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Prognostic and Therapeutic Implications of Lymphocytes in Hematological Disorders and Solid Malignancies

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Abstract

An efficient and specific cytotoxic immune response against a tumor requires a complex, rapidly evolving interaction between various immune cell types in the adaptive and innate immune system. This pliable interplay is a relentless process that has been concisely organized in three different phases: elimination, equilibrium, and escape. The identification of key immune players and molecules involved in this interplay has been crucial for the introduction of reliable prognostic factors and effective therapeutic protocols against cancers. In this chapter, we aim to depict the roles of these distinct immune cell subsets, summarize the prognostic value of immune cells in different cancer types, and discuss briefly the principles of different immunotherapeutic approaches against hematologic malignancies and solid tumors.

Keywords: lymphocytes, prognostic, therapeutic, implications, hematological, solid, malignancy

1. Introduction

Lymphocytes are a diverse population of cells that participate in both innate and adaptive immunity. There are three broad classes of lymphocyte—B cells, T cells and NK cells—and these have different developmental pathways, life span, preferred areas of settlement within the lymphoid organs, surface structure, molecular markers, and function. For many years, the cornerstones of cancer treatment have been surgery, chemotherapy, and radiation, and

more recently targeted therapies. Although these approaches have contributed to improved outcomes, most malignancies still carry a poor prognosis. Recently, cancer immunotherapy is the most exciting advancement in cancer therapy. In the following chapter, four main aspects will be addressed;

- i. Immune cell types involved in tumor recognition and rejection.
- ii. Role of different immune cells in hematological malignancies; prognostic and therapeutic implications.
- iii. Prognostic value of infiltrating immune cells in solid tumors.
- iv. Immunotherapeutic modalities for solid tumors.

2. Immune cell types involved in tumor recognition and rejection

An efficient and specific cytotoxic immune response against a tumor requires a complex, rapidly evolving interaction between various immune cell types in the adaptive and innate immune system. These cells include [1]: (i) CD8⁺ lymphocytes and Th1/Th2 subclasses of CD4⁺ T lymphocytes, traditionally referred to as cytotoxic T cells and helper T cells. They initiate the distinction between self and non-self-antigens, through recognition at the “immune synapse”, (ii) natural killer (NK) cells, characteristically, do not require antigen presentation by the major histocompatibility complex (MHC) for cytotoxic activity. Like T cells, NK cells express numerous inhibitory molecules, e.g. various killer immunoglobulin-like receptor (KIR) subtypes [2], (iii) additional cell types, such as FoxP3⁺ CD25⁺ CD4⁺ T regulatory (Treg) and myeloid derived suppressor cells (MDSCs) largely inhibit cytotoxic T lymphocyte activity [3] and (iv) macrophages differentiate into at least 2 different phenotypes: M1 macrophages, which release interferon (IFN) gamma and are responsible for phagocytosis, and M2 macrophages, which release cytokines such as IL-4, IL-10, transforming growth factor beta (TGF-beta), and dampen inflammatory responses and foster tolerance [4]. The “*immune synapse*” is the ability of T lymphocytes to distinguish self- versus non-self-antigens, which are presented by antigen-presenting cells (APCs) such as dendritic cells. Overall, the cytotoxic activity of a CD8⁺ T cell is regulated by the presence and spatial orientation of a set of stimulatory and inhibitory receptors whose expression is regulated by a myriad of cytokines. Together, this configuration is often referred to as the “immune synapse” [1]. For efficient activation of a naïve CD8⁺ T cell, its T cell receptor (TCR) must bind to a peptide presented by the MHC in the presence of a second set of costimulatory signals. This interaction leads to CD3 intracellular signaling that causes secretion of pro-inflammatory cytokines such as IL-12 and IFN gamma. In the absence of a costimulatory signal, a state of peripheral tolerance to the antigen “anergy” develops [5]. The most important costimulatory signal in naïve T cells is CD28, which binds to B7-1 and B7-2 (CD80/86) on the APC. This costimulatory process is tightly regulated by both “agonist” molecules (e.g., GITR, OX40, ICOS) and inhibitory signals on both the APC and T cells, often collectively referred to as “immune checkpoint” molecules. Examples of co-inhibitory or “immune checkpoint” molecules include cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death-1 (PD-1), TIM3, and LAG3. Chronic recognition of an

antigen may lead to feedback inhibition of effector T cell function, resulting in a phenotype termed “exhaustion” [6].

2.1. Tumor evasion of immune surveillance

The theory of ‘immunoediting,’ developed by Dunn et al. [7], emphasizes the dual role of the immune system in tumor progression, defining a very fine dynamic interplay between immune and malignant cells, which is characterized by three different phases: elimination, equilibrium, and escape (Figure 1). (i) *The elimination phase* consists of innate and adaptive

