL, et al. Intratumoral rhIL-12 administration in head and neck squamous cell carcinoma patients induces B cell activation. Int J Cancer. 2008;123(10):2354–61.
45. Santoiemma PP, Reyes C, Wang L-P, McLane MW, Feldman MD, Tanyi JL, et al. Systematic evaluation of multiple immune markers reveals prognostic factors in ovarian cancer. Gynecol Oncol. 2016;143(1):120–7.
46. Berntsson J, Nodin B, Eberhard J, Micke P, Jirström K. Prognostic impact of tumour-infiltrating B cells and plasma cells in colorectal cancer. Int J Cancer. 2016;139(5):1129–39.
47. Goeppert B, Frauenschuh L, Zucknick M, Stenzinger A, Andrulis M, Klauschen F, et al. Prognostic impact of tumour-infiltrating immune cells on biliary tract cancer. Br J Cancer. 2013;109(10):2665–74.
48. Garg K, Maurer M, Griss J, Brüggen M-C, Wolf IH, Wagner C, et al. Tumor-associated B cells in cutaneous primary melanoma and improved clinical outcome. Hum Pathol. 2016;54:157–64.
49. Yuseff M-I, Pierobon P, Reversat A, Lennon-Duménil A-M. How B cells capture, process and present antigens: a crucial role for cell polarity. Nat Rev Immunol. 2013;13(7):475–86.
50. Carmi Y, Spitzer MH, Linde IL, Burt BM, Prestwood TR, Perlman N, et al. Allogeneic IgG combined with dendritic cell stimuli induce antitumour T-cell immunity. Nature. 2015;521(7550):99–104.
51. Silva NSD, Klein U. Dynamics of B cells in germinal centres. Nat Rev Immunol. 2015;15(3):137.
52. de Wit J, Souwer Y, Jorritsma T, Klaasse Bos H, ten Brinke A, Neefjes J, et al. Antigen-specific B cells reactivate an effective cytotoxic T cell response against phagocytosed Salmonella through cross-presentation. PLoS One. 2010;5(9):e13016.
53. Deola S, Panelli MC, Maric D, Selleri S, Dmitrieva NI, Voss CY, et al. Helper B cells promote cytotoxic T cell survival and proliferation independently of antigen presentation through CD27/CD70 interactions. J Immunol. 2008;180(3):1362–72.
54. Barbera-Guillem E, Nelson MB, Barr B, Nyhus JK, May KF, Feng L, et al. B lymphocyte pathology in human colorectal cancer. Experimental and clinical therapeutic effects of partial B cell depletion. Cancer Immunol Immunother (CII). 2000;48(10):541–9.
55. DeNardo DG, Andreu P, Coussens LM. Interactions between lymphocytes and myeloid cells regulate pro-versus anti-tumor immunity. Cancer Metastasis Rev. 2010;29(2):309–16.
56. de Visser KE, Korets LV, Coussens LM. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. Cancer Cell. 2005;7(5):411–23.
57. Barbera-Guillem E, May KF, Nyhus JK, Nelson MB. Promotion of tumor invasion by cooperation of granulocytes and macrophages activated by anti-tumor antibodies. Neoplasia. 1999;1(5):453–60.
58. Olkhanud PB, Damdinsuren B, Bodogai M, Gress RE, Sen R, Wejksza K, et al. Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4+ T cells to T-regulatory cells. Cancer Res. 2011;71(10):3505–15.
59. Meshcheryakova A, Tamandl D, Bajna E, Stift J, Mittlboeck M, Svoboda M, et al. B cells and ectopic follicular structures: novel players in anti-tumor programming with prognostic power for patients with metastatic colorectal cancer. PLoS One. 2014;9(6):e99008.
60. Wirsing AM, Rikardsen OG, Steigen SE, Uhlin-Hansen L, Hadler-Olsen E. Characterisation and prognostic value of tertiary lymphoid structures in oral squamous cell carcinoma. BMC Clin Pathol. 2014;14:38.
61. Algars A, Irjala H, Vaittinen S, Huhtinen H, Sundström J, Salmi M, et al. Type and location of tumor-infiltrating macrophages and lymphatic vessels predict survival of colorectal cancer patients. Int J Cancer. 2012;131(4):864–73.
62. Shu Q-H, Ge Y-S, Ma H-X, Gao X-Q, Pan J-J, Liu D, et al. Prognostic value of polarized macrophages in patients with hepatocellular carcinoma after curative resection. J Cell Mol Med. 2016;20(6):1024–35.
63. Shimura S, Yang G, Ebara S, Wheeler TM, Frolov A, Thompson TC. Reduced infiltration of tumor-associated macrophages in human prostate cancer: association with cancer progression. Cancer Res. 2000;60(20):5857–61.
