



A New Era In Treatment Of Malignant Melanoma: Biological therapies

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ABSTRACT

Even though melanoma skin cancers represent few cases (less than 5% of all skin cancer cases), their overall mortality rate is strikingly higher, i.e., accounts for about 60-80% of deaths. The reason is that treatment of the malignant form of this disease has always been a therapeutic challenge. Until 2011, the favored therapeutic approaches for advanced melanoma included interleukin-2 and nonspecific alkylating agents and/or antineoplastic agents. However, these traditional treatment approaches have always shown a grimmer prognosis due to their relatively low survival benefit and toxicity. Over the past decades, with the better understanding and study of the effect of immunological approaches in the development of melanoma, treatment approaches have undergone changes. Accordingly, various institutions for biological modalities have shown some improvement in the treatment scenario of malignant melanoma. As a result, in this review, we discussed newer novel therapeutic agents and summarized the outcomes from recent major clinical trials.

1. Introduction

Malignant melanoma is a highly aggressive skin neoplasm arising from the malignant transformation of melanocytes. According to the appearance and localization of the tumor, it is divided into four clinical subtypes; nodular melanoma, lentigo melanoma, acral lentiginous melanoma, superficial spreading melanoma. The latter is the most frequent one that accounts for 50-70% of melanoma cases.

The global incidence of cutaneous malignant melanoma has steadily increased over the past 50 years in predominately fair-skinned Caucasians populations [1], with an annual increase of 0.6% among adults [2]. Data from the World Health Organization (WHO) demonstrated that 132,000 melanoma skin cancers occur globally each year. In 2017, new cases of melanoma were estimated

to be 87,110 in the United States, leading to 9,730 deaths from the disease [3]. Exposure to ultraviolet radiation from the sun and genetic factors exert an important role in the etiology of the disease [4]. In early stage melanoma (stages 1 and 2), the prognosis is often positive as the tumor is highly localized; thus, the tumor can be easily treated by surgical excision and adjuvant therapy. In contrast, the prognosis for advanced stage melanoma is extremely poor and the 5-year survival rate varies from 5 to 19% [5].

Until recently, only dacarbazine, hydroxyurea and interleukin-2 (IL-2) were approved by the US Food and Drug Administration (FDA) approved for the treatment of metastatic melanoma; although, none have ever shown promising results

in prolonging survival. Hydroxyurea showed only encouraging efficacy in metastatic brain lesions combined with radiotherapy [6]. Decarbazine was approved by the FDA in 1975 for the treatment of malignant melanoma. The objective response rate alone for this drug has been reported to be up to 25%. Combination therapy with other cytostatic agents did not show any significant results [7]. In 1998, the FDA approved aldesleukin, a high-dose recombinant intravenous IL-2, for the treatment of malignant melanoma. Although, high-dose IL-2 has alone been approved for the treatment of metastatic melanoma for nearly two decades, it displays relatively low efficiency with about 10% response rate and is associated with severe side effects including inflammatory response syndrome, hypotension, nausea, vomiting and diarrhea [8]. However, promising data continue to be collected regarding concomitant use of IL-2 with other therapies [9].

2. Overview of the mitogen-activated protein kinase (MAPK) pathway

It has been observed that the understanding of signaling cascades involved in the cell growth deregulation in melanoma is crucial for the development of efficient therapies. The mitogen-activated protein kinase (MAPK) pathway activation plays a key role in melanoma development pathway, making it an important therapeutic target [10]. This tightly regulated pathway consists of RAS, RAF, MEK1/2 and ERK1/2, and relays extracellular signals from cell membrane to nucleus via a cascade of phosphorylation events. Under normal circumstances, the signaling cascade is stimulated by the binding of extracellular growth factors to receptor tyrosine kinases, which induces dimerization and provokes the activation of oncogenic RAS by a GDP to GTP ligand switch. The activated RAS then triggers the formation of the "MAPK complex" by following RAF, MEK1/2, ERK1/2 and several sets of proteins initiating the MAPK cascade. Moreover, the activated RAS causes the membrane recruitment and stimulates RAF, which in turn causes the phosphorylation of MEK and ERK from the MAPK complex, subsequently resulting in activation of many transcription factors. Activation of the MAPK pathway regulates the expression of several genes encoding proteins involved in cell proliferation, differentiation, and survival. In melanomas, dysregulation of the MAPK pathway occurs frequently due to activating mutations in the BRAF and RAS genes or other genetic or epigenetic

modifications, leading to increased signaling activity promoting excessive cell proliferation, invasion, metastasis, migration and survival [11]. Extensive studies focusing on the presence of different mutations located at different level of this pathway, which were responsible for the development of melanoma, led to the discovery of new therapies. Among all mutations, BRAF is frequently mutated (about 60% of melanoma cases) [12] and among approximately 70 mutated forms of BRAF so far discovered, the BRAF V600E mutant is highly predominant