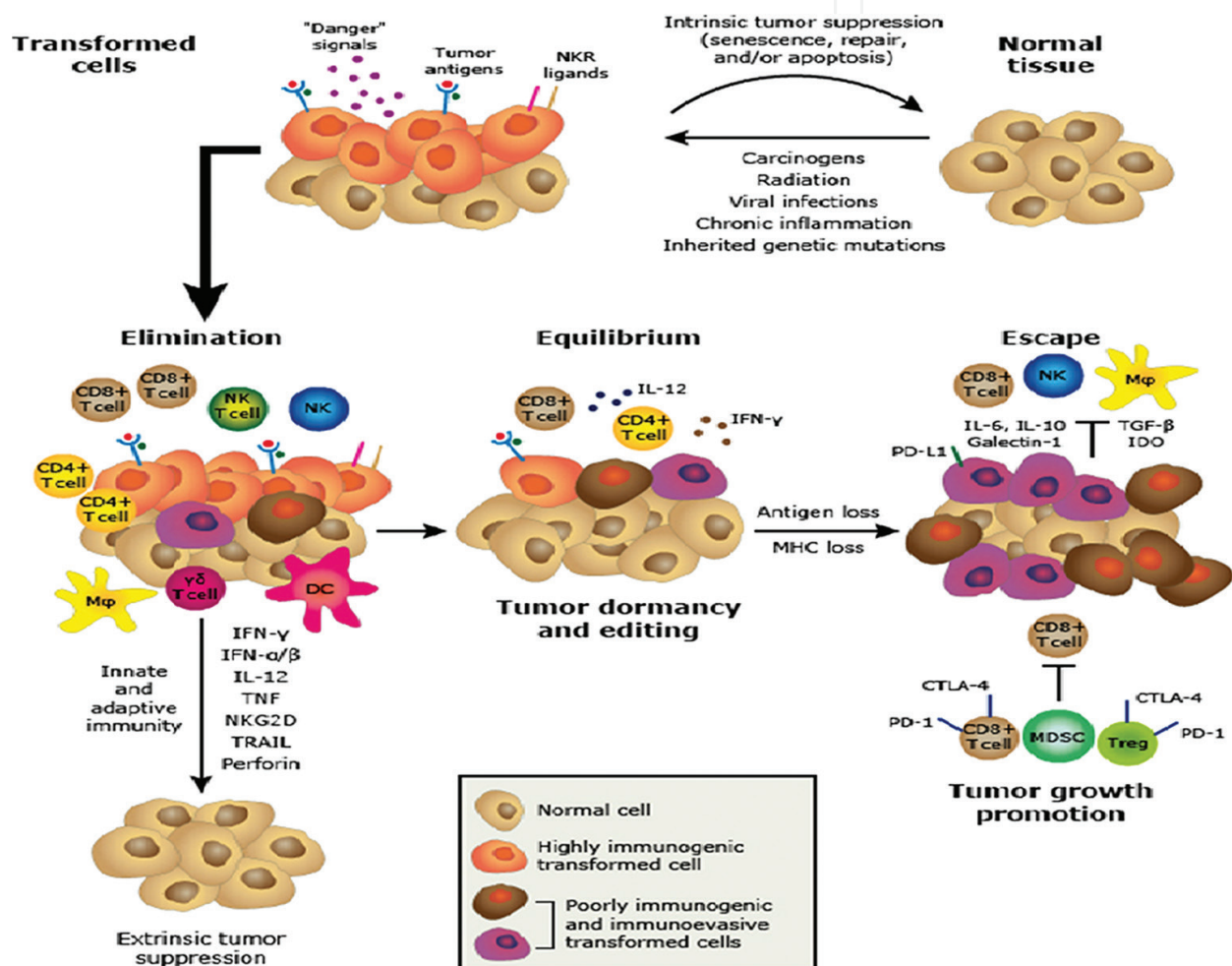


Figure 1. Cancer immunoediting. Cancer immunoediting is an extrinsic tumor suppressor mechanism that engages only after cellular transformation has occurred and intrinsic tumor suppressor mechanisms have failed. Cancer immunoediting consists of 3 sequential phases: elimination, equilibrium, and escape. In the elimination phase, innate and adaptive immunity work together to destroy developing tumors long before they become clinically apparent. If, however, a rare cancer cell variant is not destroyed in the elimination phase, it may then enter the equilibrium phase, in which its outgrowth is prevented by immunologic mechanisms. Equilibrium is a function of adaptive immunity only, where T cells, IL-12, and IFN-γ are required to maintain tumor cells in a state of functional dormancy, whereas NK cells and molecules that participate in the recognition or effector function of cells of innate immunity are not required. Editing of tumor immunogenicity occurs in the equilibrium phase. Equilibrium may also represent an end stage of the cancer immunoediting process, where outgrowth of occult cancers is restrained for the lifetime of the host. However, as a consequence of constant immune selection pressure placed on genetically unstable tumor cells held in equilibrium, tumor cells may then enter the escape phase, in which their outgrowth is no longer blocked by immunity. These tumor cells emerge to cause clinically apparent disease.

immune responses to specific tumor-associated antigens and is characterized by T, B, and NK cell effector function, which is mediated by cytokines such as IFN alpha, IFN gamma, and IL-12. (ii) *The equilibrium phase* is a balance between immune-mediated destruction by the adaptive immune system (e.g. activated CD4+ and CD8+ T cells) and persistence of rare malignant clones. (iii) *Immunologic escape* describes the phase where malignant clones have acquired the ability to evade the adaptive immune system.

3. Role of different immune cells in hematological malignancies; prognostic and therapeutic implications

3.1. Dendritic cells

Dendritic cells (DCs) are bone-marrow-derived immune cells which have a critical role in the initiation and modulation of the adaptive immune response. They support the innate immune response independently from T cells. Besides functioning as the most effective APCs within the immune system, DCs can induce tolerance in the central and peripheral lymphoid organs. Therefore, they act as suppressors rather than stimulators of the immune response. DCs can capture antigens from viable or damaged tumor cells and present the processed peptides to T-cells to prompt the generation and maintenance of an effective tumor-specific T-cell response [8]. It has been clearly established that DCs are pivotal in regulating the delicate balance between immunity and tolerance, so representing the linkage between the innate and adaptive immune responses [9].

3.1.1. Dendritic cells and cancer

DCs are crucial arbiters of the host immune response against tumors since they can regulate effector cells of innate immunity such as NK cells and NK-T cells. DCs also play a fundamental role in orchestrating adaptive immune response as APCs able to cross-present tumor-associated antigens (TAA) to CD4 and CD8 T lymphocytes in regional LNs [10]. The potential cytotoxic activity of killer DCs (KDCs) is dependent on exogenous activation signals such as IFN- α , IFN- γ , CD40L or viruses [11]. KDCs promote tumor cell death by a broad array of mechanisms including TNF- α , Fas Ligand, and TNF-related apoptosis-inducing ligand (TRAIL) dependent versus independent pathways [12]. Therefore, it is not surprising that infiltration from DCs within the primary tumor masses has been correlated with a significantly prolonged survival of patients and a reduced incidence of metastatic disease with favorable prognostic features in different types of malignant tumors [13]. On the other hand, tumor cells can deregulate DCs function by different mechanisms; including antigen down-regulation and secretion of cytokines and other factors (IL6, IL10, Vascular Endothelial Growth Factors [VEGF], Transforming Growth Factors beta [TGF- β]). These mechanisms can together adjust the immunostimulating or immunosuppressive functional plasticity of DCs [14]. Cancer microenvironment can prompt the acquisition of tolerogenic and the immunosuppressive functions of DCs turning them into a regulatory state (reg DCs (which hamper the adaptive immune response against some tumor antigens) [15]. The immuno-suppressive activity of reg DCs can be exerted through a variety of enzymatic mechanisms. Reg DCs can secrete immunosuppressive cytokines such as TGF- β and IL-10 which impair the effector immune response