64. Heller DS, Hameed M, Cracchiolo B, Wiederkehr M, Scott D, Skurnick J, et al. Int J Gynecol Cancer. 2003;13(1):67–70.
65. Čermáková P, Melichar B, Tomšová M, Zoul Z, Kalábová H, Spaček J, et al. Prognostic significance of CD3+ tumor-infiltrating lymphocytes in patients with endometrial carcinoma. Anticancer Res. 2014;34(10):5555–61.

66. Wang XL, Jiang JT, Wu CP. Prognostic significance of tumor-associated macrophage infiltration in gastric cancer: a meta-analysis. Genet Mol Res (GMR). 2016;15(4) doi:10.4238/gmr15049040.
67. Sjödahl G, Lövgren K, Lauss M, Chebil G, Patschan O, Gudjonsson S, et al. Infiltration of CD3+ and CD68+ cells in bladder cancer is subtype specific and affects the outcome of patients with muscle-invasive tumors. Urol Oncol. 2014;32(6):791–7.
68. Dong P, Ma L, Liu L, Zhao G, Zhang S, Dong L, et al. CD86$^+$/CD206$^+$, diametrically polarized tumor-associated macrophages, predict hepatocellular carcinoma patient prognosis. Int J Mol Sci. 2016;17(3):320.
69. Jensen TO, Schmidt H, Møller HJ, Høyer M, Maniecki MB, Sjoegren P, et al. Macrophage markers in serum and tumor have prognostic impact in American Joint Committee on Cancer stage I/II melanoma. J Clin Oncol. 2009;27(20):3330–7.
70. Medrek C, Pontén F, Jirström K, Leandersson K. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. BMC Cancer. 2012;12:306.
71. Colvin EK. Tumor-associated macrophages contribute to tumor progression in ovarian cancer. Front Oncol. 2014;4:137.
72. Mei J, Xiao Z, Guo C, Pu Q, Ma L, Liu C, et al. Prognostic impact of tumor-associated macrophage infiltration in non-small cell lung cancer: a systemic review and meta-analysis. Oncotarget. 2016;7(23):34217–28.
73. Becht E, Giraldo NA, Lacroix L, Buttard B, Elarouci N, Petitprez F, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Biol. 2016;17(1):218.
74. Martin-Liberal J, de Olza MO, Hierro C, Gros A, Rodon J, Tabernero J. The expanding role of immunotherapy. Cancer Treat Rev. 2017;54:74–86. doi:10.1016/j.ctrv.2017.01.008. Epub 2017 Feb 11. Review.
75. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. Lancet Oncol. 2016;17(12):e542–51.
76. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJM, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568–71.
77. Chen P-L, Roh W, Reuben A, Cooper ZA, Spencer CN, Prieto PA, et al. Analysis of immune signatures in longitudinal tumor samples yields insight into biomarkers of response and mechanisms of resistance to immune checkpoint blockade. Cancer Discov. 2016;6(8):827–37.
78. Daud AI, Loo K, Pauli ML, Sanchez-Rodriguez R, Sandoval PM, Taravati K, et al. Tumor immune profiling predicts response to anti-PD-1 therapy in human melanoma. J Clin Invest. 2016;126(9):3447–52.
79. McDermott DF, Sosman JA, Sznol M, Massard C, Gordon MS, Hamid O, et al. Atezolizumab, an anti-programmed death-ligand 1 antibody, in metastatic renal cell carcinoma: long-term safety, clinical activity, and immune correlates from a phase IA study. J Clin Oncol. 2016;34(8):833–42.
80. Wallin JJ, Bendell JC, Funke R, Sznol M, Korski K, Jones S, et al. Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. Nat Commun. 2016;7:12624.
81. Liu X-D, Hoang A, Zhou L, Kalra S, Yetil A, Sun M, et al. Resistance to antiangiogenic therapy is associated with an immunosuppressive tumor microenvironment in metastatic renal cell carcinoma. Cancer Immunol Res. 2015;3(9):1017–29.
82. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet A-L, et al. VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. J Exp Med. 2015;212(2):139–48.
83. Beuselinck B, Job S, Becht E, Karadimou A, Verkarre V, Couchy G, et al. Molecular subtypes of clear cell renal cell carcinoma are associated with sunitinib response in the metastatic setting. Clin Cancer Res. 2015;21(6):1329–39.
84. Becht E, Giraldo NA, Beuselinck B, Job S, Marisa L, Vano Y, et al. Prognostic and theranostic impact of molecular subtypes and immune classifications in renal cell cancer (RCC) and colorectal cancer (CRC). Oncoimmunology. 2015;4(12):e1049804.
85. A BIOmarker driven trial with Nivolumab and Ipilimumab or VEGFR tKi in Naïve Metastatic Kidney Cancer - full text view - ClinicalTrials.gov [Internet]. [cited 2017 Feb 28]. Available from: https://clinicaltrials.gov/ct2/show/NCT02960906
86. Fridman WH, Zitvogel L, Sautès-Fridman C, et al. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol. 2017 Jul 25. doi:10.1038/nrclinonc.2017.101. [Epub ahead of print] Review.