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REVIEW ARTICLE

The emergence of tumor-infiltrating lymphocytes in nasopharyngeal carcinoma: Predictive value and immunotherapy implications

Liu Yang [a], Guohong Liu [b], Yirong Li [a,**], Yunbao Pan [a,*]

[a] *Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan University, Wuhan, Hubei 430071, PR China*
[b] *Department of Radiology, Zhongnan Hospital of Wuhan University, Wuhan University, Wuhan, Hubei 430071, PR China*

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Abstract The clinical study of nasopharyngeal carcinoma (NPC) often reveals a large number of lymphocytes infiltrating the primary tumor site. As an important part of the tumor microenvironment, tumor-infiltrating lymphocytes (TILs) do not exist alone but as a complex multicellular population with high heterogeneity. TILs play an extremely significant role in the occurrence, development, invasion and metastasis of NPC. The latest research shows that they participate in tumorigenesis and treatment, and the composition, quantity, functional status and distribution of TILs subsets have good predictive value for the prognosis of NPC patients. TILs are an independent prognostic factor for TNM stage and significantly correlated with better prognosis. Additionally, adoptive immunotherapy using anti-tumor TILs has achieved good results in a variety of solid tumors including NPC. This review evaluates recent clinical and preclinical studies of NPC, summarizes the role of TILs in promoting and inhibiting tumor growth, evaluates the predictive value of TILs, and explores the potential benefits of TILs-based immunotherapy in the treatment of NPC.

* Corresponding author. Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan University, No.169 Donghu Road, Wuchang District, Wuhan, Hubei 430071, PR China.
** Corresponding author. Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan University, No.169 Donghu Road, Wuchang District, Wuhan, Hubei 430071, PR China.
E-mail addresses: liyirong201705@163.com (Y. Li), panyunbao@outlook.com (Y. Pan).
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Introduction

Globally, the incidence of nasopharyngeal carcinoma (NPC) has continued to rise from 2009 to 2019 increased by 45.09%. The age-standardized incidence rate (ASIR) of NPC increased from 1.81 in 2009 to 2.12 in 2019.[1] On the contrary, the age-standardized death rate (ASDR) of NPC showed a decreasing trend from 0.93 in 2009 to 0.86 in 2019 and negatively correlated with social demographic index (SDI) in most regions.[1] Morbidity and mortality rates of NPC have stabilized or declined in high-incidence areas. The incidence and mortality of NPC in males were significantly higher than those in females. The NPC patients with onset age over 50 years old accounted for the highest proportion. The World Health Organization recognizes three classes of NPC by histopathology: keratinized squamous cell carcinoma (with varying degrees of differentiation), non-keratinizing carcinoma (differentiated and undifferentiated types) and basal cell-like squamous cell carcinoma.[2,3] The etiology is not completely clear for any of these classes. The occurrence of NPC is considered as the result of the interaction of Epstein—Barr virus (EBV) infection, genetic, environmental factors (such as drinking and smoking) and consumption of salted fish,[4] with EBV infection playing a major role.[5]

At present, most NPC patients can be successfully cured by improving treatment strategies. However, due to early hidden sites and lack of specific symptoms, NPC patients usually develop to advanced stage at the time of preliminary diagnosis.[6] Chemotherapy combined with radiotherapy (stage I radiotherapy alone; stage II radiotherapy with or without simultaneous chemotherapy; stage III-IVB chemotherapy) is the first-line treatment strategy for patients with advanced NPC[7]; however, due to acquired drug resistance, the survival rate is still not ideal, resulting in recurrence, metastasis and even death. Local recurrence or metastasis of NPC remains an important challenge, requiring more effective and lasting treatment. Studies have shown that NPC patients may benefit from cancer immunotherapy because human leukocyte antigen (HLA) alleles and other immune-related genes are associated with the disease.[8,9] Lymphocytes infiltration is associated with the response of several tumor types to checkpoint inhibition,[10] which is also common in NPC specimens.[11] In recent years, tumor immunotherapy has developed vigorously and provided a breakthrough in the treatment of recurrent or metastatic diseases, creating new opportunities for NPC treatment.

As early as 1988, it was reported that tumor-infiltrating lymphocytes (TILs) play an active role in many advanced malignant tumors.[12] This means TILs as the main target for anti-tumor immunity in NPC might induce beneficial clinical reaction and become an important part of individualized therapy of NPC patients. TILs are tumor-infiltrating cells with anti-tumor effects that were discovered and isolated from mouse tumor tissue by Rosenberg in 1986. TILs include both intra-tumoral TILs in tumor cell nests and stromal TILs in the stroma.[13,14] Recent studies show that TILs are present in NPC tumors and play a critical role in the development, prognosis and treatment of advanced NPC.[15,16]

This paper reviews the latest research progress on TILs in NPC and puts forward some new ideas for the development of TILs as both tumor biomarkers and therapeutic agents, that is significant for individualized immunotherapy of NPC.

