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Review

Novel anti-melanoma treatment: focus on immunotherapy

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Abstract

Melanoma is an intractable cancer that is aggressive, lethal, and metastatic. The prognosis of advanced melanoma is very poor because it is insensitive to chemotherapy and radiotherapy. The incidence of melanoma has been ascending stably for years worldwide, accompanied by increasing mortality. New approaches to managing this deadly disease are much anticipated to enhance the cure rate and to extend clinical benefits to patients with metastatic melanoma. Due to its high degree of immunogenicity, melanoma could be a good target for immunotherapy, which has been developed for decades and has achieved certain progress. This article provides an overview of immunotherapy for melanoma.

Key words Melanoma, immunotherapy, tumor vaccine, cytokine, CTLA-4, PD-1

Melanoma is an aggressive skin cancer with a high mortality and a poor prognosis[1]. In advanced and metastatic stages, the median overall survival (OS) ranges from 6 to 9 months, and the 1-year survival rate ranges from 30% to 60%[2,3]. Traditional therapies (surgery, chemotherapy, and radiotherapy) are not effective in managing advanced metastatic melanoma and are often accompanied by inevitable adverse effects[4]. However, melanoma is known to be highly immunogenic and is therefore an attractive candidate indication for immunotherapy[5]. We review the current therapies for melanoma, with an emphasis on immunotherapeutic methods.

CTLA-4 and PD-1/L1 inhibitors

CTLA-4 inhibitors

The cytotoxic T lymphocyte-associated antigen-4 (*CTLA-4*) gene contains a cluster of T-lymphocyte immunoregulatory genes. This gene plays a key role as a negative regulator that inhibits signaling to T cells and has antitumor activity. Reports based on a murine model showed that no mice survived when the whole *CTLA-4* gene was knocked out[6]. This finding supports the idea that CTLA-4 is a "druggable target." A retrospective analysis on 100 patients treated with anti-CTLA-4 agents showed a remarkable record on disease control and progressive disease (36.1 months vs. 4.0 months) on induction treatment [7].

Ipilimumab (IPI) is a fully human monoclonal antibody (mAb) against CTLA-4 that was approved for clinical use by the Food & Drug Administration (FDA) of USA in 2011. Currently, IPI is available as a first- or second-line monotherapy for unresectable or metastatic melanoma and is effective for either the wild-type or the *B-RAF-V600E*-mutated type at a dose of 3 mg/kg of body weight[8]. This antibody is the only molecular targeted drug whose use is supported by a phase III clinical trial[9]. The large clinical trial involved 676 advanced melanoma patients treated with IPI who showed improvement in OS rate and median OS, regardless of gp100 peptide vaccination[10,11]. The adverse effects caused by IPI can be severe, with the hypophysitis incidence ranging from 0 to 17%, limiting the clinical beneficial rate[12,13]. Monotherapy with IPI has toxicity greater than the combined use with dacarbazine (DTIC) does, and even the objective response (OR) rate and OS rate are lower than those for the combination of drugs[14].

A randomized phase II study of IPI therapy combined with carboplatin and paclitaxel in 30 melanoma patients at stages III-IV showed that the response rate and disease control rate (DCR) for 14 evaluable patients at the time point of 6 months were 21.4% and 42.9% or 35.7% and 64.3%, respectively, depending on the evaluation criteria. Notably, 63% showed grade 3/4 immune-related adverse events (irAEs), such as hepatotoxity, electrolyte imbalances, myelosuppression, and infections[15].

In a retrospective analysis of approximately 193 melanoma patients initiated with *B-RAF* inhibitor therapy, with a median OS of 2.9 months, 40 patients subsequently received IPI treatment; their median progression-free survival (PFS) was 2.7 months, and their median OS was 5.0 months. The results of IPI treatment following

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B-RAF inhibitor therapy were poor, so randomized controlled trials are needed to determine whether immunotherapy is better before or after *B-RAF* inhibitor therapy, especially in the patients with *B-RAF* mutation[16].

Another retrospective study included 45 patients (23 with brain metastasis) with unresectable stage III or IV melanoma who showed a 13% overall response rate (ORR) and a median OS of 8 months[17]. In fact, OS had no difference between patients, regardless of the presence of brain metastasis and *B-RAF-V600E* mutation[17]. Compared with a placebo, IPI showed obvious clinical benefits for the relapse-free survival (RFS) of patients with completely resected stage III melanoma (26.1 months vs. 17.1 months). However, the toxicity was also significant, and the grades 3–4 irAE rate was 42%[18].

