Recent progress in immunotherapy for skin cancer

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Abstract

Skin cancer is a global health concern due to its growing incidence and high mortality rate. The most common therapeutic modalities in skin cancer include surgery, radiotherapy, and chemotherapy. However, those therapies do not specifically target cancer cells and may damage healthy tissues. Cancer induces immune response by releasing soluble antigens and danger signals caused by tumor cellular stress or death, while the immune system continuously monitor and control malignant proliferation through cancer immunoediting. Therefore, targeting this mechanism is a promising approach to manage cancer, especially those unresponsive to conventional therapies. Immunotherapy is a specific therapy that manipulates the immune system to fight the disease. Previous studies have shown promising results in its clinical use in melanoma and non-melanoma skin cancer (NMSC). However, its potential toxicity and tolerability may pose significant obstacles in developing effective cancer immunotherapy. Biomedical, immunological, and clinical research in skin cancer is still needed to elaborate further on its pathogenesis and design safe and effective therapy for each skin cancer.

Keywords: immunotherapy, melanoma, NMSC, toxicity

Background

Skin cancer is an enormous public health concern. Generally, this disease is categorized into melanoma and non-melanoma skin cancer. The incidence of melanoma in the United States (US) is only 4% of all skin cancer diagnoses, but it comprises 75% of mortality due to skin cancer.¹ In East Asia and Southeast Asia, the incidence of melanoma is approximately 0.4-0.5 per 100,000 individuals with a high regional disease burden due to the high population density in both regions and advanced disease stage at diagnosis.² Compared to melanoma, non-melanoma skin cancer (NMSC) has a higher incidence but a lower mortality rate. The incidence of melanoma, cutaneous squamous cell carcinoma (SCC), and basal cell carcinoma (BCC) at Dr. Cipto Mangunkusumo National General Hospital (RSCM) from 2014 to 2017 is 5.7%, 27.4%, and 66.9% from a total of 263 skin cancer cases, respectively.³

The approved therapeutic modalities for skin cancer treatment include surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy.¹ However, chemotherapy and radiotherapy do not specifically target cancer cells and may thereby damage healthy tissues, while surgery can only treat local tumors, hence the need for cancer cells-specific therapy with lower toxicity to increase the survival rate of patients with skin cancer. Targeted therapy directly influences the abnormal signaling pathway in tumor pathogenesis, whilst immunotherapy utilizes components of the immune system to fight the disease.⁴ The clinical use of immunotherapy in cancer is based on the observation of cancer’s natural ability to induce an immune response and the vital role of the immune system in preventing and controlling tumor cells.⁵ This literature review discusses the latest progress in immunotherapy
for skin cancer and its combination to overcome the challenges in its application.

**Immune Response to Cancer**

The immune response is generally classified into innate and adaptive immunity. Innate immunity is composed of numerous components, including epithelial barrier, phagocytic cells (neutrophil and macrophage), dendritic cells (DC), mast cells, natural killer (NK) cells, and the complement system, while the adaptive immunity consists of humoral (played by B-cell lymphocytes) and cellular immunity which is mainly mediated by T-cell lymphocytes (CD4 and CD8 T cells). Antigen-presenting activity by DC stimulates the maturation of lymphocytes and initiates its effector function to eliminate the antigen. Immune response towards cancer, including melanoma and NMSC, is induced by the release of soluble antigens and danger-associated signals caused by tumor cellular stress or death. This event activates the innate immune response, mainly played by the phagocytes and NK cells, while specialized antigen-presenting cells in the skin scavenge these damaged cells and bind the antigen. The antigen is subsequently presented in the regional lymph nodes to CD8 and CD4 T cells through the Class I and Class II MHC, respectively. Activated T cells then migrate to the tumor microenvironment and elicit an adaptive anti-tumor immune response. However, the immune response often fails to prevent tumor growth due to various pathomechanisms initiated by tumor cells to block or evade the host's immune response. 

