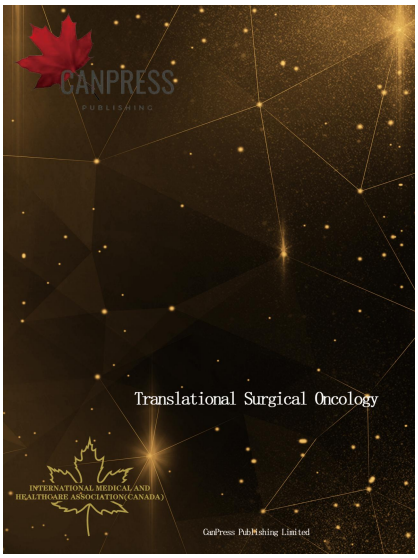
Advance in Tumor Immunotherapy: Establishing a New Paradigm for Oncological Treatment



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Abstract

Malignant tumors demonstrate distinct biological properties, typified by a pronounced proliferation rate, invasiveness, and a predilection for metastasis, which contribute to unfavorable prognosis of cancer patients. Tumor immunotherapy emerges as a therapeutic blueprint designed to marshal the immune system against tumor cells through exogenous stimuli. This strategy spans across a myriad of methods such as cytokine therapy, oncolytic viruses, tumor-specific vaccines, nanoparticle-based immunotherapy, CAR-T cell therapy, and immune checkpoint inhibitors (ICIs). In particular, ICIs have found successful clinical applications in the management of diverse solid tumors, displaying encouraging therapeutic results. This comprehensive review aims to encapsulate several prevalent tumor immunotherapy paradigms and endeavors to envisage their prospective developments.

Keywords: Malignant tumor; Tumor microenvironment; Immunotherapy; Immune checkpoint inhibitors

Introduction

It was estimated that there were 19.29 million new cases of malignant tumors and 9.95 million deaths due to malignant tumors worldwide in 2020[1]. In 2022, it is estimated to have 4.82 million new cases of malignant tumors and 3.21 million deaths due to malignant tumors in China. Comparatively, in the United States, these figures are expected to reach 2.37 million and 0.64 million, respectively[2]. Malignant tumors are caused by genomic instability, such as somatic cell mutations, chromosomal rearrangements, changes in copy numbers, and epigenetic alterations, leading to the activation of oncogenes and the loss of function of tumor suppressors. Paget proposed the "seed and soil" theory in 1889, suggesting that the formation of a tumor requires two essential conditions: one being the tumor cells, and the other being an environment conducive to the survival of tumor cells[3]. Harold Dvorak named this environment as "tumor microenvironment (TME)" in 1986. He compared tumors to "wounds that do not heal," emphasizing the similarities between the extracellular environment of tumors and that of injured tissues. He further elaborated on the role of TME in tumor progression and metastasis[4]. TME consists of various types of cells, such as tissue-resident immune cells, fibroblasts, tumor cells, and vascular endothelial cells, as well as a variety of cell factors, chemokines, and tumor matrix components secreted by these cells[5]. TME exhibits a high degree of heterogeneity, with different types of tumor cells residing in distinct TME. Consequently, the reciprocal regulatory relationship between tumor cells and TME varies as well. A clear understanding of this heterogeneity is fundamental to the development of tumor immunotherapies.

Krummel et al[6] systematically summarized the significance of immune cells in the TME and proposed the concept of "tumor immune microenvironment (TIME)" in 2018, and suggested that the key to improving the efficacy of immune checkpoint inhibitors (ICIs) such as programmed death-1 (PD-1), cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), and programmed death ligand-1 (PD-L1) monoclonal antibodies lies in increasing the infiltration of cytotoxic T lymphocytes (CTLs) in the TIME. Anderson classified the infiltration levels of immune cells in TME into

immune infiltration, immune excluded, and immune-silent types[7]. "Immune silent" refers to the absence of immune cell infiltration in tumors. "Immune exclusion" describes the distribution of CTLs only around the tumor periphery, with no immune cell infiltration within the tumor. Consequently, tumors with few or no CTL infiltration characterized by immune silent and immune excluded are referred to as "cold tumors". In contrast, tumors exhibiting immune infiltration are called "hot tumors," as CTLs are uniformly distributed within the TME. Tumor immunotherapy is essentially a stepwise process that involves the activation and mobilization of immune cells through exogenous stimuli, transforming the TME phenotype from an immune silent to immune excluded, and ultimately to immune infiltration. This review provides a comprehensive summary of the most promising clinical applications of various tumor immunotherapy approaches and strategies currently available.

1. Cytokine Therapy

Cytokines are crucial signaling molecules that mediate intercellular communication and often exert their effects through paracrine or autocrine mechanisms. The role of cytokines such as interleukin-2 (IL-2), IL-12, IL-15, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon-α (IFNα) in the development and progression of tumors has been demonstrated in animal models of cancer. The recombinant IFN-α2 has been approved for the treatment of hairy cell leukemia, making it the first cytokine globally approved for the treatment of human cancer[8]. Subsequently, IL-2 was approved for the treatment of metastatic renal cell carcinoma and advanced melanoma[9]. In the past 30 years, dozens of cytokines have been utilized in the clinical treatment of malignant tumors, and this number continues to grow.

1.1 Interferon (IFN)

Interferon (IFN) was first discovered in 1957. IFNs are often induced by pattern recognition receptors (PRRs), including type I (IFNα, IFNβ), type II (IFNγ) and type III (IFNλ)[10]. Each specific type of IFN has a corresponding receptor. For instance, the receptor for IFNα/β is the heterodimer IFNα/β receptor 1 (IFNAR1)-IFNAR2, the receptor for IFNγ is IFNGR, and the receptor for IFNλ is IFNLR (Fig. 1A). Among these, IFNAR and IFNGR are expressed in all nucleated cells, while IFNLR is only expressed in epithelial cells and certain immune cells, such as neutrophils[11]. Therefore, IFNα and IFNγ can exert effects systemically, whereas IFNλ only exerts defensive function in specific organs such as the lungs and intestines. The binding of tumor-derived DNA to cytoplasmic DNA receptors is a crucial pathway for the generation of IFNs. This pathway successively activates cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway and nuclear factor-κB (NF-κB) pathway, leading to the induction of a significant amount of IFNs[12]. Plasmacytoid dendritic cells (pDCs) are the primary source of IFNs produced through the above-mentioned pathways.

Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is one classical signaling pathway through which IFN exerts its effects[13]. When IFNα/β binds with IFNAR1-IFNAR2, tyrosine kinase 2 (TYK2) and JAK1 are activated, triggering downstream STAT1 and STAT2 phosphorylation cascade. Phosphorylated STAT1-STAT2 heterodimers, in conjunction with interferon regulatory factor 9 (IRF9), form interferon-stimulating gene factor 3 (ISGF3). ISGF3 can enter the nucleus and bind to interferon-stimulated response elements (ISREs), thereby inducing the expression of interferon-stimulated genes (ISGs) such as major histocompatibility complex-I (MHC-I), Fas cell surface death receptor (FAS), tumor necrosis factor (TNF) related apoptosis inducing ligand (TRAIL), promoting tumor cell apoptosis[10]. Additionally, IFN can promote the translation of other downstream ISGs and regulate cell cycle by activating the mammalian target of rapamycin (mTOR) pathway, the mitogen-activated protein kinase (MAPK) pathway, and the GCD-GTPases/cyclin-dependent kinases (GCD) pathway[14].

IFNγ is produced by T cells, natural killer (NK) cells, invariant NK T (iNKT) cells, regulatory T (Treg) cells, γδT cells and B cells, exhibiting both anti-tumor and pro-tumor effects[15]. CD8+ T cells are the key effector cells producing IFNγ, and the activation signal generated by the recognition of peptide MHC-I (pMHC-I) antigens by T cell receptor (TCR) can induce the expression of eomesodermin (EOMES) and promote the release of IFNγ[16]. NK cells can recognize tumor cells and produce IFNγ with the induction of IL-2 and IL-12, exerting toxic effects on targeted tumor cells[17]. FOXP3+ Treg can suppress protein kinase B (PKB, i.e. AKT) phosphorylation via forkhead box O1 (FOXO1), thereby reducing IFNG expression and attenuating the anti-tumor effects of IFNγ[18]. Similar to IFNα/β, IFNγ promotes the expression of related ISGs through the activation of JAK-STAT pathway to exert anti-tumor function. However, whether IFNγ exerts anti-tumorigenic or pro-tumorigenic effects depends on its concentration within the TME. At lower levels, IFNγ can promote tumor cell proliferation and invasion via the ICAM1-PI3K-Akt-Notch1 signaling pathway. Conversely, at higher concentrations, IFNγ may induce tumor cell apoptosis through the JAK1-STAT1-Caspase pathway[19].

It has been substantiated that IFNα enhances the median survival of patients with metastatic renal cell carcinoma, reducing the mortality risk by 28%[20]. Pegylated IFNα-2a (Peg-IFNα-2a) demonstrated good efficacy in reducing the 6-month relapse rate in patients with acute myeloid leukemia following allogeneic hematopoietic stem cell transplantation[21]. However, with the application of targeted agents and other cellular factors, IFNs have gradually become an alternative treatment for malignant tumors

1.2 Interleukin-2 (IL-2)

Interleukin-2 (IL-2) was initially identified as a growth factor that promotes the proliferation and differentiation of T cells. Upon the activation of TCR on CD4+ T cells, multiple transcription factors such as nuclear factor of activated T cells (NFAT), activator protein-1 (AP-1), NF-κB and forkhead box protein P3 (FOXP3) can be triggered to induce the production of IL-2. Conversely, the production of IL-2 can be inhibited by the T cell immunoglobulin and mucin domain-containing protein 3 (TIM3) and signaling through G protein-coupled receptors (GPCR)[22]. IL-2 receptor (IL-2R) is composed of α, β, and γ subunits (Fig. 1B). The low-affinity IL-2R is solely comprised of IL-2Rα subunit, while the intermediate-affinity IL-2R consists of both IL-2Rβ and IL-2Rγ subunits. The high-affinity IL-2R, on the other hand, is constituted by a combination of all three subunits: IL-2Rα, IL-2Rβ, and IL-2Rγ[23]. Upon binding of IL-2 to high-affinity IL-2R, signaling pathways such as JAK/STAT, extracellular signal-regulated kinase (ERK), and phosphoinositide 3-kinase (PI3K) are activated, which facilitate protein function through the phosphorylation of serine, threonine, and tyrosine at various sites[22].

IL-2 modulates the differentiation polarity of T cells. IL-2 can drive the differentiation of naive CD4+ T cells into type 1 T helper cells (TH1), TH2, TH9, Treg, and follicular regulatory T (TFR) cells, while inhibit their differentiation towards TH17 and follicular helper T (TFH) cells[24]. One of the significant contributors to tumor progression and evasion is the exhaustion of CD8+ T cells. IL-2 has the capability to reverse the exhaustion phenotype of CD8+ T cells, reactivating them to enhance anti-tumor immunity. Exhaustion of CD8+ T cells is often accompanied by the overexpression of PD-1. Consequently, the combined therapy of IL-2 and anti-PD-1 ICIs has been progressively employed in the treatment of malignant tumors.

Tregs express high-affinity IL-2R, while CD8+ T cells express intermediate-affinity IL-2R. Consequently, native IL-2 tends to bind preferentially with Tregs, thereby inducing tumor immune tolerance and evasion. NARA1 can block the IL-2Rα site and inhibit Treg differentiation mediated by CD25. This in turn promotes the binding of IL-2 with IL-2Rβ and IL-2Rγ on CD8+ T cells, thereby enhancing their cytotoxic activity[25]. Bempegaldesleukin (BEMPEG, NKTR-214), a pegylated IL-2, preferentially binds to IL-2Rβ and IL-2Rγ and selectively activates CD8+ T cells and NK cells while reducing Treg activation[26]. For the treatment of stage III/IV metastatic melanoma, the objective response rate (ORR) of BEMPEG combined with nabuliumab was 52.6%, the complete response rate (CR) was 34.2%, and the median progression-free survival (mPFS) was 30.9 months[27]. The ORR of BEMPEG combined with nabuliumab for the treatment of metastatic renal carcinoma (PIVOT-02 study) was 35.1%, CR was 18.9%, median overall survival (mOS) was 23.7 months, and mPFS was 4.1 months[28]. However, in the PIVOT-09 study (NCT03729245), this combination did not meet the primary endpoint for advanced renal cell carcinoma. Furthermore, BEMPEG has failed in various clinical studies for advanced solid tumors, leading to the discontinuation of its clinical translation[29].