NPC microenvironment

There are many reports supporting the important role of tumor microenvironment (TME) in the pathogenesis of NPC.[17] In many cases, NPC cells escape the anti-EBV immune response and need to be supported by TME. The coexistence of TILs and EBV-infected NPC cells represents a unique TME, which is considered to play an important role in supporting the growth and development of NPC (Fig. 1).[18] In the TME, while the immune system removes tumor cells, it also remodels the biological characteristics of other tumor cells (such as tumor antigenicity), which is called "immune editing".[19,20] In the process of tumor editing, tumor cells develop strategies to evade immune detection or destruction.[21] This may be due to the downregulation of major histocompatibility complex molecules and the inability to produce tumor peptides. With the loss of tumor antigens, NPC antigens are no longer effectively presented to the immune system. At the same time, the rapid growth of tumor cells after tumor formation will produce a TME that suppresses immune cells. In the NPC-TME, NPC cells release immunosuppressive molecules, such as transforming growth factor-β (TGF-β), epidermal growth factor (EGF) and interleukin-10 (IL-10). They induce Tregs to express cytotoxic T lymphocyte-associated protein 4 (CTLA-4), which suppresses other immune cells and leads to tolerance of the immune system to tumors.[22] The study shows that EBV latent membrane protein 1 (LMP1) mediated by NPC promotes myeloid-derived suppressor cell (MDSC) expansion in the TME by promoting extramitochondrial glycolysis in malignant cells. This is a case of immune escape in NPC, initially due to the large number of MDSC subsets that can be detected in tumor sections.[23]

NPC-TME consists of cellular components and extracellular matrix (ECM). The cellular components are mainly immune cells, which make TME show a reactive and inflammatory appearance. In addition, TME also contains chemokines, cytokines and other bioactive substances. The cellular components of TME play an important role in the immunosuppression, growth and invasion of NPC. The majority of lymphocytes infiltrating in NPC biopsies is $CD3^+$ T-lymphocytes.[16,18,24] Although the $CD4^+/CD8^+$ ratios vary in different NPC specimens, they generally comprise over 50% of the TILs of NPC.[25] Small subsets of B lymphocytes, monocytes, macrophages, natural killer (NK) cells and neutrophils were also detected in TME of NPC.[26] The recruitment and activity of TILs are thought to be determined by the concentration of different cytokines and chemokines present in TME. It's worth noting that there may be significant differences in the size and composition of TME between different cases of NPC. This may reflect the different degrees of attraction of chemokines to TME cells and the different degrees of activation of TME cells by cytokines and cell—cell interactions. Increased levels of IL-6, IL-8, IL-10, and interferon-γ (IFN-γ) and decreased levels of IL-2 are commonly detected in NPC patients, which may be related to the recruitment and activity of these TILs.[18]

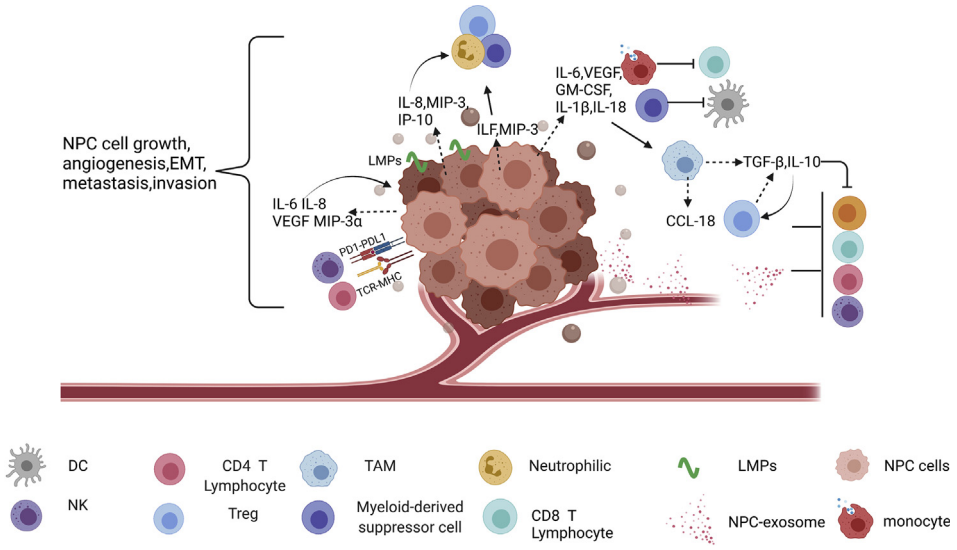

Figure 1 Illustration of the presence of multiple immune cells and cytokines in the TME of NPC.
Many proinflammatory cytokines including CCL20, MIP3-α (CCL20), interferon (IFN)-γ, IL-6/8/10/18, GM-CSF (granulocyte-macrophage colony-stimulating factor), TGF, VEGF, IL1-β are present in the TME. The presence of TGF and IL-10, as well as the up-regulation of PDL-1 and PD-1 by LMP1, and the release of exosomes containing LMP1 into TME induce the expansion of Tregs, MDSC and TAM, thereby generating immunosuppressive TME and promoting tumor escape. Mip3-α is produced by NPC cells and chemically induces lymphocytes and dendritic cells via CCR6. NPC cells also produce IL-1-β, IL-6, and GM-CSF. Transforming growth factor-β (TGF-β) and IL-10 are important immunosuppressive cytokines that promote tumor immune escape. NPC cells release a large number of exosomes expressing CCL20 to evade immune detection.

