Review Article

Immunotherapy for nasopharyngeal cancer—a review

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Abstract: Nasopharyngeal carcinoma (NPC) is associated with the Epstein-Barr virus (EBV) and characterized by peritumoral immune infiltrate. Advanced NPC has high lethality. Immunotherapy directed against EBV antigen targets has been previously explored in clinical trials, and is likely to be validated as an important target in NPC as randomized data emerges in the future. Cancer vaccines and adoptive T cell therapy have been explored in the clinic, with the latter showing the greatest success. Recent advances in gene sequencing technology now allow personalized tumor epitope mapping, whilst the advent of immune checkpoint inhibitors targeting the PD-1/PD-L1 axis offers the opportunity to activate adaptive T cell response *in vivo*. Anti-PD1 antibodies have shown promising activity in early phase clinical trials, and randomized studies against chemotherapy are underway. As immunotherapy is incorporated into standard treatment paradigms, issues of optimal combinations with targeting agents, immune adjuvants, and sequence with chemotherapy and radiation therapy will need to be addressed. Effective strategies to increase tumor antigenicity, improve immunological memory and reduce immune escape, will need to be developed to improve treatment outcomes. Here we present a brief history of the evolution of immunotherapy in NPC, and highlight key concepts relevant to its further development in the clinic.

Keywords: Nasopharyngeal cancer (NPC); Epstein-Barr virus (EBV); immunotherapy; review

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Introduction

Nasopharyngeal cancer (NPC), an Epstein-Barr virus (EBV) associated disease, has a distinct etiology and geographic distribution. It is rare in the West with incidence of less than 1 for every 100,000 people each year, but endemic in Southern China, Hong Kong, Taiwan, and Southeast Asia, where annual incidence reaches as high as 25–50 cases per 100,000 per year. Worldwide, there are 80,000 incident cases resulting in an estimated 50,000 deaths annually (1). NPC is a chemosensitive disease and 5-year survival rate in early Stage I and II disease exceeds 80%, but outcomes are very poor in stage IV disease where the 5-year survival rate is less than 10% (2). Although the disease is highly sensitive to chemotherapy, resistance invariably develops and better treatments are urgently needed (3,4). The Epstein-Barr virus (EBV) latently infects more than 90% of the world's adult human population and its association with NPC is thought to be mediated by an interplay of environmental (dietary, smoking, co-infectious) factors and genetic predisposition (high risk HLA allotypes). In NPC, the EBV virus expresses a type II latency program and is present in virtually all poorly differentiated and undifferentiated non-keratinising (WHO type II and III) NPC. The expression of viral antigens in NPC makes this disease an attractive target

for immunotherapy strategies such as virus specific adoptive cell therapies. Here in this review, we summarize the range of EBV-related and unrelated antigenic targets, and discuss the crucial role of the immune-suppressive microenvironment in NPC. Significant clinical trial data for cancer vaccines and adoptive T cell therapy trials are outlined, and we explore the potential role of immune checkpoint inhibitors in NPC, and their potential combinations with conventional chemotherapy and radiation therapy. Given the disappointing clinical outcomes of all manner of targeted therapies in advanced NPC, rational immune oncology strategies become all the more crucial.

Targets for immunotherapy in nasopharyngeal cancer (NPC)

Epstein-Barr virus (EBV) targets

EBV is associated with a variety of malignancies including Hodgkin Disease, Burkitt's Lymphoma, and NPC, with the expression of viral proteins on the tumor cell surface. The pathophysiological mechanisms involved in EBV integration with the host cell genome, latency, and transformation—is complex and incompletely understood. From a therapeutic standpoint, NPC expresses an array (albeit a limited repertoire) of EBV antigens (5,6). Hence, immunotherapeutic strategies in NPC have historically focused on EBV specific epitopes as a means of targeting this cancer.

