

# Immunoscore Guided Cold Tumors to Acquire “Temperature” Through Integrating Physicochemical and Biological Methods

Jing Liu<sup>1,a</sup>, Mengze Xu<sup>1,a</sup> and Zhen Yuan<sup>1,2,\*</sup>

## Abstract

Immunotherapy for the treatment of tumors has become the most compelling strategy after targeted treatment, especially for lung cancer and melanoma, as well as some blood cancers. For most remaining types of tumors (e.g., pancreatic, colorectal, and breast cancers), abundant immunotherapeutic strategies in the forms of immune checkpoint blockade, cancer vaccines, and CAR-T therapies produce little effect. Furthermore, the immunoreactions induced by various types of cancer and even in individual patients, differ among the single therapeutic immune checkpoint inhibitors, whose pre-existing immunoreaction remains to be optimized for cancer immunotherapy. According to the density of the infiltrating lymphocyte subsets at the invasive margin or core of primary solid tumors, the tumors were classified into four grades using the immunoscore, which is complementary to the tumor node metastasis (TNM) staging system in providing a better prognosis of cancer patients in addition to the classification of immunogenic hot tumors and non-immunogenic cold tumors. This review aimed to outline the features of the most difficult-to-treat and challenging cold tumors and potential approaches to transform “cold” tumors into “hot” tumors, because hot tumors are associated with a higher response rate to immunotherapy. We also summarized the current popular strategies for enhancing T cell trafficking, which may be helpful to provide an etiological base for a more rational design of drug delivery systems and conquer drug-resistance during cancer therapy.

## Keywords

Cold tumors, immunoscore, response to immunotherapy, T cell infiltration.

<sup>1</sup>Cancer Center, Faculty of Health Sciences, University of Macau, Macau SAR, China

<sup>2</sup>Centre for Cognitive and Brain Sciences, University of Macau, Macau SAR, China

<sup>a</sup>Equal contribution

\*Correspondence to: Zhen Yuan, Phone: +00 8538822 4989, Fax: +00 853 8822 2314. E-mail: [zhenyuan@um.edu.mo](mailto:zhenyuan@um.edu.mo)

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## Introduction

Immunotherapy has been demonstrated to be highly effective against various tumors following targeted treatment. In addition, several clinical cases have also identified that immunotherapy plays a key role in inhibiting cancer growth and metastasis. The 2018 Nobel Prize in physiology and medicine was awarded to the scientist who discovered the immune checkpoint therapy PD-1 and CTLA-4 molecules, which have been successfully applied clinically. Notably, the populations of patients who will respond to immunotherapies (e.g., immune checkpoint monotherapy) is limited to 10%–35% [1, 2]. Moreover, cancer development results from a balance between tumor invasion and host self-defense mechanisms, which mainly rely on the immune response. One study quantified the number of tumor-infiltrating T lymphocyte and cytotoxic T lymphocytes (CTL) of patients bearing stages 1–3 colon cancer and then processed the individual

data to obtain an immunoscore for each patient [3]. The connection between the immunoscore and cancer relapse rate (identified from post-surgery to recurrence) was then used to demonstrate that the immunoscore exhibited greater accuracy in predicting recurrence, disease free survival, and overall survival, especially when combined with clinical parameters [4]. In addition, the co-expression of genes related to mediating cytotoxicity and adaptive immunoreactions can be used to predict the survival of colon cancer patients, independent of the occurrence of metastases. Indeed patients with non-metastatic cancers are associated with a similarly poor prognosis as those with metastases if the adaptive immune response is low within the tumor. Conversely, patients with high intratumoral adaptive immunity were found to have a better prognosis [5]. Furthermore, it has been clinically observed that the traditional tumor staging system (i.e., Tumor Node Metastasis [TNM]) can result in different prognoses

for patients with tumors of the same phase. This indicates the TNM tumor staging criteria provides limited information for the prognosis of patients [3]. The drawbacks outlined above are far from our expectation towards obtaining a robust tumor classification method that can better predict a curative effect and prognosis. In addition, numerous findings indicate that one of the principal factors that can guarantee the expected curative effect of immunotherapy is the pre-existing immunoreaction, especially the natural intratumoral T cell infiltration and expression of immune checkpoint inhibitors [6]. The obstacles of TNM for predicting the therapeutic effect of immunotherapy and the predominant role that immunity plays in anti-cancer resulted in the development of a robust, high efficiency and expressive standard of classification to better predict the prognosis and efficacy of cancer immunotherapy.

