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TUMOR IMMUNOLOGY IN HEAD AND NECK CANCERS

Therapeutic strategy for cancer immunotherapy in head and neck cancer

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Abstract

Cancer immunotherapy is one of the new prospective treatments for head and neck squamous cell carcinoma (HNSCC). This strategy can effectively induce anti-tumor effects. However, despite surveillance by the host immune system, tumor cells can develop and acquire malignant phenotypes, for example, invasion into stromal tissue, and metastasis to lymph nodes and distant organs. Tumors with oncogenic mutations and cellular heterogeneity actively suppress the host immune system with assistance from the tumor microenvironment, including regulatory T cells, myeloid-derived suppressor cells, and type II macrophages, leading to immunoediting and immunosuppression of the anti-tumor response *in vivo*. Accumulating evidence, obtained through the use of advanced immunological technology, has elucidated the interaction between tumor cells and the host immune system. Greater understanding of this interaction has given rise to new therapeutic interventions, including cancer immunotherapy. In this review, we compile recent findings from experimental and clinical studies of cancer immunotherapy and discuss whether cancer immunotherapy has been determined to be beneficial in HNSCC patients. Cancer immunotherapy, such as cancer vaccine, dendritic cell immunotherapy, and blockade of immune checkpoints, also plays a crucial role in treatments that have contributed to improving overall survival in HNSCC patients. Moreover, due to the direct improvement of tumor- or tumor microenvironment–mediated immunosuppression in HNSCC, cancer immunotherapy in combination with targeted therapy appears to be an effective and efficient therapeutic strategy.

Keywords: *immunosurveillance*; *immunosuppression*; *tumor microenvironment*; *cancer vaccine*; *dendritic cell*; *immune checkpoints*; *chimeric antigen receptor therapy*; *targeted therapy*

In Context

The advanced genetic and immunological technologies have provided fresh insights into molecular mechanisms of tumor progression in head and neck squamous cell carcinoma (HNSCC). Sufficient effector T cell activation induces effective antitumor immune response in cancer patients. However, recent researches have shown that immune checkpoints and tumor microenvironment in which regulatory T cell and myeloid-derived suppressor cells exist repress antitumor immune response. The knowledge of cancer biology and immunology helps to develop a therapeutic theory that cancer immunotherapy induces an antitumor immune response by modulating the host immune system. In addition, identification of these immunosuppressive functions has promoted the development of the next generation of cancer immunotherapy. In this review, we summarized data from experimental models and clinical trials associated with cancer immunotherapy in several types of cancer. These data indicated that cancer immunotherapy is capable of enhancing the anti-tumor immune response in patient. Moreover, we also discussed and explored the possibility of innovative cancer immunotherapy in HNSCC. This therapeutic approach may help us to improve survival rate in patients with HNSCC.

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world. Standard treatments for HNSCC, such as surgery, chemotherapy, and radiotherapy, have exhibited a high degree of therapeutic effectiveness and safety (1, 2). However, HNSCC is often diagnosed at an advanced

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stage with a poor prognosis. Therefore, breakthrough therapeutic interventions are urgently needed.

Taking advantage of identifying immunogenic tumor antigens (TAs) and TA-specific cytotoxic T lymphocytes (CTL) in cancer patients, a number of researchers and clinicians have recently focused on translational and clinical researches of cancer immunotherapy. Novel findings from these researches have elucidated a complex link between tumor cells and the host immune system at the molecular and cellular levels (3). Immunotherapeutic interventions, such as cancer vaccines, dendritic cell (DC) therapy, immune checkpoint blockade with monoclonal antibodies (mAbs), adoptive T-cell therapy, and chimeric antigen receptor (CAR) therapy, are currently being utilized in treating several types of cancers. In particular, cancer vaccines have contributed to the improvement of clinical benefits (4). However, advanced tumors and the tumor microenvironment induce immunosuppression *in vivo* and attenuate the anti-tumor effects of cancer immunotherapy (5–7). Thus, cancer immunotherapy and therapeutic targets associated with eliciting immunosuppression *in vivo* may be valuable for the treatment of HNSCC patients.

Here, we summarize several lines of translational evidences regarding cancer immunotherapy and describe its beneficial impacts on prognosis in HNSCC patients.

Cancer immunosurveillance

Cancer is caused by multi-stage accumulation of genetic instability and epigenetic alterations, often leading to loss of normal cellular regulatory systems and acquisition of malignant phenotypes (8). Recent studies using advanced genetic technology have revealed a number of tumor-specific products encoded by mutated or oncogenic genes (9). Tumor-specific products, such as cancer–testis antigens (CTAs), differentiation antigens, mutated proteins, overexpressed antigens, oncofetal antigens, and viral proteins, are collectively referred to as TAs. TAs can be further classified as tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs): TAAs are expressed on both normal and tumor cells, whereas TSAs are expressed only in tumor cells. The first human TSA was discovered in 1991 (10). TAs were discovered in several types of cancer, including HNSCC, and play an important role in direct or indirect regulation of tumor development, invasion, and metastasis (11).

According to the theory of cancer immunosurveillance, tumor cells with oncogenic mutations are actively eliminated by TA-dependent and -independent host immune responses (Fig. 1), thereby inhibiting tumor development and progression (12–14). Elimination process includes innate and adaptive immune responses to tumor cells (13). Innate immune cells, such as natural killer (NK), NKT, γδT cells, are initially activated by inflammatory cytokines (e.g., TNF-a, IL-1, IL-10) that are released by the tumor cells, macrophages, and stromal cells (13). These component cells are recruited into tumor tissue to exert cytotoxic effects against cancer cells via the perforin–granzyme B system or apoptosis involving Fas/FasL or TRAIL/death receptor (DR)-4 or DR-5 (13, 15). This process is a TA-independent anti-tumor immune response.

Under attack by innate immune cells, many TAs are released from dying tumor cells and captured by antigen presenting cells (APCs) such as macrophages and DCs (16, 17). After processing TAs, APCs load TA-derived peptides on major histocompatibility complex (MHC) class I or MHC class II; the loaded MHCs then prime and activate effector T cells (i.e., CTLs or helper T cells) in lymph nodes (18, 19). Moreover, APCs express B7 (CD80/CD86) co-stimulatory molecules to modulate the activity of $CD4^+$ and $CD8^+$ T cells (20). These molecules bind to CD28 that produces co-stimulatory signal, or to

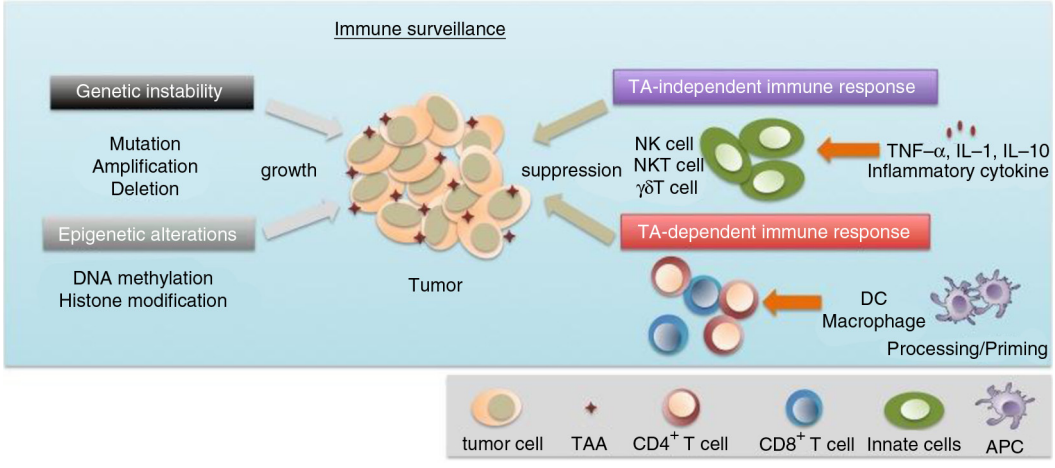

Fig. 1. Schematic illustration of immune surveillance. Acquisition of genetic instability and epigenetic alterations to drive oncogenic signals promotes tumor growth. However, anti-tumor effects induced by TAA-dependent and TAA-independent immune responses eliminate tumor cells.