EBV-associated NPC expresses a type II latency program, and tumor cells typically express the latent membrane proteins 1, 2A, and 2B (LMP1, LMP2A, and LMP2B), EBV nuclear antigen 1 (EBNA1), all of which have limited immunogenicity. In addition, several EBV non-coding RNAs primarily EBER1 and EBER2, and BamHI-A rightward transcripts (BARTs) and BamHI-A rightward frame 1 (BARF1) of EBV are expressed abundantly and are detected consistently in NPC (7-12).

EBNA1 is expressed frequently in NPC and is a dominant target for CD4 T cells. LMP1 and LMP2 are expressed in approximately 50% of NPC tumors. LMP1 may be poorly immunogenic, while the LMP2 proteins sufficiently more immunogenic and hence putative targets for EBV directed immunotherapy, such as cytotoxic T cells (8,13-15). NPC occurs in immunocompetent individuals, and it is likely that immunological pressure results in the expression of a limited array of EBV antigens. These proteins maintain cellular transformation in malignant cells and their poor immunogenicity likely plays a role in promoting immune escape by EBV-positive malignant cells (16,17). Immunotherapeutic approaches employed to target EBV are dependent upon the capacity to generate an immunological response against EBV latency response antigens, however in human hosts, these antigens have also co-evolved to evade immune recognition. LMP1 and LMP2 are known to play a role in activating and transforming cells following infection, allowing proliferation and survival of latently infected cells (18,19). The LMP antigens are oncogenic. LMP1 is a major transforming protein and behaves as a classical oncogene (20,21). LMP2A and LMP2B are likely non-essential in B cell transformation *in vitro* (22). However, LMP2A can transform epithelial cells via activation of the PI3 kinase-Akt pathway (23). These LMP antigens, particularly LMP1, are poorly immunogenic, likely due to poor antigen processing in infected cells and the subsequent limited amount of antigen available for presentation by MHC class I molecules (17). As a consequence, the LMP antigens, particularly LMP1, generate a subdominant CTL response when compared to the responses generated against lytic cycle antigens and other latent antigens, such as EBNA3 (24).

EBNA1 can be detected in all EBV-associated malignancies (15). EBNA1 is highly stable and contains a glycine-alanine repeat sequence near its N-terminus that inhibit translation and subsequent self-replication (25-27) and as result, EBNA1 is processed poorly via the MHC class I pathway. Nevertheless, the demonstration of EBNA1-specific CD8+ CTL thought to be induced via cross-presentation by professional antigen presentation cells rather than via direct recognition of infected cells (28), has established that endogenously processed EBNA1 can be detected by CD8+ T cells (29-31).

NPC cells have preserved antigen-processing function and can be recognized by major histocompatibility complex class I–restricted virus-specific CTLs *in vitro* (32), However, downregulation of major histocompatibility complex class I peptide expression is seen in NPC tumors as an immune evasion strategy (33). NPC patients also appear to have a lower prevalence of T cells that can recognize HLA-restricted epitopes in LMP2 and EBNA (34). Epidemiological studies have suggested that certain HLA allotypes have higher associations with nasopharyngeal carcinoma. These include HLA-A*11:01 and HLA-A*02:27 (35,36). A molecular explanation that cysteine at codon 99 of the Alpha2-helix of HLA-A protein is deleterious suggests a possible locus of susceptibility to NPC (36). Hence while NPC occurs across a variety of HLA allotypes, a meaningful strategy would

be to focus initial HLA specific strategies in allotypes with demonstrated susceptibilities such as HLA-A*11:01.