[15]. Moreover, reg DCs express inhibitory molecules such as programmed cell death 1 ligand 1 (PDL-1) and PDL-2, which represent a further pathway for the inhibition of an effective antitumor immunity. Overall, these data underline how malignancies can modulate the pathobiology of DCs favoring their maturation into pathways associated with an immunosuppressive function [16]. Immune dysregulation is a fundamental aspect in the pathogenesis of bone marrow (BM) failure in patients with myelodysplastic syndromes (MDS), a group of heterogeneous clonal hematopoietic disorder characterized by ineffective hematopoiesis, resulting in various degrees of peripheral blood cytopenias [16]. A remarkable genetic alteration of circulating DCs was also demonstrated in acute myeloid leukemia (AML) patients [17]. The recovery of DCs in the BM was investigated in AML patients that were mostly treated with chemotherapy. DCs were absent or at very low levels at diagnosis as compared with the average DC values obtained at complete remission (CR) after chemotherapy [18]. The occurrence and distribution of peripheral blood-derived DCs in mature lymphomas showed that in non-Hodgkin's lymphoma (NHL), DCs were reduced in frequencies and altered in functions as compared to normal and reactive LNs [19]. In chronic lymphocytic leukemia (CLL), a significant reduction in DCs proportions was demonstrated in comparison with healthy subjects, suggesting that DC development is significantly affected by the leukemic process and may contribute to immune dysregulation [20]. Finally, in the setting of an autologous hematopoietic stem cell transplantation (HSCT) for relapsed or refractory diffuse large B-cell non-Hodgkin lymphoma (DLBC NHL), improved overall survival (OS) was correlated with the higher blood pre-transplant and post-transplant levels of DC frequencies [21].

3.2. Natural killer cells

The biology of natural killer (NK) cells is complex. They have powerful cytotoxic activity, however, their activity may be eluded by the tumor microenvironment. Evading NK-cell responses to tumors may occur through immunosubversion, immunoediting or immunoselection of poorly immunogenic tumor cells and interference with tumor infiltration. Tumor cells, together with tumor-associated fibroblasts and tumor-induced aberrant immune cells (e.g. tolerogenic or suppressive macrophages, DCs and T cells) can interfere with NK-cell activation pathways or the receptor array that regulate NK-cell activation and antitumor activity. Therefore, the definition of tumor microenvironment-related immunosuppressive factors, and the identification of new classes of tissue-residing NK-like innate lymphoid cells, may represent attractive insights toward effective NK-cell-based therapies [22] (**Figure 2**).

NK cells are the first subset of lymphocytes to reconstitute after HSCT and likely play an important role in offering protection against relapse in the early months after transplant. In contrast to T cells, NK cells do not cause graft-versus-host disease (GVHD) in the allogeneic setting; indeed, a number of preclinical studies suggest that they may even protect against GVHD by targeting the recipient's dendritic cells [23]. Importantly, the role of NK cells in immune monitoring of tumors is mainly due to their non-human leukocyte antigen (HLA) restricted effect, as the absence or abnormal expression of HLA molecules induces NK-cell cytotoxicity; the so-called "missing self" hypothesis [24]. NK cells play a major role in innate defenses and are also thought to be part of the immunosurveillance against tumors. They express an array of surface receptors that mediate NK cell function. However, NK cell activation and induction of cytolytic activity and cytokine production depends on another important checkpoint, namely the expression on target cells of ligands recognized by activating NK receptors [25] (**Figure 2**).