It's reported that IL-6R is expressed in NPC, and IL-6 is produced by tumor cells in TME, which acts as a growth factor and leads to the activation of STAT3.[27] In fact, it's known that the immunosuppressive TME provides immune space for tumor growth.[28] This local tolerance is mediated by cytokines and regulatory immune cells.[29,30]

NPC cells and viral products encoded by EBV play an important role in triggering the release of various pro-inflammatory cytokines and promoting the aggregation and homing of lymphocytes to TME. NPC cells secrete IL-18[31] and CXCL-10[32] in many cases. IL-18 and CXCL-10 can activate and induce $CXCR3^+$ T cells to produce IFN-γ, which in turn may stimulate $CD68^+$ macrophages to produce IL-12 and IL-18, and then stimulate T cells and NK cells which producing IFN-γ to promote inflammation and immune cell infiltration. The IL-10 has been known as a potent immunosuppressive cytokine that can effectively inhibit the cytotoxic function of activated $CD8^+$ cells and promote EBV-infected NPC cells survival.[33] It's reported that LMP1 encoded by EBV can up-regulate a variety of cytokines and promote immune cells infiltrating into the tumor site.[23,34] Besides, LMP1 can induce the expression of CXCL-10 through NF-κ B signaling. On the other hand, endogenous LMP1 in NPC cells can up-regulate IL-8, macrophage inflammatory protein-1 α and macrophage inflammatory protein-1 β, thus attracting various types of immune cells infiltration. In addition, LMP1-mediated glycolysis increase the release of IL-1β, IL-6 and GM-CSF in TME, and finally lead to the induction and expansion of MDSC in TME.[23] Monocytes produce two powerful chemokines, CCL2 and CCL3, to attract lymphocytes to TME.[35]

The evidences show that EBV-infected NPC cells interact with TME components to promote NPC metastasis. Increased expression of $FoxP3^+$ Treg cell and $CD68^+$ tumor-associated macrophages (TAMs) was found in EBV positive NPC specimens. These two types of cells interact with NPC cells to promote tumor metastasis and are associated with poor prognosis.[36,37] The mutation of NF-κB pathway also occurs in NPC.[38] With the evidence of hypercirculating Treg cells in NPC patients, Treg cells are likely to promote the migration of primary NPC cells by producing RANKL through RANK (nuclear factor-κ B receptor activator)-RANKL signaling. TAMs are an effective producer of CCL18, which can further enhance the NF-κB signaling in NPC cells and increase the release of VEGF and GM-CSF to form a feed-forward loop which can drive NPC metastasis and support tumor growth.[39] In addition to directly secreting chemokines into TME, it's reported that malignant NPC cells can release exosomes and transport the products to TME.[40,41] The researches show that exosomes derived from NPC-TME can promote the chemotaxis of $CD4^+CD25^+FoxP3^+$ Treg cell[41,42] and carry the carcinogenic HS1-related protein Xmurl (HAX-1), which can promote tumor growth and angiogenesis in NPC.[43]

The composition of TILs in NPC-TME is complex and interacts with NPC cells. Elucidating the complex interaction between EBV-infected NPC and TME interstitial cells will be one of the future directions of NPC research. Interestingly, it's reported that the overall immune activity of NPC patients is maintained because EBV-specific CTLs can be reactivated from the patient's blood. Activated CTLs can still be transported to the tumor site, but it selectively becomes ineffective in the tumor site of NPC. Overcoming the immunosuppression of TME in NPC can provide an effective treatment option for NPC.

Inhibitory effect of TILs on anti-tumor immunity in NPC

T cells have the ability to recognize tumor antigens and stimulate tumor rejection, but this is not always the case.[44] Although it has been reported that specific cytotoxic T cells targeting EBV can be detected in peripheral blood and biopsy tissues of NPC patients, most of the patients are still in a state of immune escape (Fig. 2). Studies have shown that the expression of metallothionein and $CD3^+$ in T cells of NPC is downregulated, while the expression of Tregs and programmed death factor 1 (PD-1) is upregulated, resulting in the inability of T cells to produce an effective anti-tumor response and promoting the immune escape of NPC.[45–47] The Tregs population in NPC tissue is related to the PD-1 positive population in $CD8^+$ T cells, suggesting that the expression of PD-1 on Tregs and $CD8^+$ T cells together inhibit tumor immunity. Fogg et al[48] were able to restore the EBV-specific $CD8^+$ T cell response by removing Tregs or blocking their inhibitory function, confirming that Tregs are an important part of immune escape in NPC. It can be seen that Tregs are a T cell subset with immunosuppressive function, which represents an effective mechanism of inhibition of anti-tumor response.[49] Transcriptional factor forkhead box protein P3 (FoxP3), an important nuclear transcription factor that determines the function of Tregs, is currently recognized as a specific marker of Tregs.[50] Tregs ($CD4^+CD25^+FoxP3^+$) are a subgroup of $CD4^+$ cells that have an inhibitory effect on CD8 effector T cells and CD4 helper T cells in NPC.

The mechanisms of immune escape mediated by Tregs include: (1) After contacting with immune effector cells, Tregs directly inhibit activated effector T cells through the interaction between their surface receptors and ligands, such as CTLA-4, and then suppress immune function; (2) Tregs directly or indirectly kill effector T cells and antigen-presenting cells (APCs) through perforin and granzyme B, and so on. (3) CTLA-positive Tregs induce APC to express indoleamine 2,3-dioxygenase (IDO), then IDO inhibits T cell activation by reducing tryptophan degradation[51,52]; (4) Tregs or other cells secrete immunosuppressive factors, such as IL-10 and TGF-β, thereby interfering with antigen presentation and inhibiting the proliferation of immunoreactive cells.[48] It has been reported that the frequency of Tregs in peripheral blood increases in patients with several types of tumors, and their aggregation in the TME may be a poor prognostic factor for some types of malignant tumors.[53] The increasing number of Tregs in the peripheral circulation and in the tumor has also been shown to be associated with the progression of the tumor.[54,55] At the same time, there was a significant difference in Treg levels between patients in remission and relapse at the time of initial diagnosis. The level of Tregs in peripheral blood is highly correlated with the possibility of early recurrence in NPC patients. The increase in the number of Tregs not only interferes anti-tumor immune response but may also be a major obstacle for anti-cancer immunotherapy and active vaccination. Although great progress has been made in the treatment of NPC patients, regrettably the survival rate of NPC did not significantly improve over the past 20 years.[56]