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CTLA4 that produces co-inhibitory signal. Thus, role of B7 co-stimulatory molecules is context dependent (20). Activated T cells actively circulate in the blood and traffic to tumor sites. Especially, CTLs recognize cancer cells with targeted TA through interactions between T-cell receptor (TCR) and MHC class I, and then eliminate the targeted cells by triggering anti-tumor effects. Thus, the TA-dependent anti-tumor immune response causes release of additional TAs. These sequential processes are regarded as the cancer immunity cycle (3). Moreover, information about TAs is simultaneously memorized by adaptive B or T cell immunity (3, 21).

Immunosuppression

Regardless of whether it engages cancer immunosurveillance in a patient, a tumor matures and expands to deeper stromal tissues and distant organs. The process by which the tumor evades immune surveillance is known as immunoescape. This immune resistance is caused by attenuation of tumor immunogenicity, downregulation of human leukocyte antigen (HLA) class I on tumor cells, and secretion of inhibitory cytokines that repress the host immune system (13).

First, tumor immunogenicity depends on various phenotypic and functional cell populations within the same tumor. In particular, tumor immunogenicity is definitively affected by antigenicity and immunomodulatory factors derived from host immune-component cells or tumor cells. CTLs can recognize and eliminate tumor cells with strong immunogenic TA (18). However, tumor cells that have lost immunogenic TAs are less sensitive to immune attack by CTLs. In addition, HLA class I on tumor cells is down-regulated in order to allow the tumor to escape from immunosurveillance (22, 23). Thus, tumor cells can acquire immune resistance in spite of immune surveillance.

Next, tumor cells that have accumulated driver mutations, which are characterized by constitutive activation of mitogen-activating protein kinase (MAPK), signal transducer and activator of transcription 3 (STAT3), and the β-catenin/Wnt-signaling pathway, promote secretion of immunosuppressive cytokines such as transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), IL-6, and IL-10. Immunosuppressive cytokines directly repress NK cells and CTLs (24). Recent work has revealed that HNSCC also exhibits heterogeneity and immunosuppressive functions that allow it to evade the anti-tumor immune response (25, 26). In addition, mutated tumor cells in HNSCC express immunosuppressive molecules such as programmed death-ligand 1 (PD-L1), indoleaminepyrrole-2,3-dioxygenase (IDO), and tryptophan-2,3-dioxygenase (TDO) to repress both the innate and adaptive immune systems (27–29). Immunosuppressive cytokines secreted from tumor cells also attract cellular components associated with immune suppression: regulatory T cell (Treg), myeloid-derived suppressor cells (MDSCs), regulatory DCs, and type II macrophages (M2s) (30–33).

Tregs play important roles in immunosuppression *in vivo*. In several types of cancer, including HNSCC, Tregs have been demonstrated to promote immunoescape by tumor cells from the innate and adaptive immune responses by producing immunosuppressive cytokines such as TGF-β, IL10, and IL-35 (34, 35). In addition, Tregs further inhibit differentiation and priming functions of DCs, leading to downregulation of anti-tumor T cell response. Taken together, these data indicate that Tregs are pivotal immune suppressors in HNSCC (36).

The roles of MDSCs are characterized by a high arginase activity (which inhibits the T-cell response), production of immunosuppressive cytokines (TGF-β, IL-10), induction of $CD4^+CD25^+FOXP3^+$ Tregs, and upregulation of nitrogen oxide (NO) and reactive oxygen species (ROS) (37–44). In HNSCC patients, there are several populations of MDSCs (45–48). Among them, $CD14^+$HLA-DR^- MDSCs infiltrates head and neck tumor tissue and down-regulates T-cell proliferation and interferon-gamma [(IFN)-γ] secretion (48). Moreover, some immunomodulatory factors (i.e., CD86 and PD-L1) are highly expressed in $CD14^+$HLA-DR^- MDSCs relative to $CD14^+$HLA-DR^+ MDSCs in HNSCC (48). Similar to Tregs, MDSCs also have the important ability to suppress both the innate and adaptive immune systems in HNSCC.

M2s are an immunosuppressive population characterized by expression of CD68 and CD163 (49). M2s that infiltrate tumors, so-called tumor-associated macrophages (TAMs), secrete high levels of immunosuppressive cytokines (49). Recent work has shown that TAMs contribute to tumor invasion and poor prognosis (50). In addition, regulatory DCs are found in certain tumors (33). These DCs induce immunosuppression via some mechanisms: defective antigen presentation, secretion of immunosuppressive enzymes (IDO and L-arginase) and cytokines (IL-10 and TGF-β), negative co-stimulation via CD86-PD-L1, and induction of Tregs (33).

Taken together, these observations indicate that the immunosuppressive mechanism is very complex, and immunosuppression in HNSCC patients should be improved to enhance anti-tumor effect by cancer immunotherapy.

Roles of tumor microenvironment

The tumor microenvironment, which consists of several types of cellular and stromal components, is functionally interposed between tumor cells and the host immune system. In the tumor microenvironment, tumor cells primarily act to promote the formulation of vasculature via cellular signaling pathways (24). In HNSCC, angiogenesis and lymphangiogenesis are essential for supplying oxygen and nutrients into the tumor tissue, resulting in

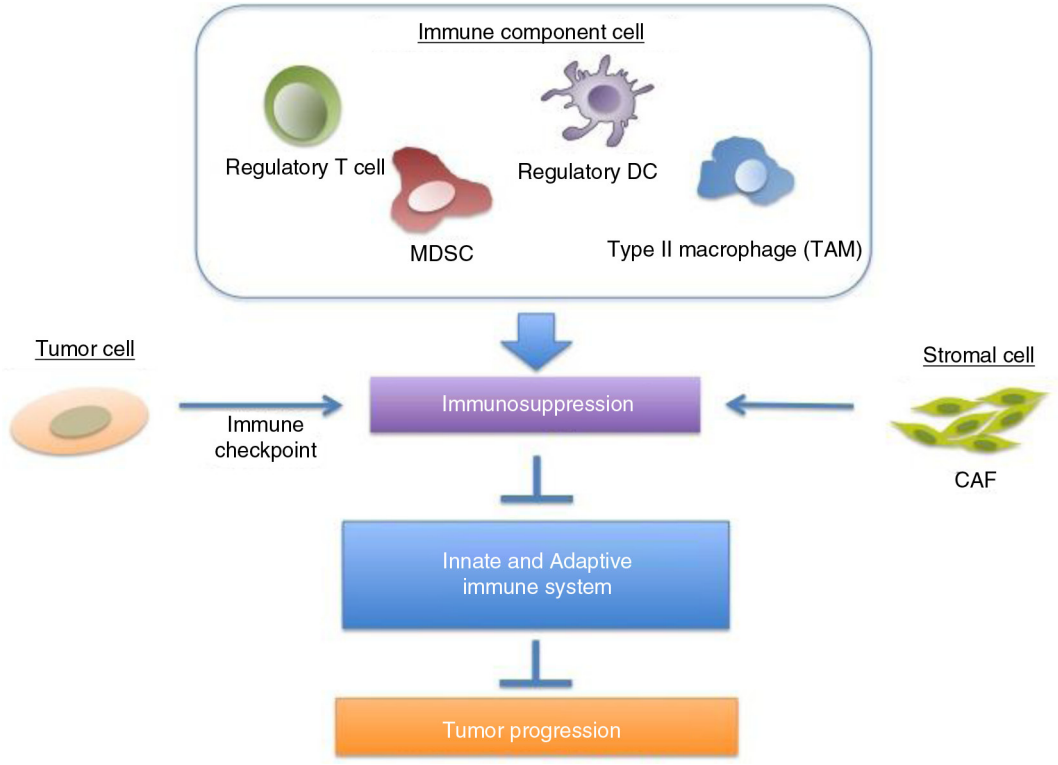

Fig. 2. The tumor microenvironment plays a suppressive role in the anti-tumor immune response. Regulatory T (Treg) cells, myeloid-derived suppressor cells (MDSCs), regulatory dendritic cells (DCs), and type II macrophages are categorized as immunosuppressive cell types in the tumor microenvironment. Moreover, via immune checkpoints, tumor and stromal cells exert potent immunosuppressive effects against the anti-tumor immune response.

growth in tumor volume. Several factors, including VEGF, platelet-derived growth factor (PDGF), and matrix metalloproteinase (MMPs), regulate both angiogenesis and lymphangiogenesis. Furthermore, the tumor microenvironment directly or indirectly suppresses the anti-tumor T-cell response through the immunosuppressive functions of tumor cells, stromal cells, and immune component cells derived from myeloid and lymphoid tissues (31) (Fig. 2).