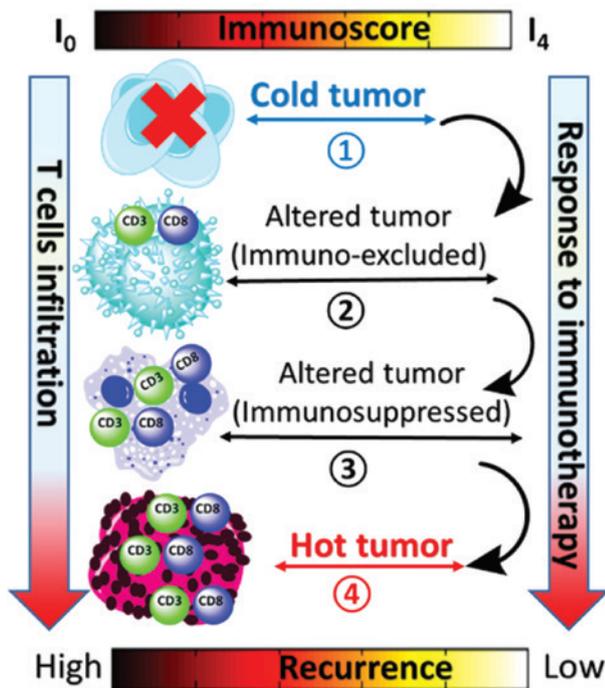
Hence, a related methodology, immunoscore, which exhibits superiority to TNM staging especially for prognosis, was put forward to quantify the immune cells in the tumor microenvironment following the antitumor immunoreaction and the impact of tumor infiltrating lymphocytes (TILs) on prognosis. The number of CD3- and CD8-expressing cells (i.e., mature T cells and cytotoxic T cells) present both at the tumor center and invasive margin are considered to be the basis of the immunoscore [3, 7]. Based on the quantification of CD3<sup>+</sup> and CD8<sup>+</sup> T cells, the immunoscore for tumors are divided into four levels, from I<sub>0</sub> (low infiltration of both types of cells, so-called non-inflamed tumor) to I<sub>4</sub> (high infiltration of both types of T cells at the edge and center of the

solid tumor, so-called inflammatory tumor). The middle two classes represent altered-excluded and altered-suppressive tumors, respectively [6]. Cold tumors are the most persistent and challenging type to treat with immunotherapy, which is the core issue to be explored in this review (Figure 1). Thus, the first step is to recognize the features of cold tumors and elucidate why they are resistant to immunotherapy, which can be used to design more reasonable and effective cancer treatment strategies.

## Features of cold tumors

### Failed T cell priming: low tumor mutational burden

The tumor mutational burden (TMB) is defined as the total mutations detected per million bases and represents a direct reflection of the neoantigen presented in tumor cells. Thus, the higher the TMB, the greater the number of neoantigens. Analysis of the lung squamous cell carcinoma database demonstrated a tight correlation between variants of DNA repair gene and TMB or the quantity of neoantigens, which contribute to the infiltration of active T cells both surrounding and within tumors. Notably, tumors with variants of DNA repair genome but no T cell infiltration are associated with an up-regulation of TGF-β and the Wnt pathway genes [8]. Hence, creating a composite analysis based on the information of the aforementioned genes leads to improved competence for predicting lymphocyte infiltration. It has been reported that the linear correlation formula for TMB and objective response rate towards immune checkpoint therapies was obtained through analyzing the pertinence between TMB in various tumor types and patient responsiveness to monoanti-PD-1/PD-L1 treatments. This tool is useful for forecasting the response rate for immune checkpoint therapies for the tumoral categories which do not yet explore anti-PD-1/PD-L1 therapies [9]. The high methylation of promoters, microsatellite transcription errors, and the mistaken splicing of exons are forms of immune epigenetics that indicate the advent of neoantigens in tumor cells [10, 11]. Paradoxically, Stefani Spranger et al. reported that there was no statistical difference between the number of non-synonymous somatic cell mutations which tightly correspond to the tumor antigen expression between cold and hot tumors [12]. This indicates that the lack of tumor antigens is not the cause of the loss of infiltrating T cells within tumors.