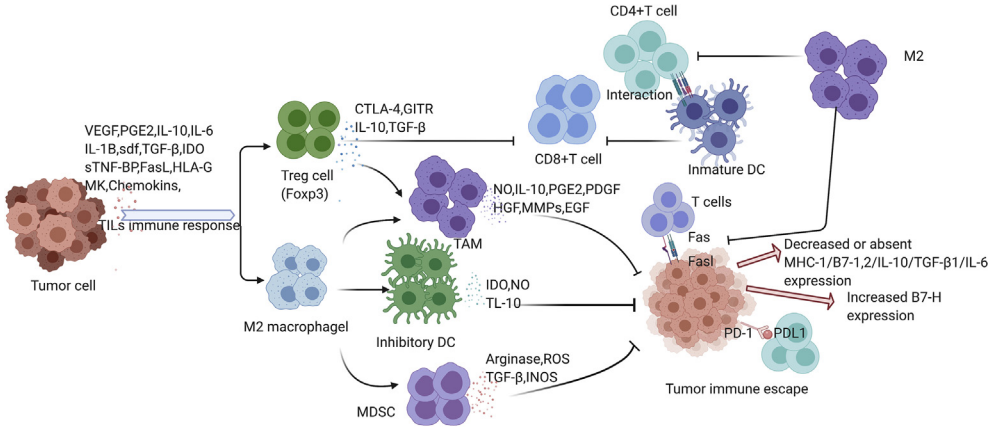

Figure 2 Tumor immune escape. The mechanisms of tumor immune escape include the following points: (1) the production of immunosuppressive cells (Tregs, TAM, MDSC, inhibitory DC); (2) the changes in the expression of tumor cell surface markers (low expression of MHC molecules in tumor cells; lack of presentation function of MHC class I molecules; apoptosis of lymphocytes mediated by Fas system; abnormal expression of B7 molecules in tumor cells); (3) lack of co-stimulatory molecules (ICAM-I, IFA-3, VCAM-1, HSA) in tumor cells; (4) tumor-derived soluble immunosuppressive factors (TGF-β, IL-10, PGE2, B7Hs, chemokines, IDO and other metabolic enzymes). In the process of tumorigenesis and development, tumor cells can escape from the immune surveillance of the body through a variety of pathways. Among them, it's considered that the main mechanism of tumor immune escape is to promote the chemotaxis of immunosuppressive cells into the tumor microenvironment to escape the immune response of the body. These immunosuppressive cells and their secreted cytokines can inhibit the anti-tumor immune response. $CD8^+$, cytotoxic T cells; $CD4^+$, T helper cells; NK, natural killer cells; DC, mature dendritic cells; Treg, regulatory T cells; MDSC, myeloid-derived suppressor cells; M, macrophage; TAM, tumor-associated macrophage; VEGF, vascular endothelial growth factor; PGE2, prostaglandin E2; IL-10, interleukin-10; IDO, indoleamine 2,3-dioxygenase; TNF, tumor necrosis factor; Fas, Fasrymndrit; HLA-G, human leukocyte antigen G; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; PDGF, platelet-derived growth factor; HGF, hepatocyte growth factor; MMPs, matrix metalloproteinases; EGF, epidermal growth factor; B7—H, serum soluble B7—H protein; TGF-β, transforming growth factor-β; INOS, inducible nitric oxide synthase; MHC-1, major histocompatibility complex-1.

At present, many studies have proved that there are a large number of Tregs in peripheral blood and within the draining lymph nodes of NPC patients, especially in local tumor tissues. These Tregs are closely related to the patients' clinical outcomes. In most solid tumors, the number of Tregs is negatively correlated with tumor progression and prognosis. In recent years, enhancing the immune status of patients by inhibiting the number or function of Tregs has become a hot research area in immunology, and Tregs are expected to become important targets of immunotherapy.

TILs in NPC prognosis

The presence of TILs is increasingly regarded as an important biomarker of head and neck squamous cell carcinoma (HNSCC). Consistent observations using various techniques showed that higher levels of TILs in the TME are associated with improved results. Many studies have investigated the prognostic value of TILs in NPC patients, but the exact role of TILs in different subtypes of NPC needs to be further clarified.[57] At present, the TNM stage (for tumor, lymph node and metastasis) and the DNA load of EBV are the main tools to determine the treatment strategy and evaluate the prognosis of NPC. However, anatomical information based on the staging system is not sufficient to obtain an accurate and definite prognosis of NPC patients. This is because treatment methods for NPC patients with the same TNM stage may be similar, while their clinical efficacy is quiet different.[58] Recently, it has been confirmed that TILs can distinguish the prognosis of different TNM stages.[59] TILs can reflect the immune heterogeneity of NPC. The distribution and number of TILs subtypes are strong prognostic factors that complement or even surpass the pathological criteria alone. Larger number of TILs in NPC patients was significantly correlated with better disease-free survival (DFS), overall survival (OS), distant metastasis-free survival (DMFS) and local-regional relapse-free survival (LRRFS).[57] It has been proved that TILs is the strongest independent predictor of DFS.[58] At the same time, new evidence suggests that TILs can predict immunotherapy responses, including CTLA-4 and PD-1/PD-L1 antibodies.[60] The current results further support the important prognostic role of TILs in NPC, and shows much potential of TILs using immunotherapeutic modalities in NPC treatment.