1.3 Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF)

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is primarily produced by lymphocytes, macrophages, fibroblasts and tumor cells, and plays a pivotal role in the differentiation and development of myeloid immune cells, including neutrophils, monocytes, macrophages and DCs[30]. However, the production of immune cells is hampered by low-dose GM-CSF, whereas high-dose GM-CSF may induce an immunocyte exhaustion phenotype, both of which promote tumor progression and metastasis[31]. GM-CSF can stimulate the formation of monocyte/macrophage inflammatory phenotype and the production of TNF-α and IL-1β by the activation of several signaling pathways such as JAK/STAT, PI3K/AKT, and MAPK (Fig. 1C). Tumor-associated macrophages (TAMs) and DCs serve as the most crucial antigen presenting cells (APCs) within the TME. GM-CSF can promote the differentiation of TAMs toward an anti-tumor M1 phenotype, thereby enhancing the presentation of both tumor-associated antigens (TAAs) and tumor neoantigens (TNAs) by TAMs and DCs. This in turn induces the reactivation of CD4+ and CD8+ T cells, which play a pivotal role in anti-tumor immune responses. Furthermore, GM-CSF can augment the expression of PD-L1 in tumor cells and PD-1 in CD8+ T cells through the activation of JAK2/STAT5 pathway[32]. Therefore, the combination of GM-CSF with PD-1/PD-L1 monoclonal antibodies can significantly slow tumor growth. Compared with the sole application of GM-CSF gene vaccine (GVAX), the combination of nivolumab monoclonal antibody and GVAX can significantly reduce the proportion of the CD8+PD-1+ T cell subpopulation in the tertiary lymph node regions, effectively improving the prognosis of patients with pancreatic ductal adenocarcinoma[33]. Additionally, the combination of GM-CSF and PD-1 monoclonal antibody has achieved favorable outcomes in the treatment of various metastatic solid tumors, including lung cancer, colorectal cancer, breast cancer and others[34].

2. Oncolytic Virus (OV)

Oncolytic viruses (OVs) refer to a class of attenuated live viruses that have been artificially modified and operate through the enhancement of anti-tumor immune responses, and selectively kill tumor cells in a targeted manner. The anti-tumor mechanisms of OVs may be associated with intratumoral viral replication and activation of antiviral response elements, activation of apoptosis-related pathways in tumor cells, and enhancement of innate and adaptive antitumor immunity[35]. OVs enter cells by recognizing specific receptors on the cellular surface. While both normal

and tumor cells express specific receptors, their expression levels vary significantly. For instance, melanoma cells often overexpress the herpesvirus entry mediator (HVEM), nectin-1 and nectin-2, which facilitate the infection of herpes simplex virus-1 (HSV-1), enhancing its oncolytic potential[35]. Breast cancer cells and multiple myeloma cells often overexpress intercellular adhesion molecule-1 (ICAM-1) and decay accelerating factor (DAF), which facilitate infection by coxsackievirus[36]. In addition, OVs have been artificially modified to target specific receptors on tumor cells. For instance, the modified adenovirus Ad5/3-Δ24 can bind with ovarian cancer cells that highly express integrins. Similarly, a modified version of the measles virus can bind with adenocarcinoma cells which overexpress carcinoembryonic antigen (CEA)[37].

The mechanisms by which OVs directly induce cytotoxicity in tumor cells and enhance anti-tumor immune responses are not yet fully elucidated. Upon recognition of the virus's pathogen-associated molecular patterns (PAMPs) by cellular elements such as protein kinase R (PKR), Toll-like receptors (TLRs), and retinoic acid-inducible gene 1-like receptors (RLRs), antiviral pathways are activated: on one hand, this can activate the NF-κB and JAK-STAT pathways, leading to the production of pro-inflammatory cytokines and IFNs; on the other hand, this can influence the cell cycle, inducing cell apoptosis[37]. However, the ability to eradicate viruses is often compromised in tumor cells due to aberrant gene expression, thus allowing OVs to easily replicate within and lyse these cells. Tumor cells, under the duress of endoplasmic reticulum stress and genotoxic stress, undergo lysis and apoptosis, which in turn releases TAAs, TNAs, and damage-associated molecular patterns (DAMPs). These entities are subsequently internalized and processed by APCs, which present the tumor antigens to CD4+ and CD8+ T cells via the MHC pathway. This culminates in the generation of CTLs that specifically target TNAs and effectively impede tumor immune evasion.

The National Medical Products Administration (NMPA) of China approved the use of the oncolytic adenovirus H101 (Oncorine) for the treatment of advanced nasopharyngeal carcinoma in 2005[38]. In 2015, Talimogene laherparepvec (T-VEC, i.e., Imlygic) became the first globally recognized OV approved for the treatment of recurrent melanoma[39]. To date, 4 OVs have been globally approved for the treatment of malignant tumors (Table 1). T-VEC utilizes the HSV-1 as its viral vector. In a phase III clinical trial, patients with stage III-IV melanoma who received T-VEC treatment demonstrated a significantly higher durable response rate (DRR) (16.3% vs. 2.1%), ORR (26.4% vs. 5.7%), and longer mOS (23.3 months vs. 18.9 months) compared to those administered subcutaneous injections of GM-CSF[40]. In a phase II clinical trial, the ORR for H101 treatment across various types of late-stage malignancies was 22.4%, with a clinical benefit rate observed in up to 77.6% of patients[41]. In a phase III randomized clinical trial, patients with head and neck squamous cell carcinoma or esophageal squamous cell carcinoma demonstrated a significantly higher ORR of 78.8% versus 39.6% with the combination regimen of H101, cisplatin, and 5-fluorouracil, compared to monotherapy[42].