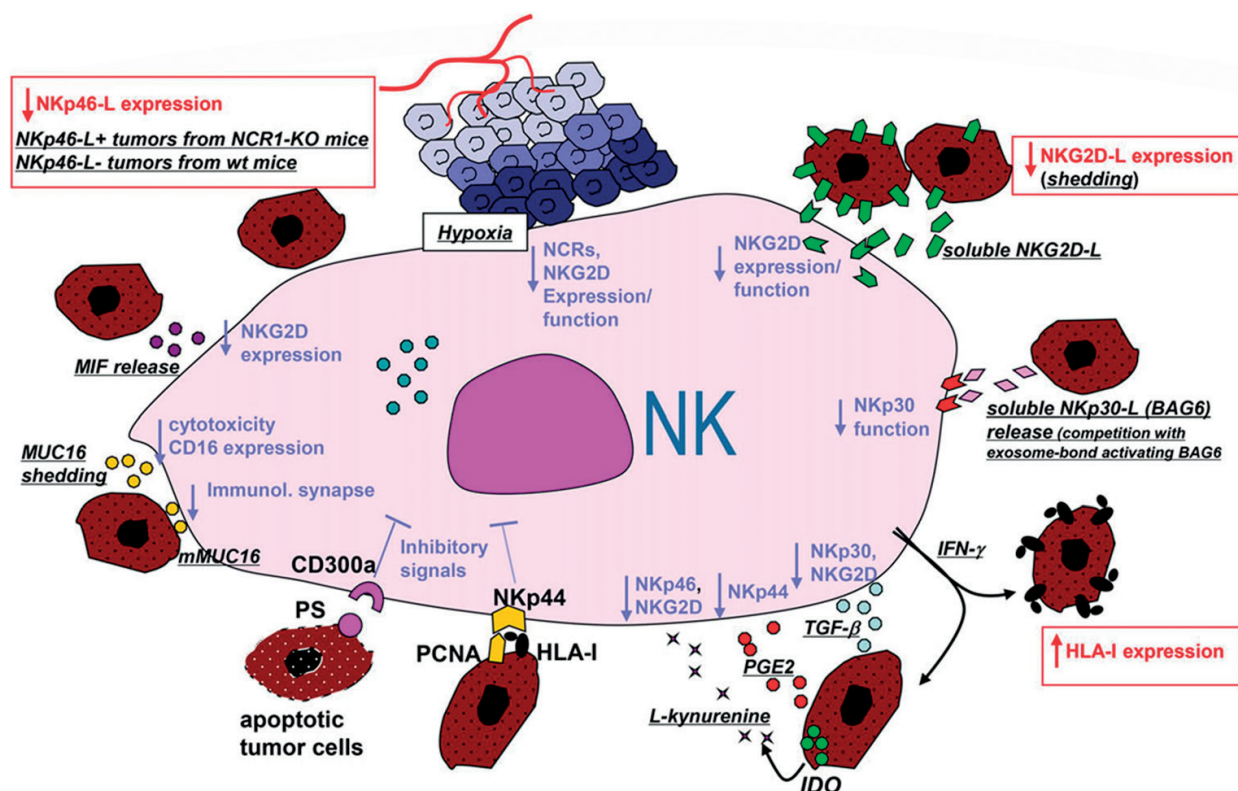


Figure 2. Mechanisms of tumor evasion from the NK-cell attack. The tumor microenvironment can suppress NK cells in different ways (indicated in blue font). Tumor cells (in brown) can induce downregulation of different activating NK-cell receptors and of NK-cell functions by different mechanisms including: the release/expression of macrophage migration inhibitory factor (MEF), MUC16, TGT- β , PGE2, IDO; the chronic engagement of NKG2D by soluble or cell surface-expressed ligands (NKG2D-L); the release of soluble NKp30- L competing for NKp30 with the activating exosome-bound NKp30-L. In addition, apoptotic tumor cells can expose phosphatidylserine (PS) at the outer cell surface. PS can induce inhibitory signals through the engagement of NKp44 by proliferating cell nuclear antigen (PCNA) associated with HLA-I molecules. The pressure of NK cells may favor the editing/selection of tumor cells expressing NK-resistant phenotypes (indicated in red font). The shedding of NKG2D-L, besides inducing NKG2D downmodulation on NK cells, also results in a reduced NKG2D-L expression on target cells and consequent reduced susceptibility to NK-cell-mediated cytotoxicity. Finally, NK cells, through IFN- γ production, can induce HLA-I expression increase on tumor cells.

Hematological malignancies are cancers that affect blood, BM, and LNs. Notably, a major difference from solid tumors, is that hematological malignancies arise from the immune system itself. The majority of adult lymphoid malignancies originate from mature B cells, while a minor proportion arises from mature T cells. Therefore, the role of immune pathways in these tumors is complex [26]. Constant immune surveillance is expected, since cells of the immune system and those of malignancy are in constant contact with each other within the hematopoietic system. Moreover, since the cellular origins of malignancy are those of the immune system, these malignant cells are considered immunostimulatory by their nature [26].

3.2.1. How do hematological malignancies escape from NK cell innate immune surveillance?

Interestingly, tumor-cells develop various escape mechanisms to NK cell surveillance and contribute to the dysfunction of NK-cell cytotoxicity [23]. Defects in NK-cell cytotoxicity have been observed in all hematological malignancies. There are general mechanisms for escape of hematological malignancies from NK cell immunity, which are common to all

immune-effector cells, and they include saturation of the immune system by rapid growth of the tumor and inaccessibility of the tumor because of deficient vascularization [26]. In addition to these general mechanisms, hematological malignancies may adopt NK cells effectors' quantitative deficiency [27]. However, in most hematological malignancies, qualitative impairment of the capacity of NK cytotoxic seems to be more important for tumor escape than quantitative defects. These qualitative defects could be achieved through increased inhibition of NK cell cytotoxicity [28], impaired activation of NK cells [29], impaired differentiation signaling of NK cells [26], and through certain cytokines [23].

3.2.2. Therapeutic approaches utilizing NK cells in hematological malignancies

The cure of high-risk leukemias in the haploidentical HSCT setting is considered the most important clinical application utilizing NK cells. In this scenario, NK cells originated from hematopoietic stem cells of HLA-haploidentical donors may express Killer Immunoglobulin-like receptors (KIRs) that are mismatched with the HLA class I alleles of the recipient. Thus, NK cells are allowed to kill leukemia blasts residual after the conditioning regimen, while sparing normal cells. Another promising approach is based on the use of anti-KIR blocking monoclonal antibodies, rendering alloreactive any KIR+NK cells [28].

3.2.3. Autologous NK cell immunotherapy

Many clinical trials initially explored the possibility of expanding and enhancing the anti-tumor activity of the native lymphocytes of patients in vivo simply by giving the patients high-dose interleukin-2 (IL-2). The use of high-dose IL-2 led to enormous expansion of NK cells in vivo and enhanced in vitro lytic activity against NK-resistant cell lines. Many researches had utilized ex vivo activated/expanded autologous NK cells along with intravenous or subcutaneous low-dose IL-2 [30]. Despite it was better tolerated, responses remained suboptimal, likely due to IL-2-induced expansion of regulatory T cells (T regs), which inhibit NK cell proliferation and function and/or due to the inhibition of autologous NK cells by the self-HLA molecules on the tumor cells. Due to these limitations, the use of allogeneic NK cells was the next logical step for scientists to explore [30].

3.2.4. Allogeneic NK cell immunotherapy in the setting of HSCT

Allogeneic HSCT creates a unique condition for NK cell alloreactivity by virtue of the "missing-self" phenomenon. As the KIR genes (on chromosome 19q13.4) and the HLA genes (on chromosome 6p21) segregate independently, a donor-recipient pair can be HLA-matched and KIR-mismatched simultaneously [31]. In fact, only 25% of the HLA-matched sibling donor/recipient pairs are KIR identical, while the probability of an HLA-matched unrelated donor (MUD)/recipient pair to be KIR identical is virtually zero [32].