**Figure 1** Features of solid tumors classified by T cell infiltration and immunoscore: the higher density of infiltrating lymphocyte subsets at the invasive margin and core of primary solid tumors, the higher the response to immunotherapy; the higher immunoscore, the lower tumor recurrence. ① Cold tumor: No T cell infiltration; Low tumor mutational burden; Poor antigen presentation; No response to immunotherapy and high risk of recurrence. ④ Hot tumor: High T cell infiltration and high immunoscore.

### Poor antigen presentation

The presentation of tumoral antigens by dendritic cells (DCs) to CD8<sup>+</sup> T cells following the processing of neoantigens is a critical process required to boost the adaptive immune response. DCs encompass the subtypes of CD304<sup>+</sup>pDCs and cDCs, the latter of which comprises the subset for CD1c<sup>+</sup>, CD16<sup>+</sup>, and CD141<sup>+</sup> DCs. Moreover, the

presentation of the tumor-derived antigens is compromised by the low frequency of these DCs [13]. The cytokine receptor, CXCR1, is only expressed on the surface of CD141+ DCs, which are dominant in presenting tumoral antigens. In addition, LKB1 signaling in DCs can suppress the activation of Treg cells and Th17 cells, leading to both an enhancement of anti-tumor immunity and maintaining the balance of the body's normal immune function. Defective DCs mature abnormally and secrete immunoregulatory molecules [14]. It has also been found that a blockade of mTOR signaling in DCs with deficient LKB1 can be used to repair the activated phenotype on abnormal DCs and suppress the development of Treg cells. Dysfunctional DCs induced by various mechanisms results in tumors avoiding recognition by lymphocytes [15]. MAPK-activated protein kinase 2, which was expected to be a target used to heal cold tumors, performs a central role in restricting CD103+ DCs and T cell infiltration. Lei Shen et al. [16] found that the loss of zinc-finger A20 in DCs positively regulates self-maturation, antigen-presentation, and inhibits the ability of Treg cells to suppress the anti-tumor response, which contributes to enhanced cytotoxic T cell and helper T cell infiltration inside solid tumors.

## Lack of co-stimulatory molecule expression on T cells and overexpression of co-inhibitory molecules

The activation of primary T cells requires co-stimulation from two signals: (1) the specific combination between antigen and TCR (T-cell receptor); and (2) the bonding between the co-stimulatory or co-inhibitory molecules on DCs and relevant ligands on T cells. Co-stimulatory molecules include, CD28, ICOS, 4-1BB, and other, among which CD28 plays a pivotal role in T cell activation. Co-inhibitory molecules include, CTLA-4, PD-1, B7-H3, TIGIT, CD96, etc. [17]. Chimeric costimulatory receptor (TIGIT-28), composed of the TIGIT exodomain fused to the signaling domain of CD28, has been demonstrated to enhance the secretion of cytokines on T cells and upregulation of activation biomarkers, which contribute to the final tumor regression [18]. The B16 melanoma mice model, modified to express the CD28 ligand, B7-1, shows a higher sensibility to IL-12 gene therapy and remarkable upregulation of CD28. Research also suggests that the CD28-scFv- $\alpha$ -erbB2-CD28- $\zeta$  domain is of great value in signal transduction [19]. Moreover, T cells of scFv-CD28- $\zeta$  deficient mice exhibit diminished cytokine (IFN- $\gamma$  and GM-CSF) production and self-proliferation [20]. Jie Fan et al. integrated monoclonal antibody agonists (mAb-AG) targeting 4-1BB and anti-CD73 therapy, which was associated with enhanced infiltration of IFN- $\gamma$ +CD8+ T cells in the tumor microenvironment [21]. In summary, multiple approaches that can be used to potentiate co-stimulatory molecules or dampen co-inhibitory molecules have been explored and have performed notably well in reversing the exhaustion/suppression of T cells responsible for the anti-tumor response.