3. Tumor Vaccines

Tumor vaccines can be broadly classified into two main categories: prophylactic and therapeutic tumor vaccines. Prophylactic tumor vaccines primarily serve as a preventive measure against the carcinogenesis of normal cells due to pathogen infections, as demonstrated by vaccines for hepatitis B virus (HBV) and human papillomavirus (HPV). Conversely, therapeutic tumor vaccines are primarily utilized to enhance the anti-tumor efficacy of immune cells. Below we provide an in-depth summary on the principles, types, clinical trials, and prospects of therapeutic tumor vaccines.

Therapeutic cancer vaccines can be classified into three categories based on their active constituents: peptide- or protein-based vaccines, cellular vaccines and genetic vaccines. In a phase III clinical trial, peptide-based vaccines did not improve the mOS or DFS in patients with advanced metastatic renal cell carcinoma[43]. However, a therapeutic vaccine based on the tumor antigen glypican-3 can significantly improve the 5-year OS rate of hepatocellular carcinoma through the activation of CTLs[44]. Sipuleucel-T, a DC vaccine for castration-resistant prostate cancer, is the first therapeutic cancer vaccine approved by FDA. Kantoff et al[45] observed that Sipuleucel-T reduced the risk of mortality in prostate cancer patients and extended the mOS by 4.1 months.

Genetic vaccines can be broadly categorized into two main classes: DNA vaccines and RNA vaccines. VGX-3100 is a synthetic plasmid expressing E6 and E7 proteins of HPV types 16 and 18. Compared to the control group, patients administered with VGX-3100 exhibited a significantly higher viral clearance rate (40.2% vs. 14.3%, P=0.003) and pathological regression rate (48.2% vs. 30.0%, P=0.034)[46]. pTVG-AR is a DNA vaccine that encodes the ligand-binding domain of the androgen receptor. Kyriakopoulos et al[47] observed that patients administered with the pTVG-AR DNA vaccine demonstrated prolonged anti-tumor immunological effects, which were mediated by both IFN-γ and granzyme B.

RNA vaccines usually use lipid nanoparticles as packaging vector and mRNA as expression vector of tumor antigen. Lipid nanoparticles not only effectively prevent mRNA degradation by ribonucleases, but also enhance the immunogenicity and cytotoxic effects[48]. Upon phagocytic uptake of lipid nanoparticles containing mRNA by APCs, APCs can present tumor antigens to CD4+ or CD8+ T cells via the MHC related pathway, enhancing the recognition and cytotoxicity to tumor cells[49]. Cafri et al[50] employed the individual tumor antigen expression profiles and corresponding gene mutation landscapes of 4 patients with metastatic gastrointestinal malignancies to inform the design of mRNA vaccines. Their findings provide solid evidence that such vaccines have the dual ability to bolster T-cell-mediated anti-tumor immune responses and to exhibit consistent safety

and immunogenicity.

It is worth noting that malignant tumors exhibit high heterogeneity. Single nucleotide variations (SNVs) represent the most common type of tumor mutation. SNVs have the potential to alter the protein structure of tumor antigens, leading to the formation of novel antigenic epitopes. This can in turn attenuate the recognition capabilities of TCR or B cell receptors (BCRs), thereby promoting immune evasion by tumors. As a result, tumor heterogeneity hampers the widespread use of therapeutic vaccine. In an effort to advance our understanding of tumorigenesis, we have conducted a comparative study of gene expression profiles in both normal and tumor cells within patients. Through the utilization of deep learning methodologies, we have specifically analyzed the sequence and structural nuances of tumor antigens[51,52]. We aim to augment both the affinity between tumor antigens and their corresponding receptors and the immunogenicity of these interactions[53]. This endeavor aims to contribute to the development of more targeted and effective therapeutic strategies in oncology. This augmentation could bolster the immune system's cytotoxic response to tumor cells, which represents a future direction in the personalized treatment of cancer through the development of therapeutic vaccines.

4. Chimeric Antigen Receptor (CAR)

Chimeric antigen receptor (CAR) refers to a specific gene sequence which is integrated into the host cell's genome through gene engineering technologies, typically via retroviral or lentiviral infection, or through gene editing techniques such as clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 and transcription activator-like effector nucleases (TALENs). This genetic integration empowers the cell with the capability to express a specific antigen. CAR comprises 4 domains: a target-binding extracellular region containing single-chain fragment of variable region antibody (scFv), an extracellular hinge region, a transmembrane region, and an intracellular signaling domain[54]. Various immune cells can express CARs, enabling them to acquire the ability to selectively target and destroy specific tumor cells. Consequently, therapies based on T cells, NK cells, and macrophages expressing CARs are referred to as CAR-T, CAR-NK, and CAR-M therapies, respectively. CAR-T therapy has demonstrated exceptional efficacy in the treatment of hematologic malignancies. Zhang et al[55] employed CAR-T therapy utilizing the CRISPR/Cas9 editing system to treat patients with relapsed/refractory aggressive B-cell non-Hodgkin lymphoma (B-NHL). Their results demonstrated an ORR of 100% in 8 patients, with a CR of 87.5% and no adverse reactions of grade 3 or higher. Jin et al[56] pioneered the use of CAR-T cells targeting human C-type lectin-like molecule-1 (CLL-1) in the treatment of adult patients with relapsed/refractory acute myeloid leukemia (AML). At present, CAR-T therapy has evolved to the fourth generation. Compared to previous three iterations, the fourth-generation CAR-T technology augments the secretion of cytokines by T cells, further promoting the activation and recruitment of T cells and other types of immune cells to enhance their ability to selectively kill tumor cells.