Taken in entirety, NPC-related EBV antigens LMP1, LMP2A/B, EBNA1, EBER, and EBV-encoded RNA each have distinct effects on growth, differentiation, and the host immune response. Collectively, they likely contribute to the development of NPC through the promotion of transformation and angiogenesis, inhibition of apoptosis, induction of stem-cell-like phenotype, and enhancement of cell motility. EBV antigens also aid in immune escape through various mechanisms, including switching off immunodominant viral antigens, impairing the HLA I or HLA II pathway, up-regulating immune-inhibitory molecules, and recruiting T regulatory cells and inducing T-cell anergy (37). Hence, an understanding of the virus–host interaction in the NPC environment is essential for successful EBV-targeted immunotherapies. Selection pressure-driven evolution constantly stimulates the emergence of new EBV variants (38,39) which may be more oncogenic and less immunogenic than the parental strain, with for example a higher tropism for epithelial cells rather than B cells, suggesting that some EBV strains may carry an increased NPC risk (40).

It is important to note that NPC, while associated with EBV and the expression EBV proteins, is an entity that encompasses a broader range of other distinct molecular aberrations that may also represent immune targets.

Non-EBV targets

Genomic alterations in NPC represent neoantigens that may be immunogenic. Studies in this area are few given the limitation of accessible tissue for interrogation in this disease and the paucity of pre-clinical models. Nevertheless, a landmark study of comprehensive sequencing analysis of 56 NPC patients (41) has shed light on cancer mutations relevant in NPC. Nine significantly mutated genes included *BAP1*, *MLL2*, *TSHZ3*, *TP53*, *PIK3CA*, *ERBB3*, *ERBB2*, *KRAS* and *NRAS*. Copy number alterations in MAPKAPK2 have been shown to be associated with NPC risk (42). Epigenetic alteration in NPC include the CpG island methylator phenotype and a high load of hypermethylated tumor suppressor genes (43). These genomic and proteomic alterations and more, can contribute to the production of oncogenic and immunogenic alterations.

Immunogenic alterations can broadly be categorized into (I) tumor specific mutations that result in neoantigens; (II) tumor specific antigens and proteins overexpressed in tumors but not expressed or are expressed at very low levels in normal cells including proteins such as surviving; (III) lineage specific antigens expressed on tumor cells as well as on normal cells such as gp100; and (IV) cancer/testis antigens including MAGE and NY-ESO-1 (44). Emerging sequencing technologies with predictive computational algorithms now offer the possibility of developing HLA-restricted epitope maps for each tumor and the corresponding mutational landscape. These technologies will accelerate neo-antigen discovery and improve efficiency of immune targeting strategies in trials.

Immune checkpoints

PD-1 is an inhibitory receptor expressed on the surface of activated T cells. PD-1 is a known marker of T-cell exhaustion in animal models of viral infection. This manifests itself as loss of effector functions such as the secretion of cytokines (IFN-γ, IL-2, and TNF-α), production of the cytolytic effector molecules perforin and granzyme B, and eventually apoptosis (45-47). The immune infiltrates of chronic inflammation frequently employ the B7-H1/PD-1 axis. Both PD-1 ligands, B7-H1 (PD-L1) and B7-DC (PD-L2) are up-regulated in peripheral tissues during an inflammatory response to infectious agents, in response to type 1 (α, β) and type 2 (IFN-γ) interferons (48). The biologic role of this upregulation is the prevention of collateral tissue damage mediated by antigen-experienced T cells during inflammation (49-52). Other immune-checkpoint molecules such as 2B4, CD160, T cell Immunoglobulin and Mucin domain-3 (TIM3), Lymphocyte Activation Gene-3 (LAG3) are upregulated in conjunction with PD-1 on "exhausted" CD8 T cells in tumor and chronic viral models (53). Programmed cell death ligand-1 (PD-L1) is highly expressed by cancer cells and tumor-infiltrating macrophages in virus-associated malignancies including NPC (54). PD-L1 expression on tumor correlates with advanced tumor stage and lymphatic metastasis (55) while PD-1 overexpression is associated with shorter overall survival and recurrence free survival and is an independent risk factor for death, treatment failure and local recurrence of NPC (56). These early studies in NPC have added to rationale to apply immune checkpoint inhibitor antibodies to this disease.