Development of immune score and TNM-immune (TNM-I) in NPC

TNM staging system is widely used to reflect the progress and predict prognosis of NPC. However, the TNM staging system does not take into account the characteristics of the immune system or the immune characteristics of the tumor infiltration area.[61] The prognosis of NPC patients receiving similar treatment in the same stage is quite different. Therefore, the TNM system may be inadequate to assess the status of the entire NPC patients or guide treatment. At present, immune score has become a useful clinical prognostic marker for a variety of cancers, such as colorectal cancer, non-small cell lung cancer and so on.[62–64] Recent studies have shown that the immune score of cancer patients has prognostic value in some cases and appears to be superior to AJCC/UICCTNM classification. Galon et al[65,66] developed a special diagnostic tool called TNM-I to quantify *in situ* immune infiltration. It's proved that immune score is highly repeatable, objective and robust when quantifying specific T cell subsets in specific tumor regions.[63] The positive prognostic value of immune score has been proved to be consistent in many cancers.[59] Immunohistochemistry was used to detect the total number of $CD3^+$ and $CD8^+$ T lymphocytes in the tumor core and tumor infiltration, from I0 (low density, neither region has two cell types) to I4 (high immune cell density at both sites), which is quantified by "TNM-I" method. The study shows that immune score has an advantage in predicting survival compared with TNM staging system.[59]

Immune score is a prognostic tool for quantifying *in situ* immune infiltration, and it shows great prospect as a supplement to TNM classification of various tumors. Part of the explanation for the statistical advantage of immune score may be that the density of $CD8^+$ T cell infiltration is negatively correlated with T stage in non-recurrent tumors. While in recurrent tumors, $CD8^+$ T cell density is lower, even in minimally invasive cancers.[67] Immune score based on the density of certain lymphocyte populations (CD3, CD8 and CD45RO) can predict the risk of individual death, disease progression and distant metastasis. Based on the threshold, each patient was given a binary score (0 for low, 1 for high) for each type of immune cell ($CD3^+$, $CD8^+$ and $CD45RO^+$) in the tumor and stroma. Immune score for each patient is obtained by adding six binary scores; the range is 0—6. Seven patient groups are defined: T cells in the tumor and stromal regions of $CD3^+$, $CD8^+$ and $CD45RO^+$ patients are classified as IS-0; one marker is classified as IS-1; and patients with two, three, four, five and six high density regions of the three markers are classified as IS-2, IS-3, IS-4, IS-5 and IS-6, respectively. The high degree of immune cell infiltration (3—6) is the high infiltration group,[68] and the low degree of immune cell infiltration (0—2) is the low infiltration group. The OS, DFS and DMFS of NPC patients with high immune score were significantly longer than those with low immune score. Studies have shown that evaluating the immune score of NPC patients could be an initial step for patient treatment. For NPC patients with low immune score, initiating therapy combined with immune checkpoint blocking inhibitors may be helpful. However, for NPC patients with high immune score, the expression information of immune checkpoints should be detected and appropriate immune checkpoint blocking inhibitors should be selected as monotherapy or combination therapy.[69,70] Combined with immune score, the expression of immune checkpoints can guide immunotherapy. This strategy is more suitable for NPC patients.

Immune score, as a marker of cancer classification, has dual advantages: firstly, it seems to be the strongest prognostic factor for DFS and OS, especially in early cancer; secondly, it provides a useful target for new treatments in NPC.[68] However, tumor infiltrating lymphocytes presented by several independent TIL-related factors are related to the prognosis of advanced NPC. The exact immune scoring system of NPC has not yet been established. There are still some challenges in the establishment of immune core, such as the standardization of immunohistochemical staining

procedure and scoring system, but the biggest challenge may be the heterogeneity of TILs in tumors. At present, as a supplement to TNM classification, there have been national and international initiatives to treat TIL infiltration in tumors. We hope to establish an immune score model for NPC patients to determine which patients are likely to benefit most from clear radiotherapy and chemotherapy, so as to provide reliable estimates of death, disease progression and distant metastasis.[68]

TILs predict survival in NPC

Although recent advances in radiotherapy techniques have improved clinical outcomes, a considerable number of patients with LA-NPC will eventually relapse. Once patients are diagnosed with a recurrent disease, it's important to determine reliable prognostic factors so as to estimate the probability of recurrence of locally-advanced NPC (LA-NPC). Recent research showed that in the process of the tumor genesis and treatment, the composition and number of TILs subsets and their location in the TME of NPC were closely related to the patients' prognosis, and there may be significant differences depending on the location and extent of the tumor.[18] According to Kaplan—Meier curves, the density of total TILs, $CD3^+$ TILs and $CD8^+$ TILs can significantly distinguish the survival time of NPC patients.[16] Wang et al[58] used H&E-stained sections to study total TILs in NPC. It was found that it's a strong correlation between total TILs and the prognosis of the disease. We have shown that low density of $CD3^+$ TILs is associated with a good prognosis for short DF and OS in locally LA-NPC. In short, $CD3^+$ TILs abundance is a potentially robust and independent prognostic factor for LA-NPC patients receiving standard chemotherapy treatment.[16] The number of $CD8^+$ cells is positively correlated with survival in the NPC.[68] In principle, $CD8^+$ TILs requires $CD4^+$ TILs to release cytokines, such as IL-2, to promote its proliferation. Thus, $CD8^+$ TILs predicting favorable prognosis may be due to widespread infiltration of $CD4^+$ TILs.[15] The greater the number of cells, the worse the prognosis, which is basically consistent with the previous studies of NPC. In addition to the traditional Th1 and Th2 helper T cell subsets, Tregs and Th17 cell subsets were also found in the total number of $CD4^+$ T cells. The new feature of Th17 cells is that they can secrete IL-17, IL-22 and IL-21 to promote the inflammatory process.[71,72] Although the number of IL-17^+ TIL seems to be associated with better OS and PFS in NPC patients, the association is not statistically significant.