However, CAR-T cell therapy has persistently failed to achieve satisfactory efficacy in solid tumors, maybe due to the shortages of tumor-specific antigens (TSAs), on-target/off-tumor toxicity, low infiltration of CAR-T cells, and the immunosuppressive microenvironment[57]. Feng et al[58] employed HER2-targeted CAR-T cell therapy in the treatment of late-stage human epidermal growth factor receptor 2 (HER2) positive advanced biliary tract cancer and pancreatic cancer. Among the patient cohort, 1 case achieved partial remission (PR), 5 cases demonstrated stable disease (SD), and another 5 cases indicated progressive disease (PD). Unlike hypoxic solid tumors, hepatocellular carcinoma (HCC) enjoys a rich blood supply, which consequently confers considerable sensitivity and responsiveness to conventional chemotherapy and biologic agents. Results from a single-arm phase II clinical trial demonstrated that HCC exhibited favorable responsiveness to CD133-directed CAR-T cells, resulting in PR or SD in at least 15 out of 21 patients (71.43%). Importantly, this therapeutic approach did not induce severe cytokine release syndrome (CRS)[59].

Unlike hematopoietic malignancies, solid tumors often present a barrier to CAR-T cell infiltration into the TME. This barrier is constructed by tumor matrix, cancer-associated immunosuppressive cells, and cancer-associated fibroblasts, among others. As a result, CAR-T cells often fail to achieve satisfactory concentrations within the TME. Increasing the number of infused CAR-T cells can lead to serious adverse reactions, such as CRS, CAR-T cell-related encephalopathy syndrome (CRES), and neutropenia[60]. In addition, the potential for on-target/off-tumor toxicity could enhance the cytotoxic effect of CAR-T cells on normal cells, posing a serious risk to the patient's life. Therefore, it is unwise to solely rely on enhancing the cytotoxic capacity of CAR-T cells without combining other immunotherapies to ameliorate the TME, particularly in solid tumors rich in tumor matrix.

5. Immune Checkpoint Inhibitors (ICIs）

Classical immune checkpoints include CTLA-4 and PD-1. Additional immune checkpoints encompass lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin and mucin domain-containing protein 3 (TIM3), indoleamine-2,3-dioxygenase (IDO), and V-domain Ig suppressor of T cell activation (VISTA) among others. ICIs are designed to competitively bind to immune checkpoint molecules on the surface of tumor cells or immune cells, thereby releasing the "brake" signal of immune checkpoints. This mechanism of action enhances the recognition and cytolytic function of CTLs towards tumor cells, aiming to improve anticancer efficacy.

CTLA-4 and PD-1 are the two most classical immune checkpoint molecules, with over 20 different types of monoclonal antibodies targeting CTLA-4 and PD-1/PD-L1

identified to date[61] (Table 2). Ipilimumab, the first ICI targeting CTLA-4, has been employed for the treatment of advanced melanoma. The combination of Ipilimumab and Nivolumab has proven effective in extending the OS to 17.1 months in patients with advanced non-small-cell lung cancer (NSCLC) who exhibit PD-L1 expression level of ≥1%[62]. NMPA granted market approval for Cadonilimab on June 29, 2022, the first ICI dual-targeting PD-1 and CTLA-4 for the treatment of recurrent or metastatic (R/M) cervical cancer[63]. Cadonilimab demonstrated a substantial ORR of 33.0% in treating R/M cervical cancer, with a CR rate of 12.0%. Additionally, patients treated with Cadonilimab showed a mOS of 17.51 months and a mPFS of 3.75 months[64].

However, not all tumor patients exhibit favorable responses to ICIs. Indiscriminate use of ICIs in patients with low expression levels of PD-1/PD-L1 or CTLA-4 may even promote tumor metastasis. Sharma et al[65] found no significant difference in the ORR to Nivolumab treatment in patients with R/M urothelial carcinoma with PD-L1 expression ≥1% versus those with PD-L1 expression <1% (24.0% vs. 26.2%, P>0.05). The results from the KEYNOTE-042 clinical trial suggest that patients with NSCLC who have a PD-L1 tumor proportion score (TPS) of ≥50% exhibit a superior response to Pembrolizumab compared to those with a 1%≤TPS<50% (HR=0.69, 95% CI=0.56-0.85 vs. HR=0.92, 95% CI=0.77-1.11)[66]. The observed variability could potentially be attributed to factors such as the type of tumor, heterogeneity, level of immune infiltration and the sensitivity of the detection platform. At present, 4 main systems for evaluating PD-L1 expression in tumors are based on combined positive score (CPS), percentage of tumor cells (TC), percentage of tumor-infiltrating immune cells (IC), and TPS. Of these, TPS and CPS are the most commonly used. The key distinction between these two lies in the cellular inclusion criteria: TPS does not consider PD-L1 positive immune cells, whereas CPS takes into account the impact of immune cells on patient prognosis. Kulangara et al[67] showed that CPS was better than TPS in predicting the ORR to Pembrolizumab in patients with gastric and esophageal cancers. However, Marchi et al[68] found a strong correlation between TPS and CPS in predicting the reactivity to PD-L1 monotherapy in NSCLC. Therefore, it is of paramount importance to select the most appropriate assessment system for evaluating the responsiveness to ICIs, taking into consideration factors such as tumor type, ICI targets, and IHC platforms. Furthermore, pathologists should establish uniform criteria for assessing the expression of immune checkpoint molecules or utilize standardized equipment to eliminate biases resulting from inter-sample variability.

6. Nanoparticle-based Immunotherapy

Although CAR-T and ICIs have demonstrated astonishing efficacy in treating hematological malignancies, the treatment of hypovascular solid tumors such as pancreatic cancer, colorectal cancer, and prostate cancer poses unique challenges. These tumors have a robust matrix that presents significant resistance to chemotherapeutic or immunotherapeutic agents. The drugs struggle to penetrate the tumor barrier, and escalating the drug dose only exacerbates systemic toxicity. Thus, improving drug delivery system to facilitate drug penetration is of significant importance. Nanomedicine refers to the encapsulation of minute vesicles with diameters ranging from 10 to 100 nm, utilizing materials such as lipid materials, polymeric nanoparticles, metallic particles, or inorganic nanoparticles[69]. Enhanced permeation and retention (EPR) effect serves as an essential mechanism in amplifying the concentration of nanomedicines within the tumor core[70]. The abnormal vascular pores within tumors facilitate the permeation of nanomedicines, while the developmental and drainage impairments of the peritumoral lymphatic network augment the retention of these nanomedicines. As a result, low doses of nanomedicines can effectively eradicate tumor cells without eliciting severe toxic reactions.