3.3. Other immunotherapeutic approaches in hematologic malignancies

3.3.1. Biology of immune inhibitory molecules and the story of check point inhibitors

For proper T cell activation, two separate signals are required. The first signal is mediated by antigen-dependent T cell receptor (TCR) binding to the MHC molecule of an APC. However,

the second signal is antigen-independent, co-stimulatory, or co-inhibitory signal delivered by the APCs, which modulates TCR signaling and determines the fate of T cells. There are several costimulatory or coinhibitory molecules on T cells with their respective ligands that are collectively known as B7-CD28 family. The prototypical co-stimulatory molecule is CD28 on resting naïve T cells, which induces interleukin-2 (IL-2) production, cell-cycle progression, and clonal expansion is constitutively expressed in resting naïve T cells. Without these co-stimulatory second signals, T cells fall into a state of anergy. Inversely, cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a co-inhibitory receptor on T cells that induces T cell tolerance [33]. CTLA-4 exerts its effect when it is present on the cell surface of CD4+ and CD8+ T lymphocytes, where it has higher affinity for the costimulatory receptors CD80 and CD86 (B7-1 and B7-2) on antigen-presenting cells (APCs) than the T cell costimulatory receptor CD28. The expression of CTLA-4 is upregulated by the degree of T cell receptor (TCR) activation and cytokines such as IL-12 and IFN gamma, forming a feedback inhibition loop on activated T effector cells. As a result, CTLA-4 can be broadly considered a physiologic “brake” on the CD4+ and CD8+ T cell activation that is triggered by APCs [33]. Additional second signal molecules include programmed death-1 (PD-1), T cell immunoglobulin and mucin domain-containing protein-3 (TIM-3), lymphocyte activation gene-3 (LAG-3), T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), or B- and T-lymphocyte attenuator (BTLA) [34].

3.3.1.1. *PD-1 and PD ligand 1/2*

The programmed cell death 1 (PD-1) receptor is expressed on activated T cells, B cells, macrophages, Tregs, and NK cells. Binding of PD-1 to its B7 family of ligands, programmed death ligand 1 (PDL1 or B7-H1) or PD-L2 (B7-DC) results in suppression of proliferation and immune response of T cells. Activation of PD-1/PD-L1 signaling serves as a principal mechanism by which tumors evade antigen-specific T-cell immunologic responses. Antibody blockade of PD-1 or PD-L1 reverses this process and enhances antitumor immune activity [35] (**Figure 3**). Many cancers exploit inhibitory molecules such as PD-1, CTLA-4, LAG-3, or TIM-3 to escape immune surveillance. Supporting evidence confirms that functions of these molecules are dysregulated in lymphoid neoplasms, myelodysplastic syndrome, plasma cell myeloma, and AML. Clinical trials had observed that PD-1 blockade is an attractive way to reinstate the host's immune function in lymphoid neoplasms, particularly in classical Hodgkin lymphoma. Serum level of soluble PD-L1 measured by enzyme linked immunosorbent assay (ELISA) can be a potential predictive biomarker in patients with refractory relapsed lymphomas or leukemias [36, 37]. Based upon prolonged overall survival in phase III trials and durable responses in phase II studies, antibodies inhibiting PD-1 (e.g. pembrolizumab, nivolumab) and PD-L1 (e.g. atezolizumab, avelumab, durvalumab) have been approved [40]. However, as the number of PD-1/PD-L1 inhibitors undergoing development is expected to rise in the foreseeable future, several important points (e.g. predictive biomarkers, mechanisms of resistance and hyperprogressors, immune-related adverse events, optimal treatment duration, treatment beyond progression, and response after prior PD-1/PD-L1 blockade), need to be taken into consideration in order to optimize the anticancer potential of this class of agents [38, 39].

3.3.2. *Manipulating T cells*

Adoptive T cell transfer broadly refers to the practice of manipulating patient-specific T cells ex vivo to make them more reactive to specific antigens.

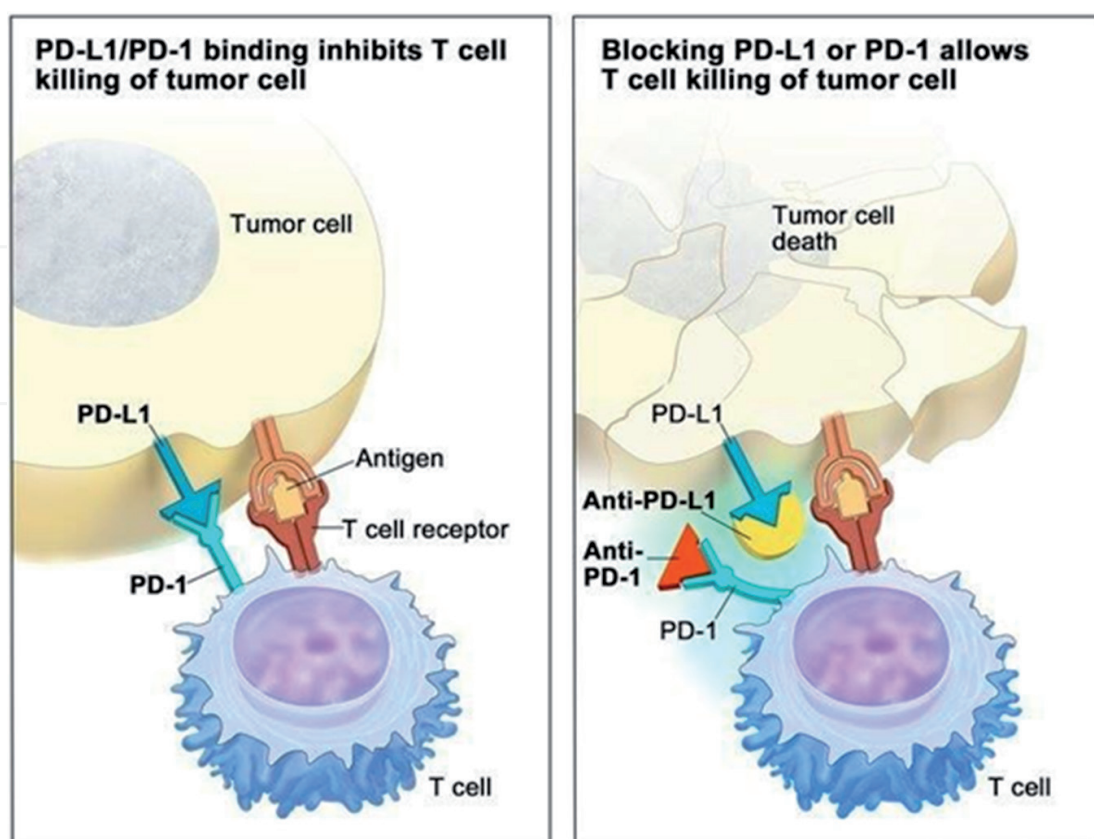


Figure 3. Programmed-death 1 receptor (PD-1, CD279). Programmed-death 1 receptor (PD-1) is one of the crucial molecules that turns down the activation of the immune response. The introduction of PD-1/PD-L1 inhibitors has a great revolution in patients with haematological malignancies.