First, tumor cells effectively inhibit the crucial functions of DCs, which are regarded as key components in the initiation of the TA-specific T-cell response (51, 52). CD47, a transmembrane protein expressed on epithelial tumors, is a negative regulator of DCs. This molecule binds to signal regulatory protein (SIRP)-α on DCs and directly represses DC phagocytosis, maturation, and production of INF-γ (53, 54). Moreover, IL-6 in tumor tissue, which is an inflammatory cytokine, can prevent DC maturation and priming of tumor-specific T cell via STAT3 signaling (55). Tumor cells further produce thymic stromal lymphopoietin (TSLP) to increase the expression of OX40 on DCs (56, 57). TSLP-mediated OX40 upregulation generates Th2 cells, which inhibit tumor cell apoptosis and promote tumor progression (57).

Several chemokines recruit immune suppressors such as Tregs, MDSCs, and M2s into the tumor microenvironment. These immunosuppressive cells also produce additional immunosuppressive cytokines to attract other immune-component cells: regulatory DCs, type II NKT cells, and suppressive γδT cells via STAT3 (24).

Recent reports have shown that cancer-associated fibroblasts (CAFs) are important non-immunocompetent cells in the tumor microenvironment. CAFs produce not only extracellular matrix (ECM) and growth factors that facilitate tumor development and initiation of invasion, but also immune suppression *in vivo* (58, 59). CAFs expressed fibroblast activation protein-α (FAP-α), which down-regulates the T-cell response via CXCL12, the ligand of CXCR4 (60). Taken together, these observations indicate that the anti-tumor response induced by TA-specific $CD8^+$ T cells is suppressed by negative immunological regulation of the tumor microenvironment.

Immunomodulation by conventional cytotoxic therapy

Both conventional chemotherapy and radiotherapy directly kill tumor cells by interfering with DNA synthesis and replication. It has been reported that these therapeutic modalities also eliminate subsets of immune cells and strongly inhibit anti-tumor immune response *in vivo* (61). However, these conventional therapies are identified

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as immunomodulators and can activate anti-tumor immune responses (62).

Cisplatin (CDDP), which is one of the cytotoxic drug most commonly used to treat HNSCC and causes cross-linking of DNA followed by inhibiting mitosis, especially has important anti-tumor immunomodulatory effects. These effects are induced by four distinguishing mechanisms, for example, upregulation of MHC class I, recruitment and proliferation of effector T cells and macrophages, enhancement of lytic activity in cytotoxic effector cells, and downregulation of MDSCs and Tregs (63). 5-FU is a thymidylate synthase inhibitor to decrease the biosynthesis of pyrimidine nucleotides. It has been reported that 5-FU increases IFN-γ production by tumor specific-$CD8^+$ T cells infiltrating tumor tissue (64). Intriguingly, neoadjuvant chemotherapy with CDDP and 5-FU increases the intra-tumoral trafficking of $CD4^+$ and $CD8^+$ T cells in both mouse models and patients with esophageal squamous cell carcinoma (65, 66). Paclitaxel is categorized as the taxane family and induces disruption of microtubule function. Paclitaxel up-regulate mannose-6-phosphate receptors on the surface of tumor cell and render them permeable to granzyme B. Moreover, these drug can induce the activation of DCs, NK cells, and tumor specific CTL via producing IL-12 and TNF-α, resulting in enhancement of anti-tumor immune response (67).

Similar to chemotherapy, radiotherapy has been used for HNSCC treatment. Recently, radiotherapy induces anti-tumor immune responses to inhibit tumor growth and to eliminate tumor cells. Radiotherapy increases type I IFNs that up-regulate the level of CXCR3 chemokine within the tumor. Moreover, type I IFNs directly enhance activation of $CD8^+$ T cell, results in induction of anti-tumor immune response (68). In contrast, chemoradiotherapy decreases the frequency of circulating $CD4^+$ T cells but increases that of $CD4^+CD39^+$ Treg in HNSCC patients (69). These data indicate that CRT-induced suppressive Treg may inhibit anti-tumor immune responses and promote recurrence in HNSCC. Taken together, strong foundation is needed to establish the best way to combine conventional therapy and immunotherapy.

Cancer vaccine

Cancer vaccines have the ability to exert anti-tumor effects, mediated by TA-specific CTLs, in cancer patients; this approach is considered to represent an attractive treatment for several types of cancers (4). To define the efficacy and safety of cancer vaccines, several clinical trials have been performed over the last decade; these studies have demonstrated both clinical efficacy and benefits for cancer patients (70–73). Although sipuleucel-T was initially approved as the immunotherapy for metastatic hormone-refractory prostate cancer patients in 2010 (70), little evidence exists that demonstrates the clinical benefits of cancer vaccine therapy in HNSCC.

TA-derived peptides are generally synthesized to bind onto MHC. After TA-specific $CD8^+$ T cells are elicited by HLA-restricted peptide vaccine, these cells can recognize peptide-MHC class I complex on tumor cells and kill them (71). Identification of highly immunogenic TAs and induction of TA-specific CTLs in cancer patients are important for achieving definite therapeutic effects. In particular, TAs (e.g., MAGE-A3, and MAGE-C1/CT7) that have not only high immunogenicity but also function to promote the survival of tumor cell, should be used preferentially for cancer vaccine therapy in the clinic (4, 74). Moreover, because multi-antigen vaccines can overcome tumor heterogeneity, vaccines based on multiple TAs are more effective at inducing CTL responses in several types of cancers than vaccines based on single TAs (75, 76). Thus, immunotherapy with cancer vaccines based on multiple peptides represents an efficient therapeutic intervention that will lead to anti-tumor effects.

A phase II trial of multiple peptide-based vaccine therapy for advanced HNSCC targeted three TAs: lymphocyte antigen 6 complex locus K (LY6K), CDCA1, and insulin-like growth factor II mRNA-binding protein 3 (IMP3). The vaccine improved overall survival in HLA-A*24: 02 (+) patients to a greater extent than in HLA-A*24: 02 (–) patients (77).

Some studies have demonstrated that stimulation of MHC class II–mediated $CD4^+$ T-cell responses helps to develop and maintain the anti-tumor effects of antigen-specific $CD8^+$ T cells in mice (78, 79). In cancer patients, immunotherapy with Trojan peptide-based vaccine induces not only MHC class I-restricted T-cell responses but also MHC class II-restricted T-cell responses (80). This vaccine is characterized by a large peptide composed of four components: MHC class I and II epitopes, HIV-1-TAT, and furin-sensitive linkers. HIV-1-TAT delivers this long peptide into APC and forms targeted peptide-MHC complexes. Melanoma antigen 3 (MAGE-A3) and human papilloma virus (HPV)-16 Trojan peptide–based vaccines were first evaluated in a phase I clinical trial of HNSCC patients (80). However, despite vaccine-induced T cell responses to the HLA-II epitope in a limited number of patients with advanced HNSCC, no Trojan-specific IgG was detected. Thus, further studies will be needed to consider cancer vaccine as a standard therapy in HNSCC.