Tremelimumab (treme) is another anti-CTLA-4 mAb that was designed with an IgG2 Fc domain, in contrast to IPI, with an IgG1 domain. Because of the different structures, treme shows less drug-related toxicities[19]. However, the evidence for treme use as a first-line therapy for metastatic melanoma is not enough, although it shows a potential benefit in refractory or relapsed melanoma, with an OR rate of 6.6% and a median OS enhanced from 6 months to 10 months[20]. Compared with standard chemotherapy in advanced melanoma patients, treme has failed to demonstrate a significant advantage regarding the OR rate[21].

PD-1/PD-L1 inhibitors

Programmed death-1 (PD-1, CD279) is an inhibitory co-receptor expressed on antigen-activated T cells, B cells, natural killer (NK) cells, and tumor-infiltrating lymphocytes (TILs) after binding to its ligand PD-L1/2. PD-1 blockade has emerged as a promising strategy for cancer therapy, and anti-PD-1 or anti-PD-L1 mAbs may improve T-cell activation and functions[22].

Lambrolizumab, an anti-PD-1 antibody, yielded a confirmed response rate of approximately 38%, a median PFS of more than 7 months, and low-grade adverse events[23]. Furthermore, re-induction anti-PD-1 therapy achieved a partial response (PR) that was maintained for 16 months off therapy[24]. As noted, most of the adverse effects were immune-related[19]. These results attracted more attention to immunomodulatory mAbs.

Nivolumab (Nivo) is a promising anti-PD-1 antibody. This fully human IgG4 mAb was designed for the treatment of cancer and is well tolerated in IPI-refractory or metastatic melanoma. The OR rate reached 25%, and clinical responses were maintained up to 140 weeks[25]. Based on a recent study, Nivo was revealed to have an OR rate of 64%, a median OS of 17.3 months, a 2-year OS rate of 48%, and a 3-year OS rate of 41% in IPI-naive melanoma patients[26]. Additionally, the safety profile was in a favorable range, with a frequency of grades 3–4 irAEs of 5%[26]. In a comparison of Nivo treatment in IPI-naive and IPI-refractory patients, the OS and PFS showed no obvious differences, and the ORR was 26% in both groups. More importantly, Nivo did not cause additional grade 3/4 irAEs beyond IPI-related ones[27]. Additionally, tumor PD-L1 expression was associated with ORR and weakly associated with OS and PFS[26].

MK-3475 (pembrolizumab), a fully human mAb against PD-1, with no cytotoxicity, has shown potent antitumor activity at different doses in patients with melanoma. In a randomized dose-evaluation phase I trial, 173 patients received pembrolizumab at 2 mg/kg (n = 89) or 10 mg/kg (n = 84). The primary endpoint ORR had no significant difference between the two dose groups, with a value of 26%. The safety profiles were also similar and both well tolerated, as only 1 case of grade 3 fatigue was reported[28]. Based on the newest data from a phase II clinical study of 411 patients with melanoma treated with pembrolizumab, the outcomes were an OR rate of 72%, a median PFS of 5.5 months, and a median OS estimated to be more than 24 months[29]. Moreover, the positive rate of tumor PD-L1 expression has been linked to PFS, but not OS[29]. Another randomized clinical trial (n = 275) of two doses of pembrolizumab for IPI-refractory or IPI-naive melanoma showed no difference within each group for the ORR, PFS, and OS by dose[30]. However, the ORRs were 26% for IPI-refractory patients and 40% for IPI-naive patients[30]. The above-mentioned research data prompted the FDA to approve pembrolizumab as a breakthrough therapy for advanced melanoma, even though further clinical trial data are still needed.

CD137 is also induced by activated lymphocytes and is a promising target for immune costimulatory mAbs[31]. Anti-CD137 antibodies prevent activation-induced death in melanoma cells. Recently, BMS-663513, a humanized anti-CD137 mAb, has entered clinical trials for immunotherapy for solid tumors, including melanoma[32].

CTLA-4 and PD-1 showed complementary actions in regulating adaptive immunity[33]. Moreover, the combination of Nivo and IPI resulted in high response rates and manageable toxicity, whether administered concurrently or sequentially[14], with tumor reduction of 80% or more and a manageable safety profile[34]. More specifically, patients with concurrent IPI/Nivo treatment had a 43% ORR, with 17% experiencing a complete response (CR) and 82% in remission. The 2-year OS rate was 79%. Nevertheless, the frequency of grade 3/4 irAEs was 62%[35].