Tumor microenvironment

NPC is characterized by substantial immune infiltrate

in the primary tumor that consists of T cells, B cells, dendritic cells, monocytes, and eosinophils. This massive lymphoid infiltrate in the primary tumor is likely favored by inflammatory cytokines produced by tumor cells (57-60). There is evidence that despite the immunogenic nature of EBV antigen expressing cancer cells, there is a marked local tolerogenic immune suppression. T regulatory cells (Treg) within the tumor site may contribute to the functional inactivation of innate cytotoxic T cell responses. Significant expansion of circulating naïve and memory CD4+CD25^{high} Foxp3+ was identified in 56 patients (61) and a smaller number was also noted to have infiltrating Treg in the tumor microenvironment. Another study of 40 untreated patients implicated the suppressive role of Treg cells with its findings of rich populations of Treg amongst tumor-infiltrating lymphocytes (TILs). A further finding in this study was that EBV-specific T cells are enriched but inactivated in the tumor microenvironment. TILs from NPC failed to produce IFN-gamma and to exert cytotoxicity when stimulated by lymphoblastoid cell lines (34). A more recent study demonstrated that both physical and pharmacologic mediated depletion of Tregs from PBMC enhances EBV-specific T cell responses in EBV-stimulated T cell lines generated from NPC (62).

A holistic immunotherapy strategy to target NPC must take into account the following:

(I) Cancer specific factors
- Genomic and proteomic differences between cancer and host, that are both EBV specific, but otherwise cancer genome specific too;
- Presence of cancer-associated antigens, that are ordinarily poorly expressed in normal tissue, including the known cancer testis antigens;
- Presence of immune-suppressive checkpoints on cancer cells;
- Immunosuppressive factors in the tumor microenvironment such as but not limited to tumor hypoxia, immune-suppressive cytokine production, the presence of myeloid derived suppressor cells, and immunosuppressive regulatory T cells;

(II) Host specific factors
- HLA Class I and II type and expression that determines presentation of peptide sequences of intracellular proteins to various subsets of immune cells;
- Immune cell population diversity and matching to tumor immune epitopes and other immunogenic cancer epitopes;
- Dendritic cell function, presentation of tumor antigens, and interaction with immune cell subsets;
- Host specific tumor permissive factors that have yet to be identified.

Immunotherapy strategies against NPC—overview

In our opinion, these strategies fall into two broad categories. The first category comprises strategies that aim to harness the host's pre-existing anti-tumor capability that may be suppressed by tumor, or to augment the host's innate ability to mount an immune response against tumor. This category of strategies assumes an innate pre-existing capacity to augment host immune response that the cancer may already have escaped, and aims to meaningfully directly impact the host immune system to mount an immune response against NPC, which represents an inflammatory cancer phenotype. Examples of these include immune checkpoint inhibitors anti-PD1, anti-PD-L1, anti-CTLA4, and anti-LAG3 antibodies to disinhibit the immune response against cancer, and cancer vaccines that attempt to stimulate and generate a host immune response.

The second category comprises therapeutic strategies that directly and preferentially target cancer cells. Chemotherapy and radiation can stimulate immunogenic cell death and this is increasingly being studied and understood for use with other immunotherapy strategies. Immune cells that target cancer cells directly include cytotoxic T lymphocytes and cytokine induced killer cells.

Host targeting agents

Immune checkpoint inhibitors

More than a fifth of patients with previously treated metastatic NPC showed an objective measurable response when treated with the pembrolizumab, according to a study reported at the 2015 European Cancer Congress. Pembrolizumab is a highly selective humanized monoclonal IgG4-kappa isotype antibody against PD-1 that is designed to block the negative immune regulatory signaling of the PD-1 receptor expressed by T cells (63). Two-thirds of patients in the study had some degree of reduction in target lesion size. The median duration of response was 10.8 months. The objective response rate with pembrolizumab in NPC was 22.2%, all partial responses. Another 15 patients had stable disease, resulting in a disease control rate of 77.8%. Forty one out of 44 patients screened for study had