## Absence of T cell trafficking to the tumor bed

The baseline level of critical anti-tumoral CD8+ T cells in the tumor microenvironment (TME) underlies the efficiency of immune checkpoint blockades like PD-1/PD-L1 [22]. There are still some roadblocks that restrict CTL migration to the TME following T cell activation by DCs. In addition, type I IFNs produced by DCs are not only tightly associated with the activation, differentiation, and migration of multi-type lymphocyte-encompassing T cells, but is also competent at enhancing the anti-tumor immune response [23]. Chemokines are low-molecular-weight cytokines that have been categorized into four subsets: CXC, CC, C, and CX3C. Through the statistical analysis of numerous primary cutaneous melanoma, Monteagudo et al. proposed that clinical cases devoid of CXCR3 (the receptor for CXCL9, CXCL10, and CXCL11) exhibit lower lymphocyte recruitment into the TME and showed a worse prognosis [24]. Harlinet et al. analyzed the quantity of chemokine receptors expressed on the surface of CD8+ T cells, and found that there was an upregulation of CCR1, CCR2, CCR5, and CXCR3, as well as a decline in the expression of CCR7 and CXCR4 on effector T cells. To gain insight into the chemokine receptors that were overexpressed on CD8+ T cells, the authors focused on the corresponding chemokines, including CCL2, CCL3, CCL4, CCL5, CXCL8, CXCL9, and CXCL10. Specifically, CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10 performed remarkably at promoting CD8+ T cell migration from normal tissues [25]. In another experiment, T cell trafficking to tumor lesions was detected in IL-12-sensitive models but not in IL-12 non-sensitive models [26]. Moreover, the migration of T cells to tumor epithelial cells is not independent of the interaction between adhesion molecules, such as VCAM-1/ICAM-1 on endothelial cells and the corresponding receptor, VLA-1/LFA-1.

## Approaches to turn “cold” into “hot”

Especially for cold tumors with the loss of CD3+CD8+ T cells, several methods, including both single and combination therapy, have been proposed to turn the “cold” tumors into “hot” tumors. Furthermore, an abundance of novel drug delivery systems were employed to potentiate the ability of drugs to heat the “cold” tumors. Substantial fractions of these treatments are somewhat specific for the different potential inhibitory factors against “hot” states.

## Radiotherapy/chemotherapy regimens: induced immunogenic cell death

After being subjected to external pressures, such as chemicals, irradiation, infection, and photodynamics, the initial

tumor cell death can consist of both immunogenic cell death (ICD) and non-immunogenic cell death. ICD involves pronounced activation of anti-tumor immune system pathways, which is responsible for the permanent success of the anti-cancer response. The suboptimal protocols which fail to trigger an innate or adaptive immune response can fail to extinguish the tumors [26]. By eliciting ICD to induce cell death and release immunogenic factors, abundant endogenous damage-related molecular patterns (DAMP) are subsequently exposed. Regardless of nonmicrobial-dependent instances of cell death, most instances of ICD share common DAMPs that rely on calreticulin (CRT), triphosphadenine (ATP), and high-mobility-group box 1 (HMGB1), which encourage DCs to present antigen to T cells [27].

## Radiotherapy

The curative effect of irradiation is also largely attributed to the body's immune system. It has been well-established that the abscopal effect. In addition to the degradation of the irradiated primary tumor, distal metastatic tumors also shrink, which is generally acknowledged to be immune-mediated. The immunogenicity of irradiation allows tumor cells to release "eat me" signals to DCs via translocating calreticulin to the cell surface, releasing ATP and HMGB1 [26, 28–30]. Furthermore, radiation promotes lymphocyte migration and infiltration around solid tumors through inducing the expression of vascular adhesion molecules, VCAM and chemokine (C-X-C motif) ligand 16 (CXCL16) [30]. With the prevalence of immunotherapy in oncology, radiotherapy is exploited more extensively as a capable partner for immunotherapy. Radiotherapy is responsible for releasing "danger" signals associated with tumor cells to the immune system to launch an immune response and improve the baseline level of immunity, whereas immunotherapy relieves immunosuppression. A variety of preclinical trials integrating irradiation and immunotherapies are ongoing. Based on the hypothesis that the abscopal effect is mediated by the immune system, New York University was the first to construct a murine model bearing syngeneic mammary carcinoma treated with cytokines FLT-3L and irradiation [31]. The same group carried out a proof-of-principle test to explore the synergism of granulocyte-macrophage colony-stimulating factor (GM-CSF) and irradiation. From a long-term detection perspective, patients who showed an abscopal effect were also associated with longer survival times [32]. In a collaboration between New York University and the University of California implemented a project orchestrating the combination of transforming growth factor  $\beta$  (TGF- $\beta$ ) and irradiation against the site of metastasis of breast cancer, which significantly annihilated the lung metastasis comparing with single therapy.