The modification of nanomedicines could regulate their targeting capabilities and drug release kinetics. NBTXR3, a hafnium oxide nanoradioenhancer, can amplify the damage induced by ionizing radiation on tumor cell DNA. It promotes the release of TAAs, TNFs, and tumor-related DAMPs through the pathway of immunogenic cell death (ICD), thereby suppressing tumor immune evasion[71]. Compared to postoperative radiotherapy alone, the combination of NBTXR3 and radiotherapy demonstrated a significantly higher CR in locally advanced soft tissue sarcomas (16% vs. 8%, P=0.044)[72]. The administration of Polo-like kinase 1 (PLK1) inhibitors as a monotherapy for NSCLC can potentially elevate the expression of PD-L1 in tumor cells, thereby facilitating immune evasion[73]. ARAC is a nanoparticle designed with a dual-modulation strategy that utilizes polyethyleneimine (PEI) and polyethylene glycol (PEG) to encapsulate a PLK1 inhibitor, and is modified with PD-L1 monoclonal antibody on the surface to target tumor cells overexpressing both PLK1 and PD-L1. The efficacy and toxicity of ARAC have been validated in lung cancer cell lines, melanoma cell lines, and breast cancer cell lines. In a murine model of metastatic lung cancer, ARAC demonstrated the capacity to reduce the effective dosage of PLK1 inhibitor and PD-L1 monoclonal antibody by 5-fold, exhibiting significant potential for clinical translation[73].

7. Conclusion

This review systematically summarizes several cancer immunotherapies with significant clinical application potential: cytokine therapy, OVs, tumor vaccines, CAR-T, ICIs and nanomaterial-based immunotherapies (Fig. 2). Tumor is highly heterogeneous, making it difficult to design a "one-size-fits-all" immunotherapy. Patients with tumors characterized by microsatellite instability-high/deficient mismatch repair (MSI-H/dMMR) often exhibit enhanced

sensitivity to anti-PD-1. However, the immunosuppressive TME contributes to the increased resistance of tumor cells to immunotherapies, inhibiting the effective activation of cytotoxic T cells and thus impairing their anti-tumor efficacy. Furthermore, some patients are prone to developing resistance to immunotherapies, leading to tumor recurrence, metastasis, and exacerbation of the disease burden. Utilizing artificial intelligence and machine learning algorithms to develop personalized treatment strategies for cancer patients and predict their responsiveness to immunotherapy could substantially improve patient prognosis. Reactivating anti-tumor immune cells through tumor vaccines to convert “cold tumors” into “hot tumors” is a promising approach. However, optimizing factors such as the types of tumor vaccines, target genes, the subpopulations of immune cells being activated, and the delivery methods are key to demonstrating the efficacy of individualized tumor vaccine therapies. Moreover, identifying novel tumor markers that can predict the response to immunotherapy or new targets that determine the malignant potential of tumors holds significant value. This will be beneficial in developing new immunotherapeutic approaches.

At present, single-cell transcriptomics help reveal the mechanisms of resistance to ICIs by comparing the differences in immune cell subsets and gene expression in the TME before and after ICIs treatment. Consequently, the combined use of immunotherapies with different mechanisms and chemotherapy regimens seems to be one solution, but this could increase the toxic effects of the drugs on the individual. In future, a multi-omics approach encompassing metabolomics, transcriptomics and proteomics could facilitate deeper understanding of individual tumor occurrence, evolution, and metastasis, and promote the development of personalized strategies to cure cancer.

Table 1. Summary of commercially available OVs

Name	Trade Name	Viral Vector	Date of First Approval	First ratifying country	Indications
H101	Oncorine	Adenoviridae	2005	China	Combined 5-FU or cisplatin chemotherapy regimen for palliative treatment of advanced nasopharyngeal carcinoma
T-VEC	Imlygic	HSV-1	2015	USA	Unresectable Melanoma
ECHO-7	Rigvir	Echovirus	2004	Latvia	Early stage (stage I-II) melanoma
Teserpaturev	Delytact	HSV-1	2021	Japan	Spongioblastoma

Table 2. Overview of ICIs

Name	Company	Trade Name	Target	Country	Date of First Approval	Indications	Clinical Trials
Ipilimumab	Bristol-Myers Squibb	Yervoy	CTLA-4	USA	2011/03/25	malignant pleural mesothelioma; metastatic melanoma; advanced renal carcinoma; metastatic colorectal cancer with MSI-H/ dMMR	NCT01654692, NCT02716272, NCT01750983
Cadonilimab	Akeso	Kai Tan Ni	PD-1/CTLA-4	China	2022/06/29	R/M cervical cancer	NCT05426005, NCT05773105, NCT05781958
Prolgolimab	BIOCAD	Forteca	PD-1	Russia	2020/04/01	U/M melanoma	NCT05757466, NCT05783882, NCT05120024
Nivolumab	Bristol-Myers Squibb	OPDIVO	PD-1	Japan	2014/07/04	Neoadjuvant therapy for resectable/metastatic NSCLC, head and neck squamous cell carcinoma, gastric cancer, esophageal cancer, urothelial cancer, malignant pleural mesothelioma	NCT04858204, NCT04507906, NCT04123925
Penpulimab	Akeso	An Ni Ke	PD-1	China	2021/08/03	R/R classic Hodgkin lymphoma	NCT05244642, NCT04970914, NCT05460481
Toripalimab	Junshi Biosciences	Tuo Yi	PD-1	China	2018/12/17	U/M melanoma, R/M nasopharynx, LA/M urothelial carcinoma that has failed platinum-containing chemotherapy, U/M esophageal squamous carcinoma, EGFR(-)/ALK(-) U/M non-squamous NSCLC	NCT04005170, NCT04627012, NCT04169672
Pucotenlimab	Lepu Biopharma	Pu You Heng	PD-1	China	2022/07/19	MSI-H/dMMR advanced solid tumor	
Zimberelimab	Gloria Biosciences	Yu Tuo	PD-1	China	2021/08/25	R/R classic Hodgkin lymphoma	NCT05632848, NCT05130177, NCT05502237
Sintilimab	Innovent	Da Bo Shu	PD-1	China	2018/12/24	R/R classic Hodgkin lymphoma, EGFR(-)/ALK(-) U/M NSCLC, U/M hepatocellular carcinoma, esophageal cancer, gastric cancer	NCT03936452, NCT04072679, NCT03812549
Dostarlimab	GSK	JEMPERLI	PD-1	USA	2021/04/21	dMMR advanced solid cancer or endometrial cancer	NCT02715284, NCT03981796, NCT03955471
Camrelizumab	Hengrui	Ai Rui Ka	PD-1	China	2019/05/29	R/R classic Hodgkin lymphoma; advanced hepatocellular cancer treated with sorafenib or	NCT04642664, NCT04047017,