In recent years, the hypothesis that the ratio between different subgroups can better predict the prognosis has attracted wide attention. The most commonly used TILs subset ratios are $CD8^+$/$FoxP3^+$ (CTL/Treg) and $CD8^+$/$CD4^+$. There are significantly statistical differences in these ratios, but there is no significant difference in survival rate. Other studies found that the $CD8^+$/$FoxP3^+$ TIL subset ratio was significantly correlated with early-stage patients' OS and PFS but was not significantly correlated for LA-NPC patients,[73] suggesting that the increase in the number of $FoxP3^+$ Tregs and the decrease in the number of $CD8^+$ CTLs can inhibit tumor growth and lymph node metastasis in early NPC (the increase of $CD8^+$ TIL number was positively correlated with N stage). That is to say, Treg cells rather than CTLs play a favorable role in the anti-tumor immunity of NPC patients. Some studies have even found that in several solid cancers such as ovarian cancer and cervical cancer, the high number of tumor-infiltrating $Foxp3^+$ Treg cells is related to poor survival.[48,74—76] However, these Treg subsets are reported to be unstable in the body and can be transformed into IL-17 secretory cells.[77—79] IL-17^+ TILs has no significant correlation with the clinicopathological characteristics and survival time of NPC patients. Since it's difficult to determine the general function of Treg cells in all types of cancer, the study suggests to analyze the function of Treg cells in different malignant tumors. Currently, the study evaluates the functions and related mechanisms of $FoxP3^+$ Tregs extracted from TILs of NPC patients *in vitro* and animal experiments.

The study has reported that OS and DFS of NPC patients with dense tumor infiltration of $CD3^+$ and $CD8^+$ T cells are significantly longer. An interesting finding is that the $CD3^+$ or $CD8^+$ lymphocyte density in the infiltrating marginal zone has nothing to do with the prognosis, but the patients with higher $CD3^+$ or $CD8^+$ lymphocyte density in the tumor center have a better prognosis than those with lower density.[80] Therefore, this result means that the immune score criteria for squamous cell carcinoma and adenocarcinoma may be different. High immune score based on the density of specific lymphocyte populations (CD3, CD8 and CD45RO) suggests the prolongation of OS, DFS and DMFS. The DFS of advanced NPC patients with high immune score is similar to that patients in the early stages of the disease, while patients with low immune score are observed to have a worse prognosis than early NPC patients.[68] This means that the immune score is a strong independent predictor of OS, DFS and DMFS in NPC patients and can predict individual death, disease progression and distant metastasis risk. Galon and his colleagues[68] had demonstrated that the immune score was highly repeatable, objective and robust in quantifying specific T cell subsets.

In addition, stratified analysis provides some clues, such as sample size and follow-up time, that may affect the results of $CD3^+$ TILs and $CD8^+$ TILs studies. Smaller studies of $CD3^+$ and $CD8^+$ TILs produce more dramatic hazard ratios than larger studies. In addition, the number of patients and the follow-up time seem to affect the results; studies with low numbers of patients and longer follow-up periods are unlikely to produce statistically significant results. Infiltration of $CD68^+$ monocytes and macrophage subsets is usually associated with poor prognosis, although this has not been shown to be statistically significant.[81] The evidence shows that cytokine and prostaglandin crosstalk can affect the number and function of TILs, especially $FoxP3^+$ Tregs. The activation of CTLs is regulated by complex immune checkpoints in the TME.[82—85] However, some studies also indicate that the correlation with prognosis is affected by the type of treatment and the stage of the disease. Different treatments suggest that the immune system may be particularly important in chemotherapy and radiotherapy, which may be due to the fact that effective cell reduction with these treatments involves the immune system. Although not reported in NPC, TILs have been shown to predict the response of breast cancer to neoadjuvant chemotherapy.[86,87]

The greater the number of TILs, the better the outcome, but this effect is more pronounced in studies of binding lymphocyte ratios. However, it's not clear whether immunotherapy can improve the prognosis of low TILs in patients with LA-NPC. Once a standardized method for evaluating TILs is developed, this indicator may be very valuable in predicting disease progression in NPC patients. It's worthwhile to conduct further prospective studies to determine the prognostic value of standardized TILs evaluation and evaluate whether they should be routinely included in the primary pathological report of NPCs as a potentially useful biomarker (Table 1).

TIL immunotherapy in NPC

At present, radiotherapy alone and concurrent chemoradiotherapy are important treatments for NPC. Compared with the good prognosis of NPC patients in the early stage of disease, the incidence of failure of LA-NPC treatment is still high.[101] Although concurrent chemoradiotherapy (CCRT) can improve the treatment outcome of these patients, about 25% of LA-NPC showed poor prognosis after receiving CCRT treatment.[102] It's reported that adjuvant chemotherapy in addition to CCRT does not significantly improve the survival rate of LA-NPC patients compared with CCRT alone, and the compliance of NPC patients is not satisfactory due to severe toxicity.[103–103] Therefore, It's urgent to find new strategies to improve DFS and reduce treatment-related toxicity. New data from the NPC immunotherapy study reveals its potential anti-tumor efficacy. The unique immune environment of EBV-related NPC provides a reasonable target for immunotherapy (Fig. 3). People are getting to know the virology of EBV and the immune escape mechanism of NPC. Immunotherapy strategies have shown encouraging efficacy and safety results in early clinical studies. To date, phase I and II studies involving adoptive metastasis of autologous EBV-specific cytotoxic T cells (EBV-CTL) have been shown to

Table 1 The categories and biological functions of TILs.