Tumor Vaccines

Tumor vaccines contain tumor antigens or tumor antigen peptides. The treatment principle is to stimulate patients' specific antitumor immune response via the introduction of tumor antigens. To date, studies of melanoma vaccines have focused on three vaccines, namely, a dendritic cell (DC) vaccine, a melanoma-associated antigen A3 (MAGE-A3) vaccine, and talimogene laherparepvec (T-VEC)[36-38].

DCs are the most efficient antigen-presenting cells (APCs)[39]. DC vaccines are composed of peripheral blood monocytes pulsed with antigens *in vitro*, and their therapeutic potential has been explored in melanoma[40]. Phase III studies of DC vaccination demonstrated that an autologous monocyte-derived DC vaccine extended median survival compared with monotherapy with DTIC[41,42]. Moreover, when cyclophosphamide (CTX) was used as an adjuvant for a DC vaccine with interleukin-2 (IL-2), the vaccine showed only mild adverse effects and was well tolerated (with a median PFS of 4.5 months and a median OS of 9.4 months)[43]. Another study, examining 24 advanced

melanoma patients [22 human leukocyte antigen (HLA)+] treated with a DC vaccine, reported exciting results, including 1 case of PR, 7 cases of stable disease (SD), and 16 cases of progressive disease (PD)[44]. The mean OS was 13.6 months in the vaccinated group compared with 7.3 months in the non-vaccinated group, and no more than grade 3 adverse effects were observed[41,44,13]. Of note, HLA+ melanoma is well known as a common genotype, and approximately 60% of Asian melanoma patients are HLA+. Further clinical trials of DC vaccination are warranted in Chinese patients of this genotype.

MAGE-A3 is a tumor-specific antigen expressed in ~76% of metastatic melanoma, but not in normal cells[45]. Certain published evidence has shown that an MAGE-A3 vaccine may provide plausible routes for inhibiting or even eliminating cancer cells in advanced melanoma[46]. The clinical responses generated by this vaccine showed a certain degree of benefit[47]. However, one trial of an MAGE-A3 cancer immunotherapy showed no significant extension of disease-free survival (DFS) compared with the placebo arm[48]. In a phase III clinical study, compared with placebo, the MAGE-A3 vaccine did not show significantly prolonged PFS in the overall patient population[49]. The secondary endpoint, PFS, has yet to mature in the MAGE-A3-positive patients and needs follow-up[49]. Thus, whether this vaccine can be used in patients needs further investigation.

Another novel oncolytic vaccine, T-VEC, which had been genetically modified from herpes simplex virus, was directly injected into tumors and selectively replicated in tumor cells until they rupture or die[38]. Additionally, T-VEC secretes the cytokine granulocyte macrophage colony-stimulating factor (GM-CSF) to enhance local and systemic antitumor immune responses[50]. Biweekly intratumoral administration of T-VEC to patients with unresectable melanoma was well tolerated, with an ORR of 26%[51]. The Oncovex (GM-CSF) Pivotal Trial in Melanoma (OPTiM), a randomized phase III trial, included 436 patients with stage IIIB/C or IV melanoma and compared biweekly intralesional T-VEC with subcutaneous GM-CSF in terms of meeting the primary endpoint, resulting in durable response rates of 16.3% and 2.1%, respectively[52]. In this trial, OS was borderline significantly prolonged, with a median of 23.3 months instead of 18.9 months, along with a frequency of serious adverse events of 13% to 26%[52]. In addition, the trial indicated that T-VEC is an effective therapy for local lesions, as the response rate was 64%[53].

Tumor vaccines have shown low toxicity in melanoma therapy, but the advantages in terms of clinical benefits are not obvious, and the possible use of monotherapy needs more proof. Certain previous reports indicated that the vaccine-induced immune response can be increased by cytokines, such as IL-2, IL-4, and GM-CSF. Combination with checkpoint protein inhibitors would also augment the clinical benefits[36]. These hypotheses were proven by a multicenter phase I trial aiming to assess the safety and validity of combining T-VEC with IPI in advanced melanoma patients (n = 18), which showed an ORR of 56%, a CR rate of 33%, a PR rate of 22%, and a SD rate of 17%, suggesting higher CR and OR rates than those of either agent alone. Of note, the adverse effects were mild, as only 3 grades 3–4 irAEs were observed for IPI[54]. Further phase II (IPI vs. T-VEC + IPI) trials are ongoing.

Cytokines

IL-2 is produced by NK cells after antigen activation and was initially described as a growth factor that is necessary and sufficient for T-cell maturation and proliferation, NK-cell activation, and immune response regulation. Since Rosenberg *et al*.[55] first combined IL-2 and CTX to treat malignant melanoma and achieved a promising result in 1988, a series of studies on IL-2 as an agent for treating melanoma patients have emerged, including a study on intralesional IL-2 therapy.