tumors that tested positive for PD-L1 expression. All but 2 of the patients had received at least 1 prior line of therapy for advanced disease, and a third of the patients had received 5 or more prior regimens. Median progression-free survival was 5.6 months. A recently opened trial uses Nivolumab (BMS), another anti-PD1 antibody, to treat patients with recurrent and/or metastatic NPC (NCT02339558). LAG3 represents another immune checkpoint that may confer immune escape. There is an ongoing Phase I clinical trial evaluating the safety and efficacy of an anti LAG3 antibody, LAG525 (Novartis), as a single agent and in combination with an anti-PD1 antibody, PDR001 (Novartis), in patients with advanced malignancies (NCCT02460224). This study includes NPC in its inclusion criteria.

Cancer vaccines

Therapeutic cancer vaccines for NPC have historically targeted EBV antigens. A study using an LMP2 vaccine has been reported. Autologous monocyte-derived dendritic cells cultured from patients with NPC and matured with cytokines were pulsed with HLA-A1101, A2402, or B40011 restricted epitope peptides from EBV-LMP2, and injected into inguinal lymph nodes. This strategy generated an expansion in the LMP2-specific response in the peripheral blood in the majority of patients, and a partial clinical response in 2 of 16 patients enrolled in the study was seen (64). A more recent Phase II study evaluated the use of dendritic cells transduced with an adenovirus-DeltaLMP1-LMP2 vector given as five biweekly intradermal injections to sixteen heavily pretreated stage 4c NPC patients. This first-in-human study demonstrated the safety of this strategy. No increase was seen in the frequency of LMP1/2-specific T cells (65). Another clinical trial using the MVA-EL vaccine has provided evidence for the effectiveness of the direct administration of a poly-specific vaccine to generate LMP/EBNA1-specific CTL responses in patients. This recombinant vaccinia virus–based vaccine, which encodes a functionally inactive fusion protein containing the CD4 epitope-rich C-terminal half of EBNA1 and CD8 epitope-rich LMP2A could induce T-cell response in 80% of patients, in some cases boosting response to both CD4+ and CD8+ mediated immunity against EBNA1 and/or LMP2 (66). This vaccine is now being evaluated in a phase II trial involving patients who have detectable plasma EBV DNA after RT or who experience optimal response to palliative chemotherapy (NCT01094405).

Cancer targeting agents

Immunogenic cell death with chemotherapy and radiotherapy: concepts from studies in other cancers

Cancer cell death can be immunogenic or non-immunogenic. Immunogenic cell death (ICD) involves changes in the composition of the cell surface as well as release of soluble mediators that occurs in a defined temporal sequence. Endoplasmic reticulum stress and autophagy result in calretuculin (CRT) exposure in the outer leaflet of pre-apoptotic cancer cells. Additionally, these pre-apoptotic cells secrete ATP, and release nuclear protein HMGB1 as membranes become permeabilized during necrosis. CRT, ATP, and HMGB1 bind to CD91, P2RX7, and TLR4 respectively, facilitating the recruitment of dendritic cells in the tumor bed, and engulfment of tumor antigens by dendritic cells and optimal antigen presentation to T cells (67). Radiation is commonly used in NPC and is known to cause ICD accompanied by CRT exposure, ATP release, and HMGB1 release. The concept of immunogenic cell death may well underpin the rationale for strategies that combine standard treatments of chemotherapy, small molecule inhibitors, and radiation therapy with immunotherapy. Studies to characterize the capacity of these treatments to cause immunogenic cell death specifically in NPC are needed.