These aforementioned strategies are early attempts to verify the complementation between irradiation and immunotherapy. Currently, more novel strategies are continuously developing. Irradiation targeting primary solid tumors induces ICD and promotes DCs and CD8+ T cells to mature and migrate into tumors through triggering pathways, which finally turn refractory cold tumors into hot tumors that are

sensitive to immunotherapies, such as immune checkpoint blockade.

## Chemotherapy

Autophagy has been found to play a crucial role in the ICD of tumor cells. Dying cells free ATP as a "find me" signal under the stress from chemicals, to draw DCs and T cells into the site of tumors, thereby contributing to ICD. In autophagy-deficient tumor cell lines, the inhibition of extracellular ATP degradation enzyme and increase extracellular ATP concentration can re-recruit immune cells and restore the efficacy of chemotherapy drugs [33]. Tumor-associated macrophages (TAMs) recruited by colony stimulating factor-1 (CSF1) facilitate the development of tumors that are dependent on their ability to enhance angiogenic, invasive, and metastasis programming of neoplastic tissue. An CSF1 blockade regime in conjunction with chemotherapy for the treatment of mammary adenocarcinomas in an animal model was found to reduce macrophage abundance, enhance CD8+ CTL infiltration, and suppress tumor development [34]. The abundance of lymphocyte infiltration in metastatic lesions in primary human colorectal cancer imparts strength to predict the efficacy of chemotherapy [35]. Breast tumor-infiltrating CD8+ T cells were significantly increased and the number of FOXP3+ Tregs showed the opposite changes, which was tightly correlated with improved relapse-free survival (RFS) and overall survival (OS) [36]. Furthermore, based on the CD8/FOXP3 ratio and American Joint Committee on Cancer (AJCC) pathological staging, patients with a long-term overall survival of 100% were separated out [36]. There are other panels of chemicals that elicit genotoxicity and enhance immunogenicity for tumors by facilitating neoantigens expressed on malignant cells. However, these neo-epitopes are routinely confronted with the limitation of stimulating the immune system resulting from the modest level of expression on tumor cells [37].

## Targeted therapies

DNA methylation is one of the earliest known epigenetic modifications. Tumorigenesis routinely originates from the silencing of tumor suppressor genes due to abnormal DNA methylation. This DNA modification scenario is able to suppress the expression of some genes, including tumor-associated antigen (TAA) genes. The use of epigenetic drugs embracing DNA methyl transferase (DNMT) inhibitors for DNA methylation upregulate TAA and MHC class I and class II molecules [38].

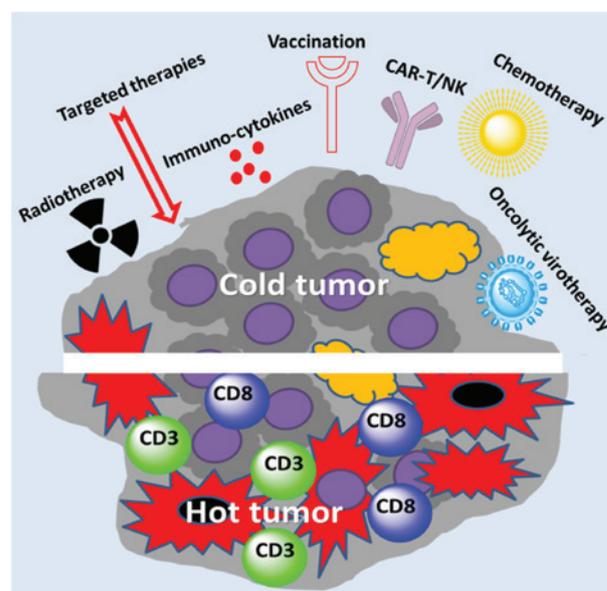
Pattern recognition receptors (PRRs) are recognition molecules expressed on the surface of innate immune cells, show a non-clonal distribution, and can recognize one or more pathogen-associated molecular patterns (PAMPs). PRRs are subdivided into three types based on their existing patterns: (1) membrane type PRRs, including major members of the Toll-like receptor (TLR) family, mannose receptors (MRs), and scavenger receptors (SRs); (2) secretory type PRRs, which consist of mannose binds lectins

(MBLs), C-reaction protein (CRP), and lipopolysaccharide binding protein (LBP); and (3) cytoplasmic PRRs, which include TLR3, TLR7/TLR8, and TLR9 from the TLR family and all members in NLR family. Across various PRRs, the TLR family represents is the most capable for distinguishing PAMPs. Moreover, imiquimod was the earliest FDA-approved TLR7/TLR8 agonist for cancer treatment [39].