1. **Cancer Immunoediting**

Cancer immunoediting is an extrinsic tumor-suppressing mechanism initiated by the cell transformation into malignant cells, in which it has not been successfully suppressed by the intrinsic mechanism. It is hypothesized that the interaction between the immune system and cancer cells occurs through three phases (termed 3E), which are the elimination phase, equilibrium phase, and escape phase (Figure 1). Normal cells, which begin to transform into malignant cells and exhibit abnormal physiologic and metabolic activities, induce the immune system. In the elimination phase, the innate and adaptive immune systems work cooperatively to eliminate these malignant cells before being clinically apparent. In certain conditions, rare tumor cell variants may survive and enter the equilibrium phase, in which tumor cells continue to proliferate and mutate against the immune selective pressure, but the immune system still maintains control over the residual tumor cell growth. In the end, the tumor may evolve into tumor cell variants that are not recognized by the adaptive immunity, insensitive to the immune effector mechanisms, or may induce immunosuppressive mechanisms. Tumor cells that successfully escape or evade the immune system can grow uncontrollably and become clinically visible.
2. **Tumor Microenvironment**

The tumor microenvironment (TME) is a complex system formed by cells and molecules which support the tumor development, including extracellular matrix (ECM), immune cells, and stromal cells such as fibroblasts, endothelial cells, and pericytes. Cancer-associated fibroblasts (CAF) are the activated fibroblasts inside the tumor. CAFs play important roles in the induction of angiogenesis, modification of ECM, inflammatory cell recruitment, secretion of growth factors and immunosuppressive cytokines, and the interaction between epithelial cells and mesenchyme. Lymphocytes that can access and accumulate inside tumor tissues are called tumor-infiltrating lymphocytes (TIL). Macrophages and neutrophils residing inside and around the tumor are termed tumor-associated macrophages (TAM) and tumor-associated neutrophils (TAN). Depending on the influencing cytokines, both cell types may polarize into proinflammatory (M1/N1) or suppressive (M2/N2) macrophages and neutrophils. Nearly all skin cancers have a high level of TAM and regulatory T cells (Treg). However, melanoma typically has a high level of migration inhibitory factor (MIF), while SCC possesses a wide array of CAF genetic variation. Therefore, in-depth knowledge of the mechanism of TME in each type of cancer is crucial for therapeutic success and the prevention of metastasis.

3. **Skin Cancer Immunogenicity**

The incidence and severity of skin cancer increase in people with T-cell immunosuppression, such as patients with HIV/AIDS or organ transplant recipients. Higher risk of skin cancer was also observed in patients treated with immunosuppressive agents, such as cyclosporine and azathioprine. Ultraviolet (UV) exposure may not only induce mutagenesis but may also evoke an immunosuppressive effect that weakens the immune system’s ability to fight skin cancer. These facts prove the importance of the immune system in controlling skin cancer and provide evidence that supports the immunogenicity of skin cancer.

The strong immunogenicity of skin cancer is derived from the expression of tumor antigens, specifically tumor-associated antigens (TAA), neoepitopes, and viral oncoproteins. Melanoma expresses various types of TAAs in excessive amounts, such as melanoma antigen gene (MAGE), melanoma antigen recognized by T cells-1 (MART1), and New York-esophageal cancer-1 (NY-ESO1). The expression of neoepitopes is caused by UV-induced mutation resulting in high tumor mutational burden (TMB) in several non-viral skin cancers, namely melanoma.
SCC, BCC, and virus-negative Merkel cell carcinoma.\(^{11}\)

Viral-associated skin cancer typically has low TMB, but its antigenicity remains high due to viral oncoprotein expression by the oncovirus (HHV-8) and Merkel cell polyomavirus (MCPyV).\(^{11,12}\) High immunogenicity of viral-associated skin cancer results in the activation of defense mechanisms to suppress immune effector function and avoid elimination. Therefore, any therapeutic modality targeting this mechanism or augmenting immune response could be a promising approach for cases of skin cancer unresponsive to conventional therapies.

**Immunotherapy for Skin Cancer**

Immunotherapy for cancer begins to develop rapidly in the last ten years. In 2018, the Nobel prize in Physiology and Medicine was awarded to James P. Allison and Tasuku Honjo for the invention of cancer therapy through inhibition of two immune checkpoint molecules expressed on T cell, cytotoxic T-lymphocyte-associated antigen (CTLA)-4 and programmed-death (PD)-1.\(^{13}\)

Previously, several types of cancer immunotherapy that harness and target different immune components in the TME have been used in skin cancer.