DC therapy

DCs play important roles in the initiation of TA-specific T cell responses in tumors. DCs are derived from bone marrow and reside in all tissues (81). Immature DCs capture exogenous TAs and migrate toward lymph nodes. Captured TAs are processed into peptides and are generally loaded onto MHC class II. DCs have the unique ability to present captured exogenous TAs on MHC class I molecules, a phenomenon known as cross-presentation. Due to cross-presentation of DCs, both naïve $CD4^+$ and

$CD8^+$ T cells are simultaneously activated and differentiated into TA-specific effector T cells by mature DCs in lymph nodes (82). Moreover, DCs have co-stimulatory molecules, such as CD40, CD80, and CD86. These factors contribute to activation of DCs and enhancement of TAs-specific T-cell responses. DC infiltration into tumor tissue is highly and significantly correlated with prolongation of survival time and repression of metastasis in patients with HNSCC (83). Therefore, DCs play important roles in eliciting tumor-specific $CD4^+$ and $CD8^+$ T cell-mediated antitumor immune responses, and could be applied to cancer immunotherapy for several types of cancer, including HNSCC.

Several therapeutic approaches using DCs have been developed and established as safe interventions (84, 85). For example, cancer vaccines using cytokine-driven DCs generated *ex vivo* have been used in cancer patients. DCs are generated *ex vivo* by culturing monocytes with cytokine combination such as IL-4 and granulocyte macrophage colony stimulating factor (GM-CSF), and these cells are then cultured with TSA-derived peptide for presentation on MHC class I and II. After maturation of DCs *ex vivo*, intra-, peri-tumoral, or intra-vascular administration is performed to induce cytotoxic effector and helper T cells. In HNSCC, Ferris and co-workers have demonstrated that DC-based wild-type p53 peptide vaccines effectively elicit anti-tumor immune responses and suppress tumor growth (86, 87).

Although DC-based vaccines can theoretically induce anti-tumor immune responses by effector T cells in patients with HNSCC, and they have been identified as potentially safe immunotherapeutic intervention, it remains unclear whether cancer vaccines are effective and contribute to sufficient clinical benefits, such as improvement of overall survival and prevention of tumor relapse or metastasis.

Immune checkpoint blockade

The anti-tumor T-cell response is influenced by a balance between co-stimulatory and inhibitory signals; certain aspects of immune-inhibitory pathways are also called immune checkpoints (88, 89). Immune checkpoints mediate not only immune tolerance in normal tissue, but also immunosuppression in tumor tissue. Basic immunological studies have shown that some receptors and ligands related to immune checkpoints are expressed in inflammatory environments *in vivo* (89, 90). In several types of cancer, immunosuppression is mainly elicited by cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed death-1 (PD-1). Both receptors are expressed on the surface of T cells (91, 92).

CTLA-4, which is expressed on the surface of T cells and has an extracellular domain similar to that of CD28 that activates T cell and promotes proliferation, combines with the CD80 (B7.1) and CD86 (B7.2) ligands and attenuates immune responses by transmitting an inhibitory signal to T cell (93, 94). Moreover, the $CD8^+$ effector T-cell activation by CD28 signaling leads to upregulation of CTLA4 expression. Thus, CTLA4 is recognized as a negative-feedback regulator in the process of T-cell activation (95). Furthermore, CTLA4 inhibits $CD4^+$ helper T cell–dependent immune responses (96–98). Therefore, CTLA4 seems to be a key factor in escape from the anti-tumor T-cell response.

PD-1, a receptor of the CD28 family, interacts with PD-L1 and PD-L2 ligands (99, 100). PD-1 is highly expressed in activated T cells, NK cells, and APCs, and plays an important role in suppression of T-cell activation induced by recognition of TAAs loaded on MHC class I (101). This suppressive function of PD-1 is associated with T-cell exhaustion in inflammatory environments and the tumor microenvironment, where T cells are continuously exposed to multiple types of antigens (93). Thus, the expression of PD-1 is considered as an exhaustion marker of T cells. However, PD-1 expression on T cell is brought to broad attention as a clinical biomarker in HNSCC. Recently, Badoual et al. have reported that presence of tumor-infiltrating $PD\text{-}1^+$ T cells in HPV-associated HNSCC has positive correlation with a favorable clinical outcome (102).

PD-L1 and PD-L2 are members of the B7 family, and are broadly expressed in several types of cell including T and B cells, NK cells, and tumor cells after exposure to IFN-γ (103). Notably, expression of PD-L1 has been observed in various types of human cancer (91), including HNSCC (25).

It has been suggested that the blockades of immune checkpoints using anti-CTLA4 and anti-PD1 mAbs exert anti-tumor effect and significant clinical benefits in several types of cancers (89, 93). In the context of HNSCC treatment, several studies have reported safety data for these monoclonal antibodies. However, it remains to be seen whether anti-CTLA4 and anti-PD-1 mAbs have clinical benefits such as improvement in overall survival and progression-free survival. A phase III trial is currently underway in patients with recurrent and metastatic head and neck cancer to investigate the clinical benefits of nivolumab as monotherapy (25). In addition to PD-1 inhibitor, two clinical trials of CTLA-4 inhibitor are also ongoing in HNSCC patients. Recent reports have shown that PD-1 inhibitor is clearly superior to the CTLA-4 inhibitor for treating advanced melanoma (104). This conclusion might be applied to treatment of HNSCC as well.

CAR therapy

In order to overcome the lower affinity of T cells for TAAs and to induce a stronger antitumor T-cell effect, immunotherapy can be performed with a CAR (105, 106). A CAR is composed of three parts: a single chain

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hypervariable fragment (scFv), a hinge region, and CD3ζ. scFv, in which the variable light (VL) and variable heavy (VH) chains of TAA-specific antibodies are arranged in tandem, has a higher affinity for TAA than the classical TCR. This fragment is linked to the CD3ζ chain, which is the intracellular T-cell signaling domain of classical TCR. This fusion complex is the so-called 'first-generation CAR'. In subsequently developed constructs, co-stimulatory molecules such as CD28, CD137, CD134, 4-1BB, and OX40 are linked between scFv and the CD3ζ chain for the purpose of up-regulating intracellular signaling and activating T cells. Second- or third-generation CARs are characterized by the inclusion of CD28 alone or CD28 plus 4-1BB, respectively (107).

In CAR therapy, the CAR is overexpressed in T cells derived from a patient using a retroviral or lentiviral vector. The CAR-modified T cells recognize a specific TAA in the cancer patient, leading to activation of the T-cell response and induction of anti-tumor effects (95). Several reports have shown that CD19 and GD2 antigen–targeted CAR therapies are clinically effective against leukemia and neuroblastoma, respectively (108–110). In HNSCC, CAR therapy targeting latent membrane protein (LMP)1-HELA/CAR (111) or chondroitin sulfate proteoglycan-4 (CSPG-4) (112) could induce anti-tumor effect *in vitro* and *in vivo*. Thus, CAR therapy might be one of the new prospective treatment modality in HNSCC.

Combination of cancer immunotherapy and targeted therapy

Several clinical trials are currently underway for cancer immunotherapy against HNSCC (Table 1). However, in well-established tumors with multiple genetic and epigenetic mutations, a potent immune-suppressive network is often formed by immune checkpoints and suppressive regulation by Tregs, MDSCs, and M2s, leading to repression of anti-tumor immunity by activated T cells. These problems must be solved in order to establish effective treatments for cancer.

Among the breakthrough therapies against tumors, there are several types of targeted therapies. Targeted therapy, which depends on our knowledge of tumor biology, inhibits tumor progression (including proliferation and metastasis) by targeting specific oncoproteins or signal transduction pathways (113). Many targeted agents are approved and are under investigation for HNSCC treatment, including epidermal growth factor receptor (EGFR) monoclonal antibody, EGFR tyrosine kinase inhibitor, vascular endothelial growth factor receptor (VEGFR) inhibitors, and phosphatidylinositol 3' kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway inhibitors (114). These targeted therapies elicit sufficient anti-tumor effects in HNSCC patients who have a single genetic mutation or signaling pathway that contributes to tumor growth and maintenance of malignant phenotypes (115).