Complementary to TLRs, the NLR family also recognizes pathogen- and damage-associated molecular patterns, among which NLRP3, excited by motolimod and mebendazole, is the most well-studied [40]. Stimulator of interferon genes (STING) protein is an important molecule that activates the body's natural immune system to resist bacterial or viral infection. Tumor-originated DNA activates cyclic GMP-AMP synthase (cGAS) to generate cGAMP binding to STING, which results in the initiation of an extensive downstream signaling pathway cascade via the secretion of IFN [41] IFNs favor the cross-presentation of tumor-associated antigens and mobilization of CD8<sup>+</sup> T cells [42, 43]. Ramanjulu et al. reported a STING small molecule agonist via linking two symmetrical aminobenzene imidazoles (ABZI) to obtain linked diABZIs with increased affinity to STING [44].

Abnormal cell metabolism as one of the 10 characteristics of a tumor, and is closely related to tumor occurrence and development. Accumulating evidence indicates that the energy metabolism based on amino acids occupies a crucial position in tumor research. A diverse number of studies have documented that DCs are also able to enhance immunosuppression and tolerance instead of provoking an effective immune response [45]. The indoleamine 2,3-dioxygenase (IDO)-guided tryptophan metabolism pathway is one of the signaling pathways that induces DC immunosuppression [45]. In clinical research, 1-methyl-D-tryptophan has been used as an IDO inhibitor and has been shown to exert a positive impact on CD8<sup>+</sup> T cell antitumor efficacy in vitro [46].

Chemokines and their receptors play an important role in the immune response against cancer. According to the position of the first two cysteines in the amino acid sequence, chemokines can be divided into four subgroups (CC, CXC, and CX3C). Chemokines may be involved in inhibiting tumor growth owing to increased tumor-infiltrating T cells [47]. Berencsi et al. constructed a tumor cell culture model, from which the data elucidated a positive correlation across CCL2 secreted by CTLs, as well as CCL2 and the anti-tumor ability of CTLs [48]. Coukos' group made it clear that CCL5 and CXCR9 mobilized CD8<sup>+</sup> T cells to migrate into solid tumors [49]. When T cells provoked by CCL5 reached solid tumors and were activated by cancer antigens, they released IFN $\gamma$ , which accelerating macrophages and DCs to secrete CXCL9, forming a virtuous cycle favoring CTL infiltration [49]. CCR5, CXCR3, CX3CR1, and CXCR6 are connected to the trafficking of CTLs towards the site of tumors or other mechanisms on changes to the inflammatory environment surrounding the tumor [25, 50–52]. Bodduluri Haribabu's team conducted an in-depth study of another class of chemokines called LTB<sub>4</sub>, whose receptor is BLT1 and found that the infiltration capacity of T cells in cancerous mice with LTB<sub>4</sub> deletion mutations were significantly affected [53]. They also demonstrated that BLT1 occupied



**Figure 2** Approaches used to turn “Cold” tumors into “Hot” tumors. Radiotherapy/chemotherapy regimens: induced immunogenic cell death and targeted therapies; immunotherapy programs: oncolytic virotherapy, vaccination, and adoptive cell immunotherapy.

a crucial position in anti-PD-1 therapy. Targeted therapies have long been a hotspot. Moreover, there are potential target molecules along each pathway which suppress tumor growth. Hence, the potential approaches that can be used to turn “Cold” tumors into “Hot” tumors is summarized in a graphic illustration of **Figure 2**.