3.3.2.1. Chimeric antigen receptors

One promising approach to cancer immunotherapy entails genetically engineering a patient's T cells to express chimeric antigen receptors (CARs) that can recognize and attack tumor cells. Upon expression by a T cell, CARs confer antigen specificity determined by the targeting domain [40]. Interestingly, in contrast to conventional T cell receptors (TCRs), which recognize antigens in a MHC-dependent manner, CARs can redirect the effector functions of a T cell toward any protein or non-protein target that is expressed on the cell surface. Therefore, this strategy avoids the need for antigen processing and presentation by the target cell and can be applicable to non-classical T cell targets like carbohydrates [41]. CAR T cells have been studied extensively in hematologic malignancies, and the final goal is to induce durable immunity against disease progression without severe adverse effects. Clinical trials targeting CD19, the pan-B cell antigen, have shown remarkable success in B cell acute lymphoblastic leukemia (B-ALL) and pre-B-cell ALL [42]. Trials in patients with CLL have also shown promising results [43]. A suicide system has been developed to eliminate gene-modified T cells when they display unwanted toxicities, such as the thymidine kinase gene of the herpes simplex virus. In order for that therapy to become routinely used, automation and robotic culture technologies should be performed during the manufacturing process instead of manual cell culture technologies. Whether this treatment option will replace HSCT or be used as a bridge to HSCT in the near future is still a debateful question [44].

3.3.3. Monoclonal TCRs

Another approach to increase effector T cell function against a particular antigen is engineering a soluble TCR (CD8) to recognize a particular antigen target and fusing this to the variable fragment that recognizes an effector target, such as CD3. The ability to engineer a TCR rather than an antibody fragment can lead to higher affinity for a given peptide chain and allow for targeting of intracellular peptide fragments. This approach must be engineered using a specific MHC class 1 molecule, but complications have occurred through TCR cross-recognition of other antigens [45].

4. Prognostic value of infiltrating immune cells in solid tumors

It was observed that the ‘pro-inflammatory’ tumor microenvironment and infiltrating CD8-expressing T lymphocytes were associated with improved clinical outcomes in a broad range of tumor types. On the other hand, the inhibitory function of other immune cells (e.g. regulatory T cells (Tregs), and myeloid-derived suppressor cells) play a major role in disrupting the capacity for the immune control of cancers, thus was associated with worse outcomes [46]. Herein, tumor-specific prognostic values of infiltrating immune cells in some solid cancers are mentioned:

4.1. Colorectal cancer (CRC)

Several large studies of CRC have shown that tumor lymphocytic reaction and T-cell subpopulations are significant prognostic biomarkers, even after adjusting for stage, lymph node count, and well-established prognostic biomarkers [47]. In a meta-analysis of nine trials, the pooled hazard ratio (HR) confirmed an OS benefit for patients with prominent TILs compared with those without, with a HR of 0.59 ($P < 0.001$) and a HR for cancer-specific survival of 0.40 ($P < 0.001$), respectively [48]. However, despite these convincing data, the host immune response is still not yet considered to represent a “standard” prognostic indicator for clinical use in CRC. This may be attributed to the need for a “standard immunoscore” to quantify the in situ immune infiltrate as a novel instrument for classification of CRC [49, 50].

4.2. Breast cancer (BC)

The presence of TILs was associated with improved prognosis in human epidermal growth factor receptor 2 (HER2) positive and triple negative breast cancers (TNBC), but not in luminal subtypes [46]. For luminal B/HER 2 negative BC treated with neoadjuvant chemotherapy (NC), high intratumoral CD8⁺ TIL expression was significantly predictive of pCR post-NC, and an independent prognostic factor for improved OS [51]. In contrast to CRC, a meta-analysis of 25 published studies comprising over 22,000 patients with BC, failed to show that immune infiltrates are associated with OS, but did find such an association in TNBC (HR: 0.79). CD8-expressing lymphocytes were associated with improved disease-free survival (DFS; HR: 0.69) and breast cancer-specific survival (HR: 0.78) in the overall population, whereas the FOXP3-expressing lymphocytes were associated with worse DFS (HR: 1.47) and OS (HR: 1.50, $P = 0.004$), respectively [52].

4.3. Melanoma

Checkpoint inhibitors were first approved in melanoma after a long history of interest in the immune response to these tumors after observation of spontaneous responses [53]. Overall, there is a large body of evidence documenting the prognostic value of the immune infiltrate in melanoma [54].

4.4. Nonsmall cell lung cancer (NSCLC)

In a meta-analysis of 29 trials with over 86,000 patients, high levels of CD8-expressing cells infiltrating the tumor or in the tumor stroma of NSCLC specimens were associated with better OS, compared with tumors without lymphocytes present. CD3 expression also demonstrated similar findings. Presence of intratumoral CD4-expressing cells between the tumor cells resulted in improved OS. FOXP3-expressing T cells in the tumor stroma had association with worse progression-free and OS [55].

4.5. Renal cell carcinoma (RCC)

There is contradictory evidence regarding the role of the immune cell infiltrate in RCC. Several studies have demonstrated a worse outcome in patients with a neutrophilic, and/or lymphocytic infiltrate [56].

4.6. Hepatocellular carcinoma (HCC)

The importance of FOXP3 in both the development and prognosis of HCC, was demonstrated in 2 large meta-analyses [57, 58]. Gabrielson et al. [59] applied the Galon Immunoscore [49] to HCC and confirmed its prognostic value, where CD3 and CD8 cell densities predicted recurrence with ORs of 5.8 and 3.9, respectively. On the other hand, PDL1 staining positively correlated with high CD3 and CD8 density and predicted a lower rate of recurrence [59].

5. Immunotherapy for solid tumors

A number of therapeutic approaches are being studied to liberate the immune system and control malignancy. In the following section, we will discuss briefly the principles of these approaches and their applications in clinical oncology. These approaches include T cells (checkpoint inhibitors, agonism of costimulatory receptors), cytokines, manipulation of T cells, oncolytic viruses, therapies directed at other cell types, and vaccines.