Recently, an analog of human recombinant IL-2, proleukin, has been discovered to play a critical role in the immune response, although the clinical benefit is not yet known[56]. Another immunocytokine, selectikine (or NHS-IL-2), is a genetically modified form of IL-2 composed of a fusion protein that has been proven to have antitumor activity in preclinical studies[57]. Either as a monotherapy or combined with radiotherapy, selectikine showed a favorable safety profile and induced biological effects in a phase I dose-escalation trial for solid tumors[58]. The results of the study revealed that the dose-limiting toxicity was a skin rash, and a dose of 0.45–0.6 mg/kg was recommended for further phase II evaluation. A phase II trial aiming to determine the maximum tolerated dose of selectikine combined with stereotactic body radiation therapy in advanced melanoma patients is now underway[59].

Intralesional therapy with IL-2 in primary cutaneous advanced melanoma would standardize clinical trials and enable new approaches to adjuvant therapy in terminal melanoma patients[60]. Furthermore, patients who receive IL-2 plus active specific immunotherapy, a patient-specific tumor stem cell vaccine derived from autologous tumor cell lines, would enjoy better OS: a longer median survival or a higher 5-year survival rate[61].

High-dose (HD) IL-2 was approved by the FDA in 1998 as therapy for unresectable melanoma. However, many aspects of IL-2 therapy in melanoma are still being studied intensively. Several studies showed that HD IL-2 has no apparent superiority over low-dose (LD) IL-2, strengthening the idea that HD IL-2 is not the prime choice for melanoma patients[62]. Furthermore, due to its unfavorable toxicity profile (25%–85%) and lower durable CR rate (4%–5%), IL-2 is not widely used as the main treatment for melanoma[63]. A phase II trial of an intratumoral IL-12 plasmid for unresectable melanoma treatment suggested that local treatment was well tolerated, with no more than grade 2 irAEs, and could induce an enhancement of systemic antitumor immunity. The ORR was 33%, the CR rate was 11%, and 62% of non-injected tumors regressed. Further evaluation of increased treatment frequency is undergoing for melanoma patients[64].

Another cytokine, interferon (IFN)-α, is the first cytokine used for melanoma therapy. With anti-angiogenic effects, IFN-α shows an advantage in increasing RFS and disease stabilization[65]. Adjuvant IFN-α significantly reduced the risk of relapse and improved OS (38.1%–46% in 5 years and 28.0%–38.5% in 10 years) in a meta-

analysis[66]. Of note, HD IFN-α2b for 1 year is the approved standard dosing regimen for stage IB–IV melanoma, although the treatment achieves RFS in only 20%-33% of patients[67]. Currently, general concerns are focused on polyethylene glycol interferon-α-2b (PEG-IFN). This formulation has been approved by the FDA for adjuvant treatment of melanoma patients, with a positive impact on RFS in stage III melanoma patients[68]. Regarding long-term results, patients with stubborn melanoma treated with PEG-IFN have received great benefits[69]. When a graded dose (7.5 pg/mL) of PEG-IFN within a reference range was used in stage IV melanoma patients, the clinical responses were a PR rate of 7% and a SD rate of 17%; median PFS and OS were 2.0 and 9.7 months, respectively[70]. Furthermore, the outcomes showed an acceptable safety profile for PEG-IFN[70]. Based on these trials, PEG-IFN might be a choice for metastatic melanoma patients and may also provide a foundation or certain novel ideas for future clinical trials.

However, according to the Dermatologic Co-operative Oncology Group (DeCOG) trial, PEG-IFN reveals no significant difference in distant metastasis-free survival (DMFS) and DFS compared to LD IFN in stage IIA–IIIB melanoma. The multicenter, open-label, randomized phase III trial for adjuvant therapy with PEG-IFN or LD IFN enrolled 909 patients, and 907 (451 PEG-IFN and 458 IFN) achieved a median follow-up time of 5 years. The data indicated the primary endpoint DMFS of 65.1% to 70.2%, secondary endpoints OS of 74.2% to 74.8%, and DFS of 57.9% to 60.8%[71]. Adverse effects were more likely in the PEG-IFN arm, such as leukopenia and an increase of liver enzymes levels [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)][71]. Fortunately, combination therapy with IPI for stage III/IV melanoma patients (n = 31) resulted in 2 cases of CR, 9 cases of PR, 3 cases of SD, and 12 cases of PD. The OR rate was 53.8%, indicating that PEG-IFN combined with IPI resulted in a great clinical benefit and a tolerable toxicity profile[72]. The data from this trial warrant further study.