## Immunotherapy programs

### Oncolytic virotherapy

Oncolytic viruses are engineered to selectively infect and annihilate tumor tissues. In another opinion article, the authors concluded that there were four steps T cells had to go through to successfully trigger an immune response: (1) T cell priming. Oncolytic viruses mimic the function of a “vaccine” for promoting the presentation and recognition of tumor-associated antigens, leading to T cell priming; (2) trafficking and infiltration. Oncolytic viruses exploit various mechanisms to augment T cell infiltration towards tumors; (3) circumventing immune suppression. To overcome the inhibition induced from a broad spectrum of immunoregulatory molecules following T cell infiltration, oncolytic viruses are expected to induce pro-inflammatory Th1 cells to contribute to the turnover against the suppressed tumor microenvironment, as well as directly kill Treg cells; (4) conjunction with neoplasm cells. The final procedure of immunotherapy is for T cells to recognize, bind to, and kill cancer cells. To bypass recognition by the immune system, neoplastic cells evolve to downregulate the pathways engaged in tumor presentation and major histocompatibility complex (MHC) class 1 expression. Oncolytic viruses may implement subversion for this landscape [54]. Oncolytic virotherapy with talimogenelaherpaprepvec in a phase 1b clinical trial testing

has been shown to be a potent immune adjuvant augmenting T cell infiltration, PD-1 protein, and IFN- $\gamma$  gene expression in several cell subsets in the tumoral microenvironment when combined with anti-PD-1 therapy [55]. The Syrian hamster pancreatic and kidney tumor models were treated with a continuous dose of oncolytic adenovirus and vaccinia virus or either of them alone, which were responsible for the introduction of a tumor-specific immunoreaction rather than an antiviral immune response [56]. Measles virus-mediated melanoma cell death is related to ICD, constituting the release of danger signal HMGB1 and inflammatory cytokine type 1 interferon, to elicit an immune reaction [57]. Currently, multiple chimeric oncolytic virotherapy platforms have been predisposed to focus on their competence to heat those refractory cold tumors through assisting T cell proliferation and trafficking into solid tumors.

## Therapeutic vaccination

Therapeutic vaccines have become a seminal breakthrough in the treatment of solid tumors because they promote T cells to attack tumors with a high degree of specificity. Extensive tumor vaccine approaches have been investigated to control the tumor burden in patients to determine the efficacy of the immunization [58]. An optimal tumor vaccine delivery platform are associated with a tendency for concentrating antigens to the HLA class I and II molecules expressed on DCs, which are responsible for the enrichment of CD4+ and CD8+ T cell populations [59]. Provenge (Sipuleucel-T) is an autoimmune DC-based vaccine therapy for metastatic castration-resistant prostate cancer [60] and the first FDA-approved therapeutic tumor vaccine. After extracting antigen-presentation cells (APC) from patients, they are loaded with prostatic cancer associated antigen, prostatic acid phosphatase (PAP), and the immune stimulatory molecule, granulocyte macrophage colony stimulating factor (GM-CSF), and then reintroduced into patients to boost the T cell response [61].

Antigens for engineered vaccines mainly constitute a mutant sequence, cancer-testis antigens, and viral antigens [59]. Tumor neoantigens derived from mutations can be viewed as totally foreign antigens, unlike tumor-associated antigens, which can lead to immune tolerance. As a function of technological advances in genic and bioinformatic analyses, neoantigens are increasingly easier to identify, which is favorable for the designation of tailored cancer vaccines [62]. In step with the progress of technology, personalized tumor vaccines has become an increasing developmental trend. Catherine Wu led a team of researchers who developed strategies to treat each patient with a peptide vaccine based on the prediction of 20 neoantigens with high immunogenicity and high expression levels, according to which corresponding peptide vaccine therapeutic strategies were developed [63]. In addition, scientists persist on developing various methods by which to amplify the effectiveness of tumor vaccines. RNA loaded liposomes can transport tumor antigens to DCs with accuracy and availability [64]. These tumor vaccine platforms carried auxiliary peptides as adjuvants do promote the stimulation of antibody and T cell

responses [65]. CD4+ helper T cells also facilitate cytotoxic T lymphocyte responses via CD27/CD70 co-stimulation [66]. Moreover, a CD27 agonist combined with a PD-1 inhibitor has been shown to improve the efficacy of a therapeutic tumor vaccine.