5.1. T cells

5.1.1. Checkpoint inhibitors

5.1.1.1. PD-1 and PD ligand 1/2

As mentioned above, the programmed cell death 1 (PD-1) receptor is expressed on activated T cells, B cells, macrophages, regulatory T cells (Tregs), and natural killer (NK) cells.

Antibody blockade of PD-1 or PD-L1 reverses this process and enhances antitumor immune activity [35]. Currently, the FDA has approved PD-1/PD-L1 inhibitors for the treatment of nine cancer types [38].

5.1.1.2. CTLA-4

As mentioned above, CTLA-4 is a co-inhibitory receptor on T cells that induces T cell tolerance [33]. CTLA-4 exerts its effect when it is present on the cell surface of CD4⁺ and CD8⁺ T lymphocytes, where it has higher affinity for the costimulatory receptors CD80 and CD86 (B7-1 and B7-2) on APCs than the T cell costimulatory receptor CD28. The anti-CTLA-4 antibody ipilimumab was the first immune checkpoint inhibitor to be approved based upon its ability to prolong survival in patients with metastatic melanoma [60].

5.1.2. Agonism of costimulatory receptors

Multiple costimulatory receptors are involved in the immune response to tumors, and hence are potential targets for cancer immunotherapy. Examples include; 4-1BB (CD137) [61] and inducible T cell co-stimulator (ICOS) [62].

5.1.3. Combination of immune checkpoint blockade

Based on the results of checkpoint inhibitors as monotherapy, multiple clinical trials are currently investigating combinations of various checkpoint inhibitors. Examples include; concurrent CTLA-4 and PD-1 blockade, ipilimumab plus nivolumab, and nivolumab plus ipilimumab [63].

5.2. Cytokines

Initial approaches to immunotherapy had utilized the numerous downstream effects of cytokines and other substances that influence immune cell activity. Examples include;

5.2.1. Interleukin (IL)-2

Interleukin (IL)-2 was initially discovered as T cell growth factor. IL-2 has pleiotropic effects on both cytotoxic T cell function as well as Treg cell maintenance. The effects partially depend upon the dose and timing of IL-2 administration [64]. High-dose IL-2 achieved durable objective responses in a minority of patients with melanoma and RCC.

5.2.2. Interferon (IFN) alfa-2b

Interferon (IFN) alfa-2b promotes Th1-mediated effector cell responses such as IL-12 secretion via STAT-1 and STAT-2-mediated downstream signaling events. IFN alfa has been used as adjuvant treatment of high-risk melanoma, although its long-term impact on OS is controversial [65].

5.2.3. *Bacillus Calmette-Guerin (BCG)*

Bacillus Calmette-Guerin (BCG), derived from attenuated *Mycobacterium bovis*, induces a robust inflammatory response when injected in the bladder and is used for the treatment and secondary prevention of superficial bladder cancer [66].

5.3. Manipulating T cells

Adoptive T cell transfer broadly refers to the practice of manipulating patient-specific T cells *ex vivo* to make them more reactive to specific antigens.

5.3.1. *Chimeric antigen receptors (CAR)*

As mentioned above, CAR T cells have been studied extensively in hematologic malignancies. However, their success in solid tumors has been limited due to many reasons, including: (i) the lack of a unique tumor-associated antigen (TAA) in most cancers; (ii) inefficient trafficking of CAR T cells to tumor sites; (iii) the inability of *ex vivo* expanded CAR T cells to persist and proliferate following adoptive transfer; (iv) heterogeneous expression of the targeted antigen (s) leading to outgrowth of antigen-negative tumor variants; (v) the presence of immunosuppressive molecules and cells; (vi) the lack of survival and growth factors (e.g., IL-2); and (vii) the metabolically hostile tumor microenvironment [67].

5.3.2. *Ex vivo expansion of tumor-infiltrating lymphocytes*

Tumor-infiltrating lymphocytes (TILs) represent an immune cell population that recognizes tumor antigen but may have developed an exhausted phenotype due to the tumor microenvironment. *Ex vivo* expansion of TILs utilizes freshly resected tumor tissue to extract TILs and co-culture with IL-2 to stimulate *in vitro* TIL expansion. Prior to reinfusion of expanded TILs, the patient receives non-myeloablative chemotherapy regimens such as cyclophosphamide or total body irradiation, which functions to deplete inhibitory Treg cells and other lymphocytes in the patient to improve the rate of *in vivo* expansion of the stimulated TILs. The *in-vitro*-stimulated TILs, largely comprised of CD8⁺ and to a lesser extent CD4⁺ T lymphocytes, are then reintroduced into patients at high doses, together with HD IL-2, where they can recognize specific tumor antigens in a microenvironment that is now less prone to induce tolerance [68].

5.3.3. *CD3-directed therapies*

5.3.3.1. *Bispecific T cell engagers*

Theoretically, bispecific T cell engager antibodies (BiTEs) function as linkers between T cells and specific target antigens in an MHC-subtype independent manner. BiTEs consist of tandem single-chain variable fragments (scFv), targeting CD3 on T cells and a tumor associated antigen, respectively. Thus, T cells are recruited to tumor cells irrespective of T-cell receptor specificity, antigen presentation, and costimulation. BiTE-mediated T-cell-tumor cell interaction triggers the formation of immunological synapses, which ultimately results

in tumor-specific cell lysis and release of TH1 effector cytokines [69]. For treatment of non-hematologic malignancies, BiTEs targeting CEA (NCT02291614), EpCAM (NCT00635596), and PSMA (NCT01723475) are in clinical development. However, engaging these antigens may cause on-target off-tumor toxicities and successful BiTE therapy of solid tumors has not been reported so far [70].

5.4. Oncolytic viruses

Oncolytic viruses mediate antitumor effects in several ways. Viruses can be engineered to efficiently infect cancer cells preferentially over normal cells, to promote presentation of tumor-associated antigens, to activate “danger signals” that promote a less immune-tolerant tumor microenvironment, and to serve as transduction vehicles for expression of immune modulatory cytokines [71]. The agent furthest along in clinical development is talimogene laherparepvec (T-VEC), which utilizes an attenuated herpes simplex virus 1 virus to overexpress granulocyte macrophage colony-stimulating factor (GM-CSF), which promotes DC-mediated antigen presentation [72]. Numerous other virus backbones are under clinical or preclinical investigation, including adenovirus, reovirus, Newcastle disease virus, and others [73].