						platinum; EGFR(-)/ALK(-) LA/M NSCLC; LA/M esophageal squamous carcinoma; A/M nasopharynx	NCT03997747
Tislelizumab	BeiGene	Bai Ze An	PD-1	China	2019/12/26	R/R classic Hodgkin lymphoma; LA/M urothelias carcinoma with high expression of PD-L1; EGFR(-)/ALK(-) LA/M NSCLC; hepatocellular carcinoma; MSI-H/dMMR advanced solid tumor	NCT03666143, NCT03430843, NCT04599777
Serplulimab	Henlius	Han Si Zhuang	PD-1	China	2022/03/22	MSI-H/dMMR advanced solid tumor; U/M NSCLC; ED-SCLC	NCT04547166, NCT05769725, NCT05742425
Cemiplimab	Sanofi	LIBTAYO	PD-1	USA	2018/09/28	NSCLC with PD-L1≥50%	NCT04177810, NCT04050436, NCT04339062
Pembrolizumab	MSD	Keytruda	PD-1	USA	2014/09/04	U/M melanoma; EGFR(-)/ALK(-) LA/M NSCLC with TPS of PD-L1≥1%; LA/M esophageal squamous carcinoma with CPS of PD-L1≥10; U/M head and neck squamous cell carcinoma with CPS of PD-L1≥20	NCT03544099, NCT02733250, NCT03609359
Sugemalimab	CStone Pharmaceuticals	Cejemly	PD-L1	China	2021/12/20	EGFR(-)/ALK(-) U/M NSCLC	NCT05623267, NCT05700448
Envafolimab	Alphamab Oncology	En Wei Da	PD-L1	China	2021/11/25	MSI-H/dMMR advanced solid tumor	NCT05448820, NCT05371197, NCT05397769
Durvalumab	AstraZeneca	Imfinzi	PD-L1	USA	2017/05/01	U/M NSCLC	NCT03829007, NCT02658214, NCT02639065
Atezolizumab	Genentech	Tecentriq	PD-L1	USA	2016/05/18	ED-SCLC; EGFR(-)/ALK(-) LA/M NSCLC; unresectable hepatocellular carcinoma	NCT05609903, NCT03645330, NCT04862949
Avelumab	Merck	BAVENCIO	PD-L1	USA	2017/03/23	metastatic Merkel cell carcinoma; LA/M urothelias carcinoma	NCT03330405, NCT03403777, NCT02625623

Abbreviations: MSI-H, microsatellite instability-high; dMMR, defective match repair; R/M, recurrent/metastatic; U/M, unresectable/metastatic; R/R, relapsed/refractory; LA/M, locally advanced/metastatic; A/M, advanced/metastatic; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ED, extensive-disease; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