## Adoptive cell immunotherapy

Adoptive cell immunotherapy is stratified into non-specific (lymphokine-activated killer cells, CD3 monoclonal antibody-activated cells, and cytokine-induced killer cells) and specific (tumor infiltrating leukocyte, gene modified TCR, and chimeric antigen receptor T cells) therapies. The so-called “adoptive” approach removes cells from the human body and cultures them *in vitro* for amplification before transferring them back into the patient. In the 1980s, Rosenberg and his team found that splenic lymphocytes from a mice model generated an extant anti-tumor phenotype following stimulation with a T cell growth factor. The activated T cells were termed lymphokine activated killer (LAK) cells, which shed light on a new era of tumor immunotherapy. Subsequently, LAK therapy integrated with IL-2 showed distinct usefulness in the battle against melanoma with pulmonary metastasis [67]. LAK therapy exhibits hallmarks of a broad spectrum of anti-tumor responses and MHC independence, as well as its non-specificity. Rosenberg further exploited TIL strategies, which demonstrated a higher level of accuracy and efficacy [68]. However, TILs still are routinely confronted with scenarios in which they cannot recognize cancer cells. Hence, oncologists expect to proactively send information to T cells. Aimed at MHCs, the domain recognized by TCR expression on T cells, gene-encoding TCR technology specific for melanomas-associated antigen, MART-1, was engineered [69]. Tumors continuously co-progress with our scientific research and have evolved sophisticated mechanisms to antagonize external pressure. Tumor cells may autonomously reduce their immunogenicity by abandoning their MHC expression, to which the TCR-T strategy was vulnerable. Chimeric antigen receptor T cell (CAR-T) technology is a unique strategy that is not restricted by MHCs. Genomic expression vectors for CAR-T cells containing antibody variable domains alongside TCR genes can competently attack tumor cells [70]. Compared with immune checkpoint therapy, adoptive cell therapy develops relatively slowly. However, it is clear that there is consensus regarding the notion that CAR-T therapy is a promising immunotherapy. To turn “cold” tumors into “hot” tumors, CAR-T faces the following challenges: (1) a lack of tumor specificity or downregulation of antigens; (2) lack of necessary pro-inflammatory stimulating molecules in the immunosuppressive tumor microenvironment; (3) refractory physical barrier that is difficult to penetrate; (4) malicious factors from the tumor environment are harmful for CAR-T survival. Accumulating evidence indicates that oncolytic virotherapy can be regarded as a good adjuvant for CAR-T through reshaping the tumor microenvironment to improve the capacity for the recruitment and effector function of T cells [71–73]. The aberrant tumor vasculature is considered to be the main barrier to T cell infiltration [74]. CAR-T cells

targeting vasculature antigens embracing VEGF, CD276 and endothelin B receptor have been identified to normalize tumor microvessels and strikingly delay tumor growth, also be effective with combination with CAR-T therapy [75, 76].

Localized delivery CXCL11 into mice bearing a subcutaneous tumor via vaccinia virus vector pronouncedly amplified the number of tumor infiltrating T cells following a CAR-T cell injection and significantly promoted the anti-tumor response [77]. In addition, another promising regimen for increasing the trafficking of CAR-T into solid tumors is the development of CAR-T targeting FAP (fibroblast activation protein), which plays a role in epithelial-mesenchymal transition in multi-type malignancies [78]. It is recommended that more intriguing designation be integrate with CAR-T rather than using CAR-T therapy alone in clinical trials.

## Conclusion and future outlook

Immunoscore facilitates the development of personalized cancer treatment based on individual patient and tumor types with different immunoscores, despite the ongoing challenges of reversing the absence of T cells within the center and edge of solid tumors. However, it remains difficult to determine the source of the lack of T cells in cold tumors, which is required to integrate multi-disciplinary measurements to design a therapeutic platform that is adaptive to the specific physiology of the tumor. Furthermore, it can be inferred that the colder the tumor is, a more complicated and integrated regiment will be needed through taking advantage of

physicochemical and biological methods. Recently, a growing number of studies have employed immune combination therapies which have performed well in turning “cold” tumors into “hot” tumors. One strategy combining triple-RNA for mediating the expression of immunomodulatory factors with immune checkpoint therapy has entered phase I clinical trials. However, more accurate and robust measurements of cancer-immune interplay parameters whose relative “weight” varies considerably across individual patients are also crucial. In a word, immunoscore based on accurate and high-sensitive detection means is vital to guiding personalized precise medicine for immunotherapy, which also depends on interdisciplinary therapeutic methods like the most typical synergistic therapy of chemotherapy, phototherapy and immunotherapy.

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## Competing financial interests

The authors declare no competing financial interests.

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