5.5. Oncolytic virus plus checkpoint inhibition

Injection of oncolytic viruses may synergize with checkpoint inhibitors by increasing CD8+ T cell infiltration and IFN gamma signaling as well as upregulating PD-L1 in the microenvironment [74].

5.6. Therapies directed at other cell types in the tumor microenvironment

Cell types other than tumor-specific and circulating T cells contribute to an effective versus a suppressed immune response, and thus represent additional targets for immunotherapy beyond T cells.

5.6.1. NK cells

As discussed previously, in the section of hematologic malignancies.

5.6.2. Macrophages

The presence of intratumoral macrophages can portend a poor prognosis. Although an oversimplification, the general categorization of macrophages into classically activated phenotype (M1) and alternatively activated (M2) suggests that in the context of malignancy, M2 macrophages play a pro-tumoral role due to their involvement in immunosuppression, angiogenesis, and tumor cell activation [75]. Intratumoral macrophages are largely recruited by C-C chemokine ligand 2 (CCL2) or colony-stimulating factor 1 (CSF-1), and pre-clinical and clinical data have focused on targeting the CSF-1/CSF-1 receptor axis. The antitumor impact of CSF-1 receptor (CSF-1R) inhibition in pre-clinical models varies, but there are promising data in combination with other modalities such as chemotherapy, radiation therapy, angiogenic inhibitors, adoptive cell transfer, as well as when used in conjunction with CTLA-4 and PD-1 blockade in the challenging setting of pancreatic adenocarcinoma [76].

5.6.3. IDO

Indoleamine 2,3-dioxygenase 1 (IDO1) catalyzes the rate-limiting step in the conversion from the essential amino acid L-tryptophan (Trp) into L-kynurenine (Kyn). IDO1 expression by tumors can promote evasion of immune surveillance by suppressing T cell function and impairing immune surveillance [77].

5.7. Vaccines

There is a long history of attempting to utilize the adaptive immune recognition of a cancer-related antigen to effect antitumor responses. A simplistic way to view vaccine development method is that varying types of antigens, administration schedules, and accompanying immune adjuvants can influence an adaptive immune response. Antigen choices range from simple peptides, which are easy to administer but affect a narrow antigen spectrum and are often restricted by specific HLA class I molecule expression that allows efficient antigen presentation, to whole cell preparations that offer a broader range of antigens but are more costly and time-consuming to prepare [78]. The only currently approved vaccine-based therapy for advanced cancer is sipuleucel-T, which is an autologous dendritic-cell preparation engineered to target prostatic acid phosphatase (PAP) that demonstrated an overall survival benefit in men with castrate-resistant prostate adenocarcinoma [79]. Given the increasing understanding of the importance of immune recognition of multiple patient-specific, tumor-specific antigens, efforts are ongoing to explore the use of individualized pooled antigens. This suggests that patient-specific vaccination approaches may be feasible, particularly in immunogenic tumors such as melanoma, NSCLC, mismatch-repair deficient CRC, and bladder carcinoma [80].

6. Conclusions

The immune response against a tumor requires a complex, rapidly evolving interaction between various immune cell types in the adaptive and innate immune system. The identification of key immune players and molecules involved in this interplay has been crucial for the introduction of reliable prognostic factors and effective therapeutic protocols against cancers. Tumor-infiltrating lymphocytes have been identified as important prognostic factors in many solid tumors. A number of therapeutic approaches are being studied to liberate the immune system and control malignancy. Checkpoint inhibition has already become a primary treatment modality for patients with a broad diversity of cancers, resulting in significantly prolonged survival in some patients. Trials exploring other malignancies and a wide variety of immunotherapy combinations are in progress and should improve these results.

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Conflict of interest

The authors had no conflict(s) of interest for this work.

List of abbreviations

APC	antigen presenting cell
AML	acute myeloid leukemia
BM	bone marrow
B-ALL	B cell acute lymphoblastic leukemia
BC	breast cancer
BiTEs	bispecific T cell engager antibodies
BTLA	B- and T-lymphocyte attenuator
CARs	chimeric antigen receptors
CCL2	C-C chemokine ligand 2
CD	cluster of differentiation
CLL	chronic lymphocytic leukemia
CML	chronic myeloid leukemia
CRC	colorectal cancer
CSF-1	colony-stimulating factor 1
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
DC	dendritic cell
DFS	disease-free survival
DLBC	diffuse large B-cell
ELISA	enzyme linked immunosorbent assay
FOXP3	forkhead box P3
GM-CSF	granulocyte macrophage colony-stimulating factor
GVHD	graft-versus-host disease
HER2	human epidermal growth factor receptor 2
HLA	human leukocyte antigen
HR	hazard ratio
HSCT	hematopoietic stem cell transplantation

HPV	human papilloma virus
ICOS	inducible T cell co-stimulator
IDO1	Indoleamine 2,3-dioxygenase 1
IFN	interferon
IL	interleukin
KDCs	killer DC
KIR	killer immunoglobulin-like receptor
LAG-3	lymphocyte activation gene-3
LN	lymph node
MDS	myelodysplastic syndrome
MDSCs	myeloid derived suppressor cells
MHC	major histocompatibility complex
NC	neoadjuvant chemotherapy
NHL	non-Hodgkin's lymphoma
NK	natural killer
NSCLC	non-small cell lung cancer
OS	overall survival
PAP	prostatic acid phosphatase
PD-1	programmed cell death-1
PDL-1	programmed cell death 1 ligand 1
scFv	single-chain variable fragments
TAA	tumor-associated antigens
TIM-3	T cell immunoglobulin and mucin domain-containing protein-3
TCR	T cell receptor
TGF- β	transforming growth factor beta
TILs	tumor-infiltrating lymphocytes
TNBC	triple negative breast cancer
TRAIL	TNF-related apoptosis-inducing ligand
Treg	regulatory T cells
VEGF	Vascular Endothelial Growth Factor
VISTA	V-domain Ig suppressor of T cell activation

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