Targeted Therapy with Small Molecules

B-RAF inhibitors and MEK inhibitors

B-RAF and MEK are both involved in the RAS-RAF-MEK-ERK cascade signaling pathway, regulating several important cellular functions, such as cell survival, proliferation, and apoptosis resistance[73]. *B-RAF* has been treated as a driver oncogene in melanoma, as nearly 50% of melanoma cases exhibit a mutated *B-RAF* gene and as drugs targeting *B-RAF* seemed to be a potential effective way to treat patients[74].

Vemurafenib and dabrafenib are the first generation of selective B-RAF inhibitors. These inhibitors were approved as a single drug for metastatic or unresectable melanoma patients by the FDA in 2011 and 2013, respectively. Although these inhibitors showed improvement in PFS and OS in advanced melanoma with *V600*-mutant *B-RAF*, the adverse effects caused by the combined treatment should not be ignored[75-77]. The small-molecule MEK inhibitor trametinib was approved by the FDA in 2013 for *B-RAF-V600E*-mutated or *B-RAF-V600K*-mutated unresectable melanoma patients. Compared with B-RAF inhibitors, trametinib has serious adverse effects and low OR rates[78]. B-RAF inhibitors and MEK inhibitors are both blockers of the MAPK signaling pathway; one acts upstream, and the other acts downstream. This association in *B-RAF*-mutant melanoma obviously improves PFS when adverse events occur more commonly[79].

Other small-molecule therapies

Hot shock protein 90 (HSP90) has emerged as a potential therapeutic target in many cancers. Ganetespib is a novel potent HSP90 inhibitor, and data have illustrated that targeting both *B-RAF-V600E* and HSP90 provided a combinatorial benefit *in vitro* and *in vivo*[80]. Oblimersen is the sixth exon open reading frame of an antisense oligonucleotide targeting Bcl-2[81]. The combination of oblimersen, nab-paclitaxel, and temozolomide had a DCR of 75% and a 6-month PFS rate of 34.4% in advanced melanoma patients, and more importantly, the treatment-related adverse events, which were commonly grade 1 or 2, were well tolerated[82].

Ganglioside (GD2) is a cell surface glycosphingolipid that is highly expressed on cancer cells and that has been chosen as an attractive target for immunotherapy[83]. Certain previous studies suggested that the anti-melanoma activities of GD2 inhibitors in a xenograft mouse model did not show any obvious neurotoxicity, and preclinical trials showed that trifunctional bispecific antibody therapy did not break tolerance to auto-antigens[84].

According to these findings, it is necessary to conduct further clinical studies on the therapeutic use of ganetespib, oblimersen, and GD2, either as a single agent or a combinatorial partner, in melanoma.

Summary

Recent years have witnessed tremendous progress in immunotherapy for melanoma. Many therapeutic approaches have been promoted, including cancer vaccines, cytokines, immune checkpoint blockade, targeted therapy with molecules, and combined drugs. However, the clinical benefits of immunotherapy are accompanied by limitations, and we are still facing many uncertainties.

As no single agent can cure melanoma both safely and effectively, combined drug therapy within individualized medicine is now a trend. For instance, compared with monotherapy, combining a cytokine with immunomodulatory agents may lead to fewer irAEs. Meanwhile, clinical trials of combined therapies are still in early stages, and more details of the clinical benefits need to be confirmed. Similarly, the combined effects of vaccines and cytokines (IL-2 and IFN-α) are still unclear. Excitingly, *CTLA-4* and *PD-1/PD-L1* show massive potential for treating advanced melanoma, and the co-administration of anti-CTLA-4 and anti-PD-1 offers a durable response. The FDA has approved pembrolizumab as a breakthrough therapy for stage III/IV melanoma. The high response rate and long OS following concurrent Nivo/IPI was validated, although with notable

irAEs. Additionally, IPI-naive patients may respond better than IPI-refractory patients do after PD-1 blockade. Likewise, as an adjuvant treatment, IPI has prominent clinical benefits for stage III melanoma, and the additional effect of the T-VEC vaccine cannot be ignored. At present, how to choose an appropriate regimen from the various combinations is also a major challenge. Whether concurrent or sequential checkpoint protein blockades will increase toxicity must be verified, and the results of a phase II trial of pembrolizumab and Nivo is a focus in this research area. Overall, immunotherapy will be an indispensable part of the clinical treatment of malignant melanoma in the future.

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