1. **Immunotherapy for Melanoma**

   a. *Bacillus Calmette-Guérin*

   *Bacillus* Calmette-Guérin (BCG) is a live attenuated strain of *Mycobacterium bovis* that can stimulate a strong nonspecific immune response. According to an initial study, the administration of high dose BCG to eight metastatic melanoma patients resulted in a 90% regression of local nodules and 20% regression of metastatic nodules, which proved the influence of immune system manipulation in the clinical course of melanoma.\(^{14}\)

   Intrallesional BCG injection is categorized into Level of Evidence 2B in the 2020 National Comprehensive Cancer Network (NCCN) guideline for local therapy of unresectable stage III melanoma (clinical satellite/in-transit) (Appendix 1).\(^{15}\) An in vitro study in 2017 by Lardone et al. concluded that BCG exerts its function by programming macrophages inside the TME into a transcriptionally and functionally distinct cell population (M2-BCG). M2-BCG enhances IFN-γ production by CD4 T cells and increases granzyme B-producing CD8 T cells, thus augmenting antitumor immunity against melanoma.\(^{16}\)

   BCG can be injected directly into the nearest axillary and inguinal region from the malignant melanoma lesion at a dose of 0.1-0.5 mL every week for one year, every two weeks for six months, and monthly afterward.\(^{14}\) Currently, BCG is not widely used for melanoma, especially because of its high incidence of post-administration adverse effects (fever, abscess, lymphadenitis, and systemic infection) and the approval of dacarbazine chemotherapy (DTIC) for melanoma around the same period.\(^{11,14}\)

   b. **Cytokine therapy**

   IFN and IL-2 are two of the most studied cytokines for antitumor therapy. IFN-α may inhibit melanoma cell proliferation, induce apoptosis, decrease the secretion of vascular endothelial growth factor (VEGF), and increase class I MHC expression. Extrinsically, IFN-α enhances clonal expansion and cytotoxic effect of CD8 T cells, induces NK cells, optimizes DC maturation and function, and stimulates polarization into T helper (Th)1 response.\(^{11,17}\)

   In Europe, IFN-α is approved as adjuvant therapy for stage II and stage III melanoma, and post-excision of the primary tumor in which metastatic involvement was confirmed by lymph node dissection or sentinel lymph node biopsy. IFN-α can also be used in combination with other therapies, such as chemotherapy and checkpoint inhibitors. However, its success was hampered by the low response rate and high toxicities observed by previous studies (neutropenia, diarrhea/colitis, liver enzymes elevation, rash, and anxiety/depression).\(^{17}\)

   IL-2 is produced by mature T cells, NK cells, and DC. It plays a vital role in the differentiation and activity enhancement of those cells in an autocrine manner.\(^{18}\) IL-2 has been shown to induce tumor regression in melanoma patients. Together with BCG, it is included as category 2B in the 2020 NCCN guideline for local therapy of unresectable stage III melanoma (clinical satellite/in-transit) (Appendix 1).\(^{15,18}\) As a monotherapy, IL-2 did not exert a significant response in metastatic melanoma, mainly due to its high-dose toxicities (*vascular leak syndrome*, pulmonary edema, hypotension, and heart disorder) and the paradoxical nature of IL-2 in stimulating both CD8 T cell and Treg expansion.\(^{11,18}\)
c. Adoptive cell therapy

Tumor cells may bring about various mechanisms to escape T cells. This pathomechanism may be potentially addressed using the adoptive cell therapy (ACT), in which T cells with high avidity towards tumor antigen are isolated from the patient, expanded in vitro, genetically engineered, or activated ex vivo to obtain stronger antitumor ability. ACT that is currently being developed for skin cancer are TIL and transgenic T cells.\(^{11}\)