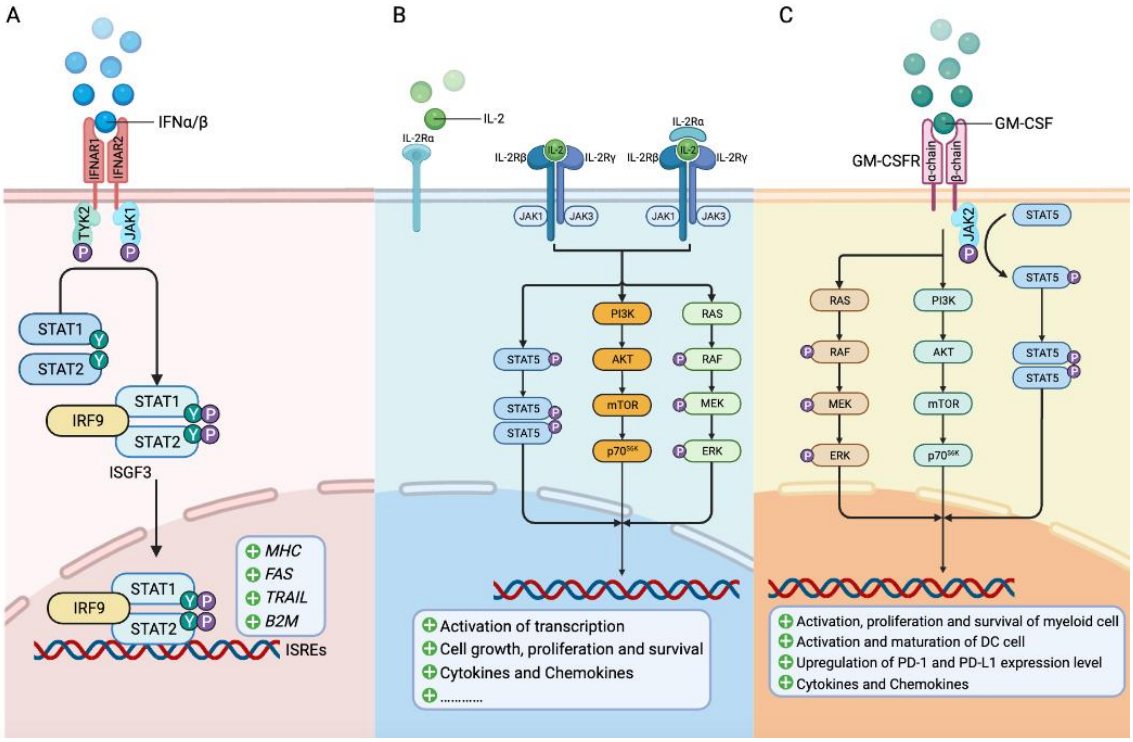

Fig. 1 The mechanisms of different cytokines. (A) IFNα/β signaling pathway. IFNα/β exerts the effect by activating the downstream JAK/STAT pathway. Upon the binding of IFNα/β to the heterodimeric IFNAR1-IFNAR2 complex on the cell membrane, TYK2 and JAK1 become phosphorylated. Subsequently, the STAT1-STAT2 heterodimer is phosphorylated and forms ISGF3 in conjunction with IRF9. Once ISGF3 enters the nucleus, it interacts with ISREs and induces ISGs such as FAS, TRAIL, and B2M, which promote apoptosis in tumor cells. (B) IL-2 signaling pathway. The low-affinity IL-2R is a monomer composed of IL-2Rα, while the intermediate-affinity IL-2R is a heterodimer composed of IL-2Rβ and IL-2Rγ. The high-affinity IL-2R is a trimer made up of IL-2Rα, IL-2Rβ, and IL-2Rγ. Upon binding of IL-2 with the intermediate and high-affinity receptors, JAK1 and JAK3 are phosphorylated and activated. Subsequent activation of downstream pathways, such as JAK/STAT, PI3K/AKT, and MAPK leads to enhanced cytokine release, cell proliferation, migration, and gene transcription. (C) GM-CSF signaling pathway. GM-CSFR is comprised of two chains, α and β. Upon the binding of GM-CSF to GM-CSFR, the phosphorylation of JAK2 is activated, which subsequently stimulates downstream pathways such as JAK/STAT, PI3K/AKT, and MAPK. This cascade of events promotes the maturation and activation of myeloid immune cells, facilitates the secretion of cytokines, and upregulates the expression of PD-1 and PD-L1. Abbreviation: IFN, interferon; JAK, Janus kinase; STAT, signal transducer and activator of transcription; IFNAR, IFNα receptor; TYK2, tyrosine kinase 2; ISGF, interferon-stimulating gene factor; ISRE, interferon-stimulated response element; FAS, Fas cell surface death receptor; TRAIL, TNF-related apoptosis-inducing ligand; B2M, β2-microglobulin; ISG, interferon-stimulated gene; IL-2R, IL-2 receptor; PI3K, phosphoinositide 3-kinase; AKT (i.e. PKB), protein kinase B; mTOR, mammalian target of rapamycin; p70S6K, p70 kinase; RAS, rat sarcoma virus; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; GM-CSF, GM-CSF receptor.

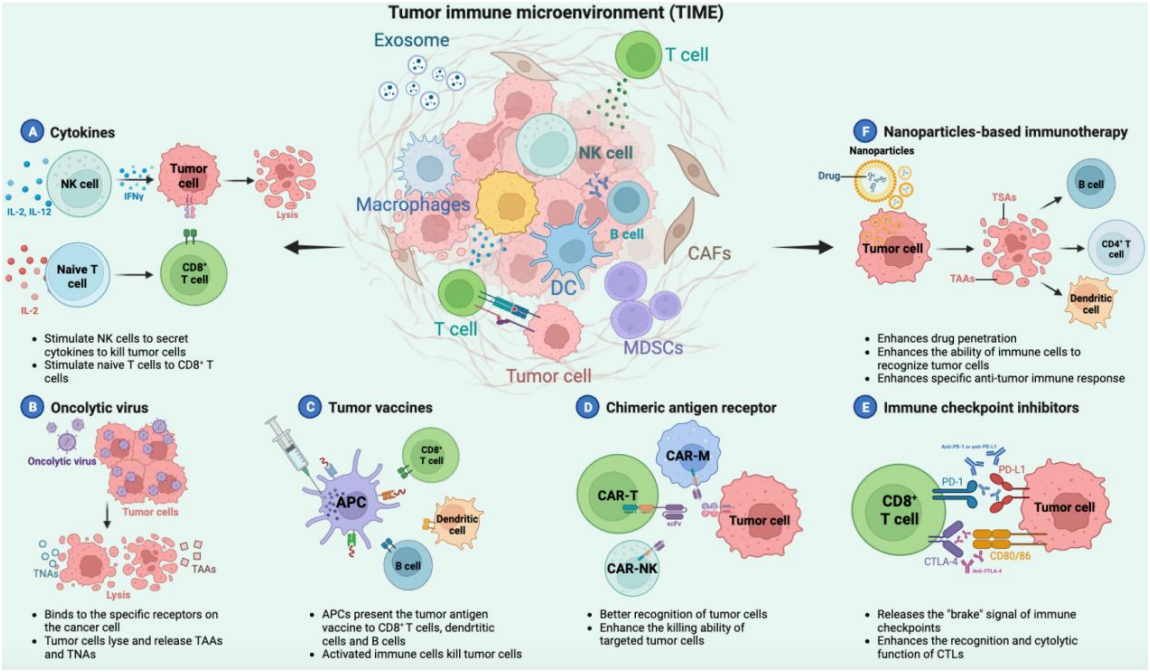

Figure 2. Composition of TIME and tumor immunotherapy. TIME is composed of tumor stroma, tumor cells, stromal cells, and a variety of immune cells, including cytotoxic T cells, macrophages, NK cells, B cells, DCs, and others. Currently, a variety of immunotherapeutic methods demonstrating promising therapeutic effects are available for different types and stages of tumors. These include cytokine therapy, OVs, cancer vaccines, CARs, ICIs, and nanoparticles-based immunotherapy. Abbreviation: TIME, tumor immune microenvironment; OV, oncolytic virus; CAR, chimeric antigen receptor; ICI, immune checkpoint inhibitor.

Author Contributions

BHY and KC designed this review and drafted the manuscript. BHY, XXL, WKL and YSM searched the related literature. BHY prepared the tables and figures. XDT and YMY revised and polished the manuscript and approved to submit manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare no conflict of interest.

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