Table 1. Recent clinical trials of cancer immunotherapy in head and neck cancer

Cancer vaccine (including DC vaccine)
• MAGE-A3 + HPV16 Trojan cancer vaccine →Phase I
• Multi-peptides (LY6K, CDCA1, IMP3) vaccine →Phase II
• Wild type-p53 peptide vaccine →Phase I
• Epstein-Barr virus–LMP2 peptide vaccine →Phase I
• NY-ESO-1 peptide vaccine →Phase I
• HPV-DNA vaccine →Phase I
CAR therapy and adaptive T-cell therapy
• Targeted LMP1-CAR therapy →Pre-clinical study
• Epstein-Barr virus-specific adaptive T cell therapy →Pilot study
• Targeted CSPG-4-CAR therapy →Pre-clinical study
The blockade of immune checkpoints
• Anti PD-1 antibody (Nivolumab) →Phase III
• Anti CTLA-4 antibody (ipilimumab) →Phase I

DC, dendritic cell; MAGE-A3, melanoma associated antigen 3; LY6K, lymphocyte antigen 6 complex locus K; IMP3, insulin-like growth factor II mRNA-binding protein 3; LMP2, latent membrane protein 2; CSPG-4, chondroitin sulfate proteoglycan-4; HPV, human papilloma virus; CAR, chimeric antigen receptor; PD-1, programmed cell death-1.

Additionally, a number of analyses have shown that several oncogenic signaling pathways also affect regulation of the host immune system against tumor cells (1). Cetuximab, which is a neutralizing antibody against EGFR, inhibits tumor growth signals and exerts clinical benefits in HNSCC (116).

Cetuximab significantly up-regulates not only expressions of MHC class II on HNSCC cells followed by enhancing anti-tumor recognition by the EGFR-reactive $CD4^+$ helper T cells (117) but also producing TGF-b and PGE2 as immune suppressors from HNSCC cells (118). Moreover, co-stimulatory molecules (i.e., CD40, CD80/CD86) on DCs are increased by treatment of cetuximab (119). DCs that are co-cultured with tumor cells and cetuximab effectively promote DC priming and induce tumor-specific T-cell responses *in vitro*, result in enhanced anti-tumor immune response (120). Additionally, cetuximab facilitates NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC), macrophage-mediated antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC), which may promote anti-tumor effect (121).

Furthermore, small-molecule inhibitors targeting VEGFR signaling decrease the activity of Tregs or MDSCs and dampen tumor-induced immunosuppression (122, 123). Bevacizumab and sunitinib are categorized as VEGFR inhibitors. Bevacizumab has the ability to promote the

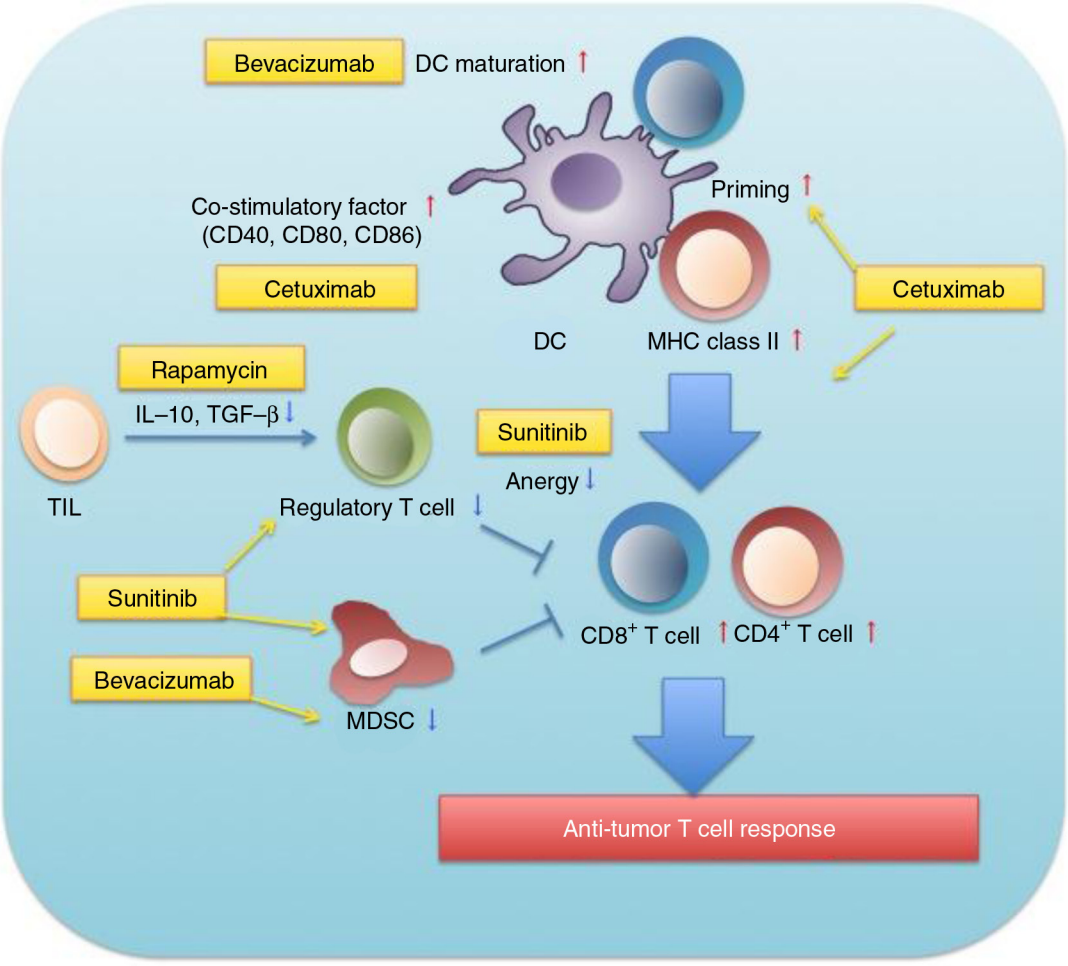

Fig. 3. Several targeted therapies may have immunomodulatory functions on tumor-mediated immunosuppression. Targeted therapies enhance effective dendritic cell (DC) maturation and T-cell priming to induce the anti-tumor T-cell response. In addition, targeted therapy has the possibility to attenuate the immunosuppressive side effects of cancer immunotherapy.

maturation of DCs and block the expansion of MDSCs (124). Sunitinib acts as a multi-tyrosine kinase inhibitor to block VEGFR function, and suppresses the number and function of Tregs and MDSCs. Moreover, sunitinib can skew the immune response toward Th1, and it represses secretion of IL-10 and TGF-b to induce Tregs. In addition, sunitinib enhances production of IFN-γ from tumor-infiltrating T cells (125, 126). Matsushita et al. have conducted a phase I clinical trial using combination treatments of sunitinib plus autologous tumor lysate–loaded DC immunotherapy in metastatic renal cell carcinoma patients (127).

Rapamycin, an inhibitor of mTOR, is an effective immunosuppressant (128). However, a recent report demonstrated that rapamycin activates effector and memory T cells (129). Moreover, administration of rapamycin after vaccination to stimulate host immune system suppress the levels of tumor-infiltrating lymphocytes (TILs)-induced Tregs (130). Taken together, these data show that targeted therapies help to enhance the anti-tumor immune response induced by cancer immunotherapy (Fig. 3).

Conclusion

In recent years, our understanding of oncogenic mechanisms has given rise to new therapeutic modalities. Cancer immunotherapy is among the novel therapies that can confer clinical benefits by inducing an anti-tumor T-cell response. However, the clinical effectiveness of cancer immunotherapy with targeted therapy has not been fully studied in treatment of HNSCC. Additionally, little is known about the safety of cancer immunotherapy. Some immunotherapies can up-regulate anti-tumor immune response and at the same time broadly activate the host immune system. Because of individual immunological variability, some patients received cancer immunotherapy might experience mild and localized side effects, and the others severe and systematic.

Further translational and clinical studies of cancer immunotherapy in HNSCC will be needed to provide

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evidence of effective and harmless treatment. Moreover, combinatorial therapeutic strategies may offer a number of strong synergies.

Authors' contributions

HI and ST wrote the manuscript. HI and KM discussed the context and contributed to the final manuscript.

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Conflict of interest and funding

The authors report no conflicts of interest.