\[\text{TCR-T cells are only suitable for patients who are MHC-compatible and patients with high expression of identical target antigens. Moreover, the tumor may eventually adapt by decreasing its MHC expression. To counteract this mechanism, CAR-T cell technology was developed, in which it fuses antibody single-chain variable fragment (scFv) with the intracellular signaling domain of T cell. This method produces T-cells capable of antigen recognition independent of MHC or antigen presentation by antigen-presenting cells (APC).}\(^{19}\)

\[\text{The biggest challenge in applying ACT for solid tumors is the TME. The fluctuation of chemokine secretion of abnormal neovascularization may inhibit and disturb T-cell mobilization into the tumor. The secretion of inhibitory cytokine, such as transforming growth factor (TGF)-β, expression of immune checkpoint molecules, and transformation of tumor metabolic environment can suppress the function of T-cell effectors. Furthermore, Treg and MDSC populations in the TME may naturally suppress immune responses.}\(^{20}\)

\[\text{d. Checkpoint inhibitor}\]

Activated T cells express surface inhibitory receptors, namely CTLA-4 and PD-1. T-cell activation needs the binding of TCR with MHC and a costimulatory signal from the binding of CD28 on T cells with its ligands on APC, B7-1, and B7-2. CTLA-4 acts as a competitor of CD28 by binding with B7-1 and lowering T-cell response.\(^{22}\) The binding of PD-1 with its two ligands: PD-L1, which is overexpressed on tumor cells; and PD-L2, expressed by APC, delivers
inhibitory signals to T cells. CTLA-4 and PD-1 work as immune checkpoints which prevent T-cell hyperactivation, but it is often misused by tumors to escape from T cells. Checkpoint inhibitors (CPI) bind these receptors, block their bonds with ligands, and relieve the “brakes” towards antitumor activities of T cells (Figure 2). In 2011, the anti-CTLA-4 antibody (ipilimumab) was the first CPI approved by the Food and Drug Administration (FDA) for its use in melanoma, while anti-PD-1 antibodies (pembrolizumab dan nivolumab) were approved in 2014. Numerous initial clinical studies showed better overall survival (OS), progression-free survival (PFS) and OR in melanoma patients treated with CPI compared to those in the control group. However, the best result was obtained by combining both of these CPI, with 11.5 months of PFS (compared to 2.9 months with ipilimumab monotherapy) and 56.6%-58.9% tumor responses (compared to 19%-45% with ipilimumab or nivolumab monotherapy).

Figure 2. Checkpoint inhibitor therapies for cancer (Ayoub NM, Al-Shami KM, Yaghan RJ. Immunotherapy for HER2-positive breast cancer: Recent advances and combination therapeutic approaches. Breast Cancer Targets Ther. 2019;11:53-69).

CTLA-4 blockade disturbs the natural inhibition of autoreactive T cells, causing the risk of immune-related toxicities in the cutaneous system (pruritus, vitiligo, rash), gastrointestinal system (diarrhea, colitis), liver (hepatitis), and endocrine glands (hormonal imbalance). Anti-PD1 is less toxic than anti-CTLA-4 or chemotherapy, and the combination of both agents results in higher toxicity compared to monotherapy. In the 2020 NCCN guideline, ipilimumab was included as category 2A for second-line therapy in metastatic melanoma, while nivolumab and pembrolizumab were included as category 2A for adjuvant therapy in stage III melanoma, and category 1 for first-line therapy in metastatic melanoma (Appendix 1).

The efficacy of checkpoint inhibitors is dependent on the availability of functional tumor-specific T cells, while adoptive cell therapy needs an optimal environment to maximize its antitumor effect. Consequently, several trials have explored these therapies' potency when combined to exert higher effectiveness and long-lasting antitumor effects. Preclinical trial of CAR-T cell and anti-PD-1 combination in transgenic Her2 mice showed enhanced proliferation and functional capabilities of Her2-specific T cells, in addition to stronger tumor regression. Another trial came up with a chimeric protein that fused the extracellular and transmembrane domains of CTLA-4 or PD-1 with a cytoplasmic activating signal domain, such as CD28 (PD-1/CD28). When this novel protein binds to its ligand, the inhibitory signal of CTLA-4 or PD-1 transforms into a stimulatory signal which maximizes T cells' anti-tumor function.
Non-melanoma skin cancer (NMSC) comprises several types of skin tumors; the two most commonly reported types are BCC and SCC. The basic concept of immunotherapy in NMSC is similar to that in melanoma; however, most immunotherapies are currently applied exclusively in melanoma. Melanoma is the most common cause of skin cancer-related mortality worldwide. Its immunogenicity is also stronger than that of NMSC due to the overexpression of TAA and UV-associated mutations resulting in higher TMB.11 Trials regarding immunotherapy in melanoma showed promising results, particularly CPI. Therefore, researchers have begun to explore the use of immunotherapy in NMSC.