TIL types	Mechanism	Biological effect	Prognosis
$CD3^+$ lymphocyte	The surface marker of CD3 cell and TCR form the TCR-CD3 complex.	Transduce antigen signals into the cells.	Good prognosis[15,25,88]
Th1	Activated by reacting with polypeptide antigens presented by MHCII; secrete cytokines such as IFN-γ, TNF- α, and IL-2	Mediate cellular immunity, enhance the killing ability of NK cells and cytotoxic T cells; inhibit the proliferation of Th2.	Good prognosis[25,89,90]
Th2	Secrete cytokines such as IL-4, IL-5, IL-6, IL-10, and IL-13	Angiogenesis, tumorigenesis, immune regulation	Poor prognosis[91]
Th17	Secrete cytokines such as IL-17, IL-6, TNF-α	Promote the inflammatory response in tissue.	Ambiguous[91,92]
$CD45RO^+$	Regulate the signal transduction process after TILs are stimulated	Promote the development and maturation of lymphocytes	Good prognosis[68]
$CD20^+$ B	Regulate the flow of calcium ions into B cells and participate in BCR signal transduction; participate transmembrane calcium flow	Regulate B cell proliferation and differentiation.	Good prognosis[92]
$CD8^+$ Cytotoxic cell	secrete perforin, granzyme and other substances; Combine with the receptors on the surface of target cells by expressing FasL or secreting TNF-α, respectively	Induce apoptosis of target cells.	Good prognosis[15,25]
$FoxP3^+$ Treg	Secrete cytokines such as IL-2, IL-4, TNF-α, IL-17, IL-10, IFN-γ	Inhibit the function of effector cells or effector molecules.	Poor prognosis[37,73,94]
CD56 NK	Recognize NKG2D homodimers and natural cytotoxic receptors	Cytotoxic functions without prior sensitization	Good prognosis[11,95,96]
M1	Express CD163, CD206 and CD204 markers, and secrete Th2 cytokines	Promoting anti-tumor TH1 and TH17 immune responses	Good prognosis[97]
M2	Secrete TH1 cytokines such as TNF-α, IL-12, and small amounts of IL-10 and IL-4	Supporting angiogenesis, tumor progression, and metastasis;	Poor prognosis[36,98]
DC	Activate the initial T cells and $CD8^+$ T cells and secrete a variety of cytokines and chemokines	Binary immune regulatory function shaped by TME.	Ambiguous[99]
MDSCs	Differentiating into TAMs, secreting cytokines (TGF-β/IL-10) and inhibiting T cell activation	Promote tumor growth	Poor prognosis[23,100]

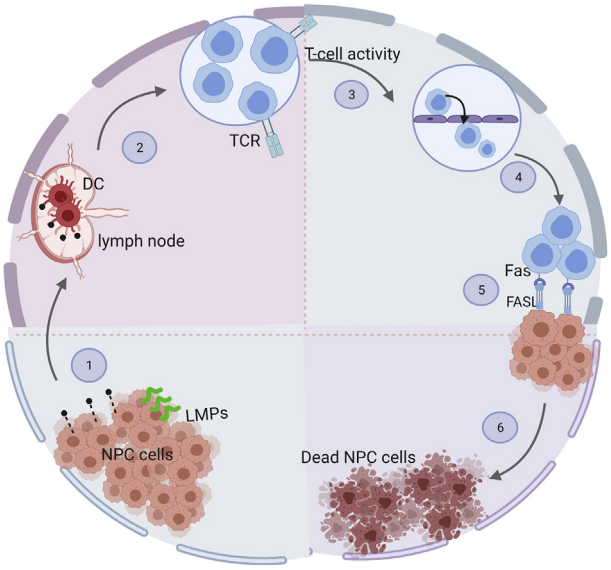

Figure 3 NPC-immunity cycle. 1) NPC cells express and release antigens; 2) Presentation of NPC antigens on the major histocompatibility complex class (MHC) by antigen-presenting cells; 3) Recognition of NPC antigens on the MHC by the T cell receptor, initiating and activating TILs; 4) TILs infiltrating to the NPC site; 5) Recognition of NPC antigens on the MHC within NPC; 6) Attack on NPC cells, resulting in NPC cell injury/death.

be safe and potentially effective for EBV-associated malignant tumors, including NPC.[9,106,107] The evidence indicates that TILs selected for tumor identification and massive amplification *in vitro* is particularly effective in the treatment of NPC patients.[108]