References

1. Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. Lancet. 2008;371:1695–709.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55(2):74–108.
3. Chen DS, Mellman I. Oncology meets immunology: The cancer-immunity cycle. Immunity. 2013;39(1):1–10.
4. Kono K. Current status of cancer immunotherapy. J Stem Cells Regen Med. 2014;10(1):8–13.
5. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454:436–44.
6. Chew V, Toh HC, Abastado JP. Immune microenvironment in tumor progression: Characteristics and challenges for therapy. J Oncol. 2012; 2012: 608406. doi: 10.1155/2012/608406.
7. Pak AS, Wright MS, Matthews JP, Collins SL, Petruzzell GJ, Young MR. Mechanisms of immune suppression in patients with head and neck cancer: Presence of CD34+ cells which suppress immune functions within cancers that secret granulocyte-macrophage colony-stimulating factor. Clin Cancer Res. 1995;1(1):95–103.
8. Tian T, Olson S, Whitacre JM, Harding A. The origins of cancer robustness and evolvability. Integr Biol (Camb). 2011; 3:17–30.
9. Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. Nature. 2013;501:328–37.
10. Van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde BJ, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science. 1991;254:1643–7.
11. Hoffmann TK, Bier H, Donnenberg AD, Whiteside TL, De Leo AB. p53 as an immunotherapeutic target in head and neck cancer. Adv Otorhinolaryngol. 2005;62:151–60.
12. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: From immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991–8.
13. Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. Immunology. 2007; 121(1):1–14.
14. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. Science. 2011;331:1565–70.
15. Smyth MJ, Godfrey DI, Trapani JA. A fresh look at tumor immunosurveillance and immunotherapy. Nat Immunol. 2001;2:293–9.
16. Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, Archambault JM, et al. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. J Exp Med. 2011;208(10):1989–2003.
17. Fuertes MB, Kacha AK, Kline J, Woo SR, Kranz DM, Murphy KM, et al. Host type I IFN signals are required for antitumor CD8+ T cell responses through $CD8^+$ dendritic cells. J Exp Med. 2011;208(10):2005–16.
18. Boon T, Cerottini JC, Van den Eynde B, can der Bruggen P, Van Pel A. Tumor antigens recognized by T lymphocytes. Annu Rev Immunol. 1994;12:337–65.
19. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;392:245–52.
20. Taylor PA, Lees CJ, Fournier S, Allison JP, Sharpe AH, Blazar BR. B7 expression on T cells down-regulates immune responses through CTLA-4 ligation via T-T interactions. J Immunol. 2004;172:34–9.
21. Zhang X, Sun S, Hwang I, Tough DF, Sprent J. Potent and selective stimulation of memory-phenotype CD8+ T cells *in vivo* by IL-15. Immunity. 1998;8:591–9.
22. Chang CC, Campoli M, Ferrone S. Classical and nonclassical HLA class I antigen and NK cell-activating ligand changes in malignant cells: Current challenges and future directions. Adv Cancer Res. 2005;93:189–234.
23. Campoli M, Ferrone S. HLA antigen changes in malignant cells: Epigenetic mechanisms and biological significance. Oncogene. 2008;27:5869–85.
24. Shurin MR, Umansky V, Malyguine A, editors. The tumor immunoenvironment. New York: Springer; 2013. p. 307–24.
25. Swanson MS, Sinha UK. Rationale for combined blockade of PD-1 and CTLA-4 in advanced head and neck squamous cell cancer – Review of current data. Oral Oncol. 2015;51:12–5.
26. Wallis SP, Stanfford ND, Greenman J. The clinical relevance of immune parameters in the tumor microenvironment of head and neck cancers. Head Neck. 2014; 37: 449–59. doi: 1002/hed.23736.
27. Cho YA, Yoon HJ, Lee J, Hong SP, Hong SD. Relationship between the expressions of pd-1 and tumor-infiltrating lymphocytes in oral squamous cell carcinoma. Oral Oncol. 2011;47:1148–53.
28. Gildener-Leapman N, Ferris RL, Bauman JE. Promising systemic immunotherapies in head and neck squamous cell carcinoma. Oral Oncol. 2013;49:1089–96.
29. Laimer K, Troester B, Kloss F, Schafer G, Obrist P, Perathoner A, et al. Expression and prognostic impact of indoleamine 2,3-dioxygenase in oral squamous cell carcinomas. Oral Oncol. 2011;47(5):352–7.
30. Lindau D, Gielen P, Kroesen M, Wesseling P, Adema GJ. The immunosuppressive tumour network: Myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. Immunology. 2013;138(2):105–15.
31. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008;27(45):5904–12.
32. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: An immunologic functional perspective. Annu Rev Immunol. 2009;27:451–83.
33. Tesone AJ, Svoronos N, Allegrezza MJ, Conejo-Garcia JR. Pathological mobilization and activities of dendritic cells in tumor-bearing hosts: Challenges and opportunities for immunotherapy of cancer. Front Immunol. 2013; 4: 435. doi: 10.3389/fimmu.2013.00435.
34. Byrne WL, Mills KH, Lederer JA, O'Sullivan GC. Targeting regulatory T cells in cancer. Cancer Res. 2011;71(22):6915–20.
35. Chikamatsu K, Sakakura K, Whiteside TL, Furuya N. Relationships between regulatory T cells and CD8+ effector populations in patients with squamous cell carcinoma of the head and neck. Head Neck. 2007;29(2):120–7.