Cell based therapies

EBV is associated with several cancer namely, post-transplant lymphoproliferative disease (PTLD), Hodgkin lymphoma (HL), Burkitt lymphoma, tumors in HIV-infected patients, T cell lymphoma, NK/T cell lymphoma, gastric cancers, and NPC (5). Following primary infection, EBV persists for life as a latent infection which is controlled by cytotoxic T lymphocytes (CTL) (68). Adoptive immunotherapy was first developed for the treatment of PTLD and has now been successfully utilized for over ten years using autologous EBV-immortalized LCLs to stimulate the expansion of EBV-specific CTLs (69). CTL therapies in NPC were developed on the basis of this evidence.

CTL can be heterogeneous, primarily with regards to their differentiation status and homing properties. Following antigen encounter, a naïve or memory T cell will proliferate and acquire an increasing number of effector functions, resulting in fully differentiated effector cells which display the full array of effector functions (70,71). However, differentiation into effector cells significantly

alters the trafficking properties of the T cell (72). There is now evidence that this change in homing properties can be tissue-specific, whereby stimulation in different lymphoid organs can influence trafficking to particular peripheral tissues (73,74). Effective immunotherapeutic treatment of NPC may be dependent upon the capacity to generate CTL that can home in to nasopharyngeal tissue and other sites of metastatic disease. It also remains to be elucidated what impact the differentiation status of CTL has upon survival post-transfer. Although terminal differentiation may generate greater effector function, poor survival of these T cells post-transfer may reduce the number of cells accessing tumor sites. There is evidence that less differentiated T cells retain a greater capacity to expand following antigen encounter *in vivo* and provide greater protection following transfer (75). Therefore, treatment with non-terminally differentiated CTL may have some benefit in prolonging their survival and proliferation capacity following adoptive transfer.

Current strategies used to generate CTL that rely upon long-term *in vitro* cultures will generate cells with a late-stage effector phenotype. Lymphodepletion prior to adoptive transfer may provide another mechanism to enhance survival and proliferation of transferred CTL. In addition to the benefits associated with the removal of Treg cells, there is evidence that lymphodepletion can enhance the efficacy of CTL-based therapy by removing T cells which compete for homeostatic cytokines, such as IL-15 and IL-7, and thus creating 'space' in the lymphoid system to accommodate transferred T cells (76,77). However, some recent observations have suggested that whilst lymphodepletion may promote T cell engraftment (78) it may not improve the clinical outcome following T cell therapy (79). Our group had previously shown that a delayed graft-versus-NPC effect was demonstrable in three of 21 heavily-pretreated advanced NPC patients who received a conditioning regimen of subablative cyclophosphamide, *in vivo* T cell lymphodepletion with iv thymoglobuline and thymic irradiation followed by sibling HLA-matched and one-antigen mismatched allogeneic peripheral blood stem cells. The delayed objective responses were coincident with rising donor haematopoietic chimerism and better survivors correlated with chronic graft-versus-host disease. These results indicated to us that a potentially powerful immune alloresponse was operative against even bulky, progressing, and chemoresistant NPC disease (80).

We proceeded to conduct and complete a phase II trial exploring the role of cytoreductive chemotherapy followed by autologous CTL in previously untreated patients with advanced EBV-associated NPC. The patients received four cycles of gemcitabine and carboplatin followed by six doses of EBV-specific T cells (81).This combination therapy was well tolerated and resulted in an encouraging response rate of 71.4% with 3 complete and 22 partial responses. Moreover, the median overall survival of 29.9 months and the 2- and 3-year overall survival rates at 62.9% and 37.1%, respectively, were significantly higher than those observed in historical controls receiving chemotherapy alone (11–22 months). The study was the first in which a chemotherapy regimen followed by a planned cell-therapy is given as frontline therapy for any cancer, allowing timely delivery of adequate CTL cells following chemotherapy completion. The study also had a high overall completion rate, with 35 of the 38 enrolled patients receiving the planned consolidation with EBV-specific T cells with no attendant grade III or IV toxicities with CTL therapy. A multicenter Phase III randomized control trial (NCT02578641) using this protocol is underway.