At present immunotherapy of NPC is one of the important methods of tumor biotherapy. The core idea is that the occurrence of tumors is due to the suppression of the auto-immune system so that cancer cells can not be eliminated. Removing endogenous auto-immune suppression with immune checkpoint inhibitors[109] or exogenously supplementing immune cells won't be suppressed through adoptive immunotherapy. It's possible to treat or even cure cancer. TILs immunotherapy has aroused people's interest, mainly because the lymphocytes that infiltrate into the tumor are more meaningful in anticancer effect than circulating lymphocytes and more accurately reflect the immune response state of the interaction between host tumors. They are autologous lymphocytes, there is no immune rejection. The results of experimental study and preliminary clinical application show that the body can tolerate a therapeutic dose of TILs without serious side effects. Various types of immunotherapy have been actively investigated in recent years, including adoptive immunotherapy, therapeutic vaccines, immune checkpoint inhibitors, solution-induced therapy and viral immunotherapy. Since latent EBV infection provides a unique immune target for NPC cells, adoptive T cell therapy targeting EBV antigens is promising in the treatment of NPC. The principle is to use immune effector cells to recognize immunogenic antigens so as to activate or enhance the immune system. For example, immunotherapy with autologous TILs has become a powerful treatment option for patients with metastatic melanoma.[110] In recent years, adoptive autologous T cell immunotherapy in NPC patients has used the EBV antigen-reactive T cells from patients' peripheral blood mononuclear cells (PBMCs),[111] including EBV-CTLs, and E1-LMPpoly-generated T cells and T cells transduced with the EBV antigen-specific TCR gene.These T cells can survive in a patient's tumor tissue, vary depending on the tumor-associated virus antigen of each patient and reduce the plasma EBV load in the body. The current phase I clinical trial evaluates the adoptive immunotherapy of NPC with concurrent chemotherapy and radiotherapy (CCRT) for the locally advanced EBV-related NPC patients. Amplified TILs are used to assess the safety of radiotherapy and antitumor activity, and evaluate toxicity, survival, clinical, and immune responses in all treated patients.[112] TILs from NPC patients has high frequency of $CD4^+$ T cells and always produces high frequency $CD4^+$ IFNγ-releasing cells in response to EBNA1 stimulation,[113] which can induce the expansion of EBNA1-specific T cells *in vivo* and may contribute to tumor regression as shown in clinical evaluation. According to studies, the clinical benefits to patients don't depend entirely on the number of TILs and $CD8^+$ T cells.[47] A number of clinical studies have explored the efficacy of EBV-specific cytotoxic T lymphocytes in patients with refractory NPC. It can be observed that the EBV-DNA copy number decreases and the CTL level increases in these clinical studies.

It's worth noting that a significant advantage of TILs therapy is that compared with the single specificity and limited coverage of major histocompatibility complexes of newer TCR and chimeric antigen receptor transduction techniques, TILs have a wide range of recognition characteristics for all possible major histocompatibility complexes, rather than a single specificity. For example, double-positive ($CD3^+CD56^+$) T cells induced by cytokines can kill tumors, and they are not restricted by MHC.[114] At the same time, IL-2 is involved in the proliferation and activation of T cells. After chemotherapy, NPC patients tolerated TILs combined with IL-2 injection well, and there was no treatment-related death, infection, fever, or allergic reaction. Therefore, T cells can rapidly expand in cultured condition containing IL-2, which is consistent with the previously reported TILs combined with IL-2 injection for the treatment of NPC.[80]

Although TILs therapy has shown some clinical success, it still has some limitations. Its generation from peripheral blood is complex, laborious and lengthy (12—16 weeks). During this period, NPC patients may have developed and become unsuitable for infusion. The main problem is that lymphocytes injected into the body have a short time to work. The number of TILs increases significantly after one week of infusion (the increase of LMP-1, LMP-2 and EBNA-1 specific T cells can be detected), but it reduces to the normal range within 6 months after infusion. In the small-scale phase 1 study, the researchers cannot find a statistical correlation between the clinical benefit and the number of EBV antigen-specific T cells in the peripheral blood of treated patients.[108] At the same time, mild adverse events consistent with immune-related reasons are also observed, including grade 3 neutropenia, some patients experience

DFS of more than 12 months and objective anti-tumor reactions.[108] Therefore, Li and his colleagues are studying the use of TILs for rapid expansion (4—5 weeks). The advantage is that TILs contain high rate of EBV antigen-specific T cells. The expansion process is relatively efficient and can infiltrate into TME. Further modification of T cell expansion methods to enhance their homing and cytotoxicity capability may pave the way for clinical application.

In the phase I clinical study of cancer adoptive cell therapy (ACT) with autologous TILs in patients with local recurrence and metastasis of NPC,[115] the studies did not clearly define the results and efficacy of TIL immunotherapy. Getting more about immunological mechanism of EBV-induced NPC carcinogenesis and its interaction with the host immune system will allow the improvement of immunotherapeutic strategies. While the success of this approach has so far been limited to melanoma patients, we still have a long way from offering this treatment to patients with NPC.[108] In short, TILs-based immunotherapy strategies related to EBV are still in the early stage. Large, randomized phase II studies are needed to further determine the efficacy of TIL immunotherapy and support further clinical studies on the use of *in vitro* amplified TIL in the treatment of NPC patients.

Conclusion and perspective

Numerous studies have confirmed the role of TILs in the immune microenvironment. The study emphasizes that TILs are not only a powerful independent predictor of DFS in NPC patients, but also an important regulator of NPC progression. TILs are considered as an auxiliary way of TNM classification to improve the diagnosis, treatment and prognosis of patients. However, some studies have reported the association between lymphocytes and OS or DFS, the exact role of TILs in different NPC subtypes has not been further elucidated. There is no consensus on how lymphocyte infiltration affects tumor progression and prognosis. Therefore, the main objective of clinical studies is to evaluate the role and function of TILs in NPC and to improve the clinical benefit.

In summary, as a new therapeutic target of NPC, adoptive therapy with TILs combined with traditional therapy (such as surgery, radiotherapy and chemotherapy) and emerging therapy (immune checkpoint inhibitors and genetic modification) is the direction of clinical treatment in the future. It's highly necessary for us to further study the subsets and functions of TILs in NPC, which is the basis for developing new approaches to tumor immunotherapy.

Conflict of interests

The authors have no conflicts of interest to declare.

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