36. Strauss L, Bergmann C, Szczepanski M, Gooding W, Johnson JT, Whiteside TL. A unique subset of CD4+ CD25highFoxp3+ T cells secreting interleukin-10 and transforming growth factor-beta1 mediates suppression in the tumor microenvironment. Clin Cancer Res. 2007;13:4345–54.
37. Bronte V, Serafini P, Mazzoni A, Segal DM, Zanovello P. L-arginine metabolism in myeloid cells controls T-lymphocyte functions. Trends Immunol. 2003;24(6):302–6.
38. Filipazzi P, Valenti R, Huber V, Pilla L, Canese P, Iero M, et al. Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. J Clin Oncol. 2007;25(18):2546–53.
39. Obermajer N, Muthuswamy R, Lesnock J, Edwards RP, Kalinski P. Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells. Blood. 2011;118(20): 5498–505.
40. Tartour E, Pere H, Maillere B, Terme M, Merillon N, Taieb J, et al. Angiogenesis and immunity: A bidirectional link potentially relevant for the monitoring of antiangiogenic therapy and the development of novel therapeutic combination with immunotherapy. Cancer Metastasis Rev. 2011; 30(1):83–95.
41. Huang B, Pan PY, Li Q, Sato AI, Levy DE, Bromberg J, et al. Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. Cancer Res. 2006;66(2): 1123–31.
42. Serafini P, Mgebroff S, Noonan K, Borrello I. Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. Cancer Res. 2008;68(13):5439–49.
43. Yang L, DeBusk LM, Fukuda K, Fingleton B, Green-Jarvis B, Shyr Y, et al. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. Cancer Cell. 2004;6(4):409–21.
44. Kusmartsev S, Nefedova Y, Yoder D, Gabrilovich DI. Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. J Immunol. 2004;172(2):989–99.
45. Almand B, Clark JI, Nikitina E, van Beynen J, English NR, Knight SC, et al. Increased production of immature myeloid cells in cancer patients: A mechanism of immunosuppression in cancer. J Immunol. 2001;166(1):678–89.
46. Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, et al. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cell. J Immunol. 2009;182(9):5693–701.
47. Brandau S, Trellakis S, Bruderek K, Schmaltz D, Steller G, Elian M, et al. Myeloid-derived suppressor cells in the peripheral blood of cancer patients contain a subset of immature neutrophils with impaired migratory properties. J Leukoc Biol. 2011;89(2):311–7.
48. Chikamatsu K, Sakakura K, Toyoda M, Takahashi K, Yamamoto T, Masuyama K. Immunosuppressive activity of CD14+HLA-DR- cells in squamous cell carcinoma of the head and neck. Cancer Sci. 2012;103(6):976–83.
49. Puig-Kröger A, Sierra-Filardi E, Domínguez-Soto A, Samaniego R, Corcuera MT, Gómez-Aguado F, et al. Folate receptor beta is expressed by tumor-associated macrophages and constitutes a marker for M2 anti-inflammatory/regulatory macrophages. Cancer Res. 2009;69(24):9395–403.
50. He KF, Zhang L, Huang CF, Ma SR, Wang YF, Wang WM, et al. CD163+ tumor-associated macrophages correlated with poor prognosis and cancer stem cells in oral squamous cell carcinoma. Biomed Res Int. 2014; 2014: 838632. doi: 10.1155/2014/838632.
51. Dhodapkar MV, Dhodapkar KM, Palucka AK. Interactions of tumor cells with dendritic cells: Balancing immunity and tolerance. Cell Death Differ. 2008;15(1):39–50.
52. Dhodapkar MV, Dhodapkar KM. Recent advances and new opportunities for targeting human dendritic cells *in situ*. Oncoimmunology. 2014;3(8):e954832.
53. Sarfati M, Fortin G, Raymond M, Susin S. CD47 in the immune response: Role of thrombospondin and SIRP-alpha reverse signaling. Curr Drug Targets. 2008;9(10):842–50.
54. Chao MP, Alizadeh AA, Tang C, Myklebust JH, Varghese B, Gill S, et al. Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma. Cell. 2010;142(5):699–713.
55. Chomarat P, Banchereau J, Davoust J, Palucka AK. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. Nat Immunol. 2000;1(6):510–4.
56. Steinbrink K, Wlofl M, Jonuleit H, Knop J, Enk AH. Induction of tolerance by IL-10-treated dendritic cells. J Immunol. 1997;159(10):4772–80.
57. Aspord C, Pedroza-Gonzalez A, Gallegos M, Tindle S, Burton EC, Su D, et al. Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4+ T cells that facilitate tumor development. J Exp Med. 2007;204(5):1037–47.
58. Bello IO, Vered M, Dayan D, Dobriyan A, Yahalom R, Alanen K, et al. Cancer-associated fibroblasts, a parameter of the tumor microenvironment, overcomes carcinoma associated parameters in the prognosis of patients with mobile tongue cancer. Oral Oncol. 2011;47(1):33–8.
59. Costea DE, Hills A, Osman AH, Thurlow J, Kalna G, Huang X, et al. Identification of two distinct carcinoma-associated fibroblast subtypes with differential tumor-promoting abilities in oral squamous cell carcinoma. Cancer Res. 2013; 73(13):3888–901.
60. Feig C, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergies with anti-PD-L1 Immunotherapy in pancreatic cancer. Proc Natl Acad Sci USA. 2013;110(50):20212–7.
61. Kuss I, Hathaway B, Ferris RL, Gooding W, Whiteside TL. Decrease absolute counts of T lymphocyte subsets and their relation to disease in squamous cell carcinoma of the head and neck. Clini Cancer Res. 2004;10:2755–62.
62. Bracci L, Schiavoni G, Sistigu A, Belardelli F. Immune-based mechanisms of cytotoxic chemotherapy: Implications for the design of novel and rationale-based combined treatments against cancer. Cell Death Differ. 2014;21:15–25.
63. de Biasi AR, Villena-Vargas J, Adusumilli PS. Cisplatin-induced antitumor immunomodulation: A review of preclinical and clinical evidence. Clin Cancer Res. 2014;20:5384–91.
64. Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T-cell-dependent antitumor immunity. Cancer Res. 2010;70:3052–61.
65. Predina JD, Judy B, Aliperti LA, Fridlender ZG, Blouin A, Kapoor V, et al. Neoadjuvant *in situ* gene-mediatedcytotoxic immunotherapy improves postoperativeoutcomes in novel syngeneic esophageal carcinoma models. Cancer Gene Ther. 2011;18:871–83.
66. Tsuchikawa T, Md MM, Yamamura Y, Shichinohe T, Hirano S, Kondo S. The immunological impact of neoadjuvant chemotherapy on the tumor microenvironment of esophageal squamous cell carcinoma. Ann Surg Oncol. 2012;19:1713–9.

67. Chang CL, Hsu YT, Wu CC, Lai YZ, Wang C, Yang YC, et al. Dose-dense chemotherapy improves mechanisms of antitumor immune response. Cancer Res. 2013;16:119–27.
68. Lim JY, Gerber SA, Murphy SP, Lord EM. Type I interferons induced by radiation therapy mediate recruitment and effector function of CD8(+) T cells. Cancer Immunol Immunother. 2014;63:259–71.
69. Schuler PJ, Harasymczuk M, Schilling B, Saze Z, Strauss L, Lang S, et al. Effects of adjuvant chemoradiotherapy on the frequency and function of regulatory T cells in patients with head and neck cancer. Clin Cancer Res. 2013;19:6585–96.
70. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411–22.
71. Huppa JB, Davis MM. T-cell-antigen recognition and the immunological synapse. Nat Rev Immunol. 2003;3(12):973–83.
72. Aranda F, Vacchelli E, Eggermont A, Galon J, Sautes Fridman C, Tartour E, et al. Trial watch: Peptide vaccines in cancer therapy. Oncoimmunology. 2013;2(12):e26621.
73. Cecco S, Muraro E, Giacomin E, Martorelli D, Lazzarini R, Baldo P, et al. Cancer vaccines in phase II/III clinical trials: State of the art and future perspectives. Curr Cancer Drug Targets. 2011;11(1):85–102.
74. Figueiredo DL, Mamede RC, Spangnoli GC, Silva WA, Jr, Zago M, Neder L, et al. High expression of cancer–testis antigens MAGE-A, MAGE-C1/CT7, MAGE-C2/CT10, NY-ESO-1, and gage in advanced squamous cell carcinoma of the larynx. Head Neck. 2011;33:702–7.
75. Kono K, Iinuma H, Akutsu Y, Tanaka H, Hayashi N, Uchikado Y, et al. Multicenter, phase II clinical trial of cancer vaccination for advanced esophageal cancer with three peptides derived from novel cancer-testis antigens. J Transl Med. 2012;10:141.
76. Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med. 2012;18(8): 1254–61.
77. Yoshitake Y, Fukuma D, Yuno A, Hirayama M, Nakayama H, Tanaka T, et al. Phase II clinical trial of multiple peptide vaccination for advanced head and neck cancer patients revealed induction of immune responses and improved OS. Clin Cancer Res. 2015;21(2):312–21.
78. Zwaveling S, Vierboom MP, Ferreira Mota SC, Hendriks JA, Ooms ME, Sutmuller RP, et al. Antitumor efficacy of wild-type p53-specific CD4(+) T-helper cells. Cancer Res. 2002; 62(21):6187–93.
79. Hoffmann TK, Dworacki G, Tsukihiro T, Meidenvauer N, Gooding W, Johnson JT, et al. Spontaneous apoptosis of circulating T lymphocytes in patients with head and neck cancer and its clinical importance. Clin Cancer Res. 2002; 8(8):2553–62.
80. Voskens CJ, Sewell D, Hertzano R, DeSanto J, Rollins S, Lee M, et al. Induction of MAGE-A3 and HPV16 immunity by Trojan vaccines in patients with head and neck carcinoma. Head Neck. 2012;34(12):1734–46.
81. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature. 2007;449(7161):419–26.
82. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer. 2012;12(4):265–77.
83. Ma Y, Shurin GV, Peiyuan Z, Shurin MR. Dendritic cells in the cancer microenvironment. J Cancer. 2013;4(1):36–44.
84. Ueno H, Palucka AK, Banchereau J. Harnessing human dendritic cell subsets for medicine. Immunol Rev. 2010;234(1): 199–212.
85. Palucka K, Ueno H, Roberts L, Fay J, Banchereau J. Dendritic cells: Are they clinically relevant? Cancer J. 2010;16(4):318–24.
86. Gnjatic S, Cai Z, Viguier M, Chouaib S, Guillet JG, Choppin J. Accumulation of the p53 protein allows recognition by human CTL of a wild-type p53 epitope presented by breast carcinoma and melanomas. J Immunol. 1998;160(1):328–33.
87. Schuler PJ, Harasymczuk M, Visus C, Deleo A, Trivedi S, Lei Y, et al. Phase I dendritic cell p53 peptide vaccine for head and neck cancer. Clin Cancer Res. 2014;20(9):2433–44.
88. Zou W, Chen L. Inhibitory B7-family molecules in the tumor microenvironment. Nat Rev Immunol. 2008;8(6):467–77.
89. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
90. Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ. Adaptive immunity maintains occult cancer in an equilibrium state. Nature. 2007;450(7171):903–7.
91. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996; 271:1734–6.
92. Okazaki T, Chikuma S, Iwai Y, Fagarasan S, Honjo T. A rheostat for immune responses: The unique properties of PD-1 and their advantages for clinical application. Nat Immunol. 2013;14(12):1212–8.
93. Hathcock KS, Laszlo G, Dickler HB, Bradshaw J, Linsley P, Hodes RJ, et al. Identification of an alternative CTLA-4 ligand costimulatory for T ell activation. Science. 1993; 262(5135):905–7.
94. Azuma M, Ito D, Yagita H, Okumura K, Phillips JH, Lanier LL, et al. B70 antigen is a second ligand for CTLA-4 and CD28. Nature. 1993;366(6450):76–9.
95. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharp AH. Loss of CTLA-4 leads to massive lymphoproliferation and gatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity. 1995;106:67–75.
96. Lenschow DJ, Walunas TL, Bluestone JA. CD28/B7 system of T cell costimulation. Annu Rev Immunol. 1996;14:233–58.
97. Wing K, Onishi Y, Prieto-Martin P, Yamanguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science. 2008;322(5899):271–5.
98. Peggs KS, Quezada SA, Chambers CA, Korman AJ, Allison JP. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med. 2009;206(8):1717–25.
99. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11: 3887–95.
100. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192: 1027–34.
101. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, et al. Autoimmune dilated cardiomyopathy in PD-1 receotor-deficient mice. Science. 2001;291(5502): 319–22.
102. Badoual C, Hans S, Merillon N, Van Ryswick C, Ravel P, Benhamouda N, et al. PD-1-expressing tumor-infiltrating T cells are a favorable prognostic biomarker in HPV-associated head and neck cancer. Cancer Res. 2013;73:128–38.
103. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. Annu Rev Immunol. 2005;23:515–48.
104. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced

melanoma. E Engl J Med. 2015. doi: 10.1056/NEJMoa1503093.
105. Burns WR, Zhao Y, Frankel TL, Hinrichs CS, Zheng Z, Xu H, et al. A high molecular weight melanoma-associated antigen specific chimeric antigen receptor redirects lymphocytes to target human melanomas. Cancer Res. 2010;70(8):3027–33.
106. Kochenderfer JN, Yu Z, Frasheri D, Restifo NP, Rosenberg SA, Ferrone S, et al. Adoptive transfer of syngeneic T-cells transduced with a chimeric antigen receptor that recognizes murine CD19 can eradicate lymphoma and normal B cells. Blood. 2010;116(19):3875–86.
107. Gao J, Bernatchez C, Sharma P, Radvanyi LG, Hwu P. Advances in the development of cancer immunotherapies. Trends Immunol. 2013;34(2):90–8.
108. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371(16):1507–17.
109. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365(8):725–33.
110. Nishio N, Diaconu I, Liu H, Cerullo V, Caruana I, Hoyos V, et al. Armed oncolytic virus enhances immune functions of chimeric antigen receptor-modified T cells in solid tumors. Cancer Res. 2014;74(18):5195–205.
111. Tang X, Zhou Y, Li W, Tang Q, Chen R, Zhu J, et al. T cells expressing a LMP1-specific chimeric antigen receptor mediate antitumor effects against LMP1-positive nasopharyngeal carcinoma cells *in vitro* and *in vivo*. J Biomed Res. 2014;28(6):468–75.
112. Geldres C, Savoldo B, Hoyos V, Caruana I, Zhang M, Yvon E, et al. T lymphocytes redirected against the chondroitin sulfate proteoglycan-4 control the growth of multiple solid tumors both *in vitro* and *in vivo*. Clin Cancer Res. 2014;20:962–71.
113. Le QT, Giaccia AJ. Therapeutic exploitation of the physiological and molecular genetic alterations in head and neck cancer. Clin Cancer Res. 2003;9(12):4287–95.
114. Dorsey K, Agulnik M. Promising new molecular targeted therapies in head and neck cancer. Drugs. 2013;73(4):315–25.
115. Psyrri A, Seiwert TY, Jimeno A. Molecular pathways in head and neck cancer: EGFR, PI3K, and more. Am Soc Clin Oncol Educ Book. 2013;246–55. doi: 10.1200/EdBook_AM.2013.33.246.
116. Ang KK, Berkey BA, Tu X, Zhang HZ, Katz R, Hammond EH, et al. Impact of epidermal growth factor receptor expression on survival and pattern of relapse in patients with advanced head and neck carcinoma. Cancer Res. 2002;62(24):7350–6.
117. Kumai T, Matsuda Y, Oikawa K, Aoki N, Kimura S, Harabuchi Y, et al. EGFR inhibitors augment antitumour helper T-cell responses of HER family-specific immunotherapy. Br J Cancer. 2013;109:2155–66.
118. Kumai T, Oikawa K, Aoki N, Kimura S, Harabuchi Y, Celis E, et al. Tumor-derived TGF-β and prostaglandin E2 attenuate anti-tumor immune responses in head and neck squamous cell carcinoma treated with EGFR inhibitor. J Transl Med. 2014;12:265.
119. Srivastava RM, Lee SC, Andrade Filho PA, Lord CA, Jie HB, Davidson HC, et al. Cetuximab-activated natural killer and dendritic cells collaborate to trigger tumor antigen-specific T-cell immunity in head and neck cancer patients. Clin Cancer Res. 2013;19:1858–72.
120. Correale P, Botta C, Cusi MG, Del Vecchio MT, De Santi MM, Gori Savellin G, et al. Cetuximab ± chemotherapy enhances dendritic cell-mediated phagocytosis of colon cancer cells and ignites a highly efficient colon cancer antigen-specific cytotoxic T- cell response *in vitro*. Int J Cancer. 2012;130(7):1577–89.
121. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer. 2012;12:237–51.
122. Farsaci B, Higgins JP, Hodge JW. Consequence of dose scheduling of sunitinib on host immune response elements and vaccine combination therapy. Int J Cancer. 2012;130(8):1948–59.
123. Ko JS, Zea AH, Rini BI, Ireland JL, Elson P, Cohen P, et al. Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. Clin Cancer Res. 2009;15(6):2148–57.
124. Alfaro C, Suarez N, Gonzalez A, Solano S, Erro L, Dubrot J, et al. Influence of bevacizumab, sunitinib and sorafenib as single agents or in combination on the inhibitory effects of VEGF on human dendritic cell differentiation from monocytes. Br J Cancer. 2009;100(7):1111–9.
125. Ozao-Choy J, Ma G, Kao J, Wang GX, Meseck M, Sung M, et al. The novel role of tyrosine kinase inhibitor in the reversal of immune suppression and modulation of tumor microenvironment for immune-based cancer therapies. Cancer Res. 2009;69(6):2514–22.
126. Kao J, Ko EC, Eisenstein S, Sikora AG, Fu S, Chen SH. Targeting immune suppressing myeloid-derived suppressor cells in oncology. Crit Rev Oncol Hematol. 2011;77(1):12–9.
127. Matsushita H, Enomoto Y, Kume H, Nakagawa T, Fukuhara H, Suzuki M, et al. A pilot study of autologous tumor lysate-loaded dendritic cell vaccination combined with sunitinib for metastatic renal cell carcinoma. J Immunother Cancer. 2014;2:30.
128. Araki K, Ellebedy AH, Ahmed R. TOR in the immune system. Curr Opin Cell Biol. 2011;23(6):707–15.
129. Araki A, Youngblood B, Ahmed R. The role of mTOR in memory CD8 T-cell differentiation. Immunol Rev. 2010;235(1):234–43.
130. Wang Y, Camirand G, Lin Y, Froicu M, Deng S, Shlomchik WD, et al. Regulatory T cells require mammalian target of rapamycin signaling to maintain both homeostasis and alloantigen-driven proliferation in lymphocyte-replete mice. J Immunol. 2011;186(5):2809–18.