Anti-PD-1 has been reported to be the most promising antitumor response in NMSC. A case report by Ascierto et al. showed durable tumor response following the administration of pembrolizumab in a metastatic SCC patient.27 An objective response (OR) of 34% following pembrolizumab administration was also observed in recurrent or metastatic SCC patients, while a phase II study examining its use as first-line monotherapy for SCC yielded an OR of 41%.7 This result was in line with the phase I clinical trial of anti-PD-1 agent (cemiplimab) in 26 advanced stages SCC patients, which observed a 50% partial response (PR), as well as the subsequent phase II clinical trial in 59 patients which showed a 47% OR and a good safety profile.28 In 2018, FDA approved cemiplimab as a therapeutic agent for patients with locally advanced or metastatic SCC who are not candidates for curative surgery or radiation.7,27 Pembrolizumab was then approved by FDA in 2020 as a therapeutic agent for patients with recurrent or metastatic SCC ineligible for surgery or radiation.7

Merkel cell carcinoma (MCC) is aggressive skin cancer caused by Merkel cell polyomavirus. A clinical trial of an anti-PD-L1 agent (avelumab) in 39 patients with metastatic MCC showed an OR of 62.1%.29 This result was supported by the phase II clinical trial of pembrolizumab in 50 patients with advanced-stage MCC which showed a 56% OR.30 Both trials observed a strong and durable antitumor response with tolerable adverse effects, but discontinuation of therapy and death were observed in a minority of patients. In 2017, FDA approved avelumab as a therapeutic agent for patients with metastatic MCC.31

Studies regarding immunotherapy for other types of NMSC are still scarce. In a prospective trial in 2019 consisting of nine BCC patients treated with pembrolizumab, an OR of 44% was noted. Administration of nivolumab in one BCC patient with multiorgan metastasis who previously received kinase inhibitor and chemotherapy resulted in almost full recovery of liver metastasis. Another case report showed stable disease without any worsening disease using anti-PD-1 therapy for BCC and metastatic basosquamous carcinoma.31 A phase II study on cemiplimab for metastatic BCC and locally advanced BCC showed ORs of 21% and 29%, respectively. These results led to the approval of cemiplimab by the FDA in 2021 for patients with advanced BCC, which has been treated with or not suitable for Hh pathway inhibitors (HHI) treatment.7 Anti-PD-1 and anti-PD-L1 therapies were also studied in other tumor types, and reports showed that responses in skin cancers were better than in solid tumors of other systems.11

Conclusion

Skin cancer is an immunogenic tumor. Immunotherapy has a potential role as an alternative treatment for patients with advanced-stage skin cancer unresponsive to conventional therapies. Until recently, CPI therapy showed the most promising result compared to other immunotherapies, and it has been approved as a therapy for skin cancers. Nevertheless, a small percentage of patients experienced toxicities due to post-immunotherapy reactivation and immune system enhancement. The appropriate combination of immunotherapy and other therapeutic modalities may potentially result in better long-term efficacy and tolerability. Further biomedical, immunological, and clinical studies in skin cancer are warranted to better understand the pathogenesis and to design safe and effective combined treatment according to the characteristics of each type of cancer and the patient’s conditions.

References


### Appendix 1. NCCN categories of evidence and consensus.*

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<tr>
<th>Category</th>
<th>Description</th>
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<tbody>
<tr>
<td>Category 1</td>
<td>Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate</td>
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<tr>
<td>Category 2A</td>
<td>Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate</td>
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<tr>
<td>Category 2B</td>
<td>Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate</td>
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<tr>
<td>Category 3</td>
<td>Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate</td>
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