The Italian group had previously treated ten advanced NPC patients progressing after conventional therapy, using autologous EBV-specific T cells generated from EBV-infected LCLs as antigen presenting cells to stimulate a polyclonal response to latent EBV antigens. They observed partial responses in two patients and stable disease in four others (82). The Baylor group previously observed 10 responses in 15 patients treated with active disease (5 complete responses, 2 partial responses, and 3 with stable disease) (83,84). An additional eight patients were treated in their second or subsequent remission, and five remained free of disease with follow-up of six years. Both groups have attempted to improve these results by pretreating patients with lymphodepletion using either chemotherapy with cyclophosphamide and fludarabine (79) or CD45-depleting antibodies (78) but neither added approach improved the overall response rate. In the studies by both groups, the LCL-induced EBV-specific T cells contain T cell clones that target all nine latent-cycle antigens of EBV as well as some of the virus's lytic antigens. The majority of the T cells, however, are responding to the most immunogenic antigens, including EBNA3 and the lytic-cycle antigens such as BZLF1, which are not expressed by EBV-infected NPC cells. Instead, the tumor cells express antigens associated with the type II latency pattern, including LMP1, LMP2, EBNA1, and BARF, which are less immunogenic and are present at a lower frequency in polyclonal LCL-induced EBV-specific T cells. It is therefore notable that both groups have identified an association between measurable benefit

of EBV-specific T cells and the presence in the product of LMP2-reactive clones that expand in the patient after infusion (82,83). This observation was also seen in the Phase II study reported by Chia *et al*., who showed a strong association of benefit with specificity for EBV-LMP2 in the infused line (P=0.04) (81).

Hence current studies are enriching lines for cells that recognize the EBV antigens expressed in NPC and other type II latency tumors, using either overlapping peptide pools pulsed on dendritic cells (85) or an adenoviral construct termed AdE1-LMPpoly that encodes EBNA1 fused to CD8+ T cell epitopes from LMP1 and LMP2 to stimulate T cells (86). The second approach has been tested in 16 patients with recurrent and metastatic NPC who received EBV-specific T cells generated by stimulation with AdE1-LMPpoly. After adoptive transfer, there was a transient increase in the frequency of T cells responding to LMP1, LMP2, and EBNA1. The median overall survival of these patients was 523 days, compared with 220 days in patients who did not receive T cells (86).

Currently, several novel strategies to improve the activity of CTL in NPC are being explored in clinical trials. MALTED is testing closely matched allogeneic CTL (NCT01447056), and RESIST-NPC is testing CTL cell that additionally express Dominant Negative Receptor that confers them resistance to TGFbeta, a factor secreted by cancer cells that confers immune suppression to CTL and allows immune escape (NCT02065362).

Cytokine-induced killer (CIK) cells represent a heterogeneous population of immune cells that have been expanded from peripheral blood mononuclear cells using cytokines. These have shown *in vitro* killing in a variety of cancers (87). NPC patients who received autologous CIK cell transfusion in combination with gemcitabine plus cisplatin chemotherapy had a higher overall survival and progression-free survival rates than patients with gemcitabine plus cisplatin chemotherapy (88) CIKs have also demonstrated tumor killing capacity against putative cancer stem cells of nasopharyngeal cancer, in pre-clinical models. This was demonstrated to be mediated somewhat via NKG2D–ligands as blocking by anti-NKG2D antibody significantly but partially abrogated CIK cell-mediated cytolysis against putative NPC cancer stem cells (89).

Future directions

The broad and potent responses of immune checkpoint inhibitors in a wide variety of tumors, is deepening our understanding of tumor immunogenicity and spearheading a resurgent interest in immunotherapy for NPC. As the complex interplay of EBV and NPC continues to be unraveled, it is likely that immunotherapeutic strategies will merge into mainstream clinical practice and offer durable remissions in patients with advanced NPC who are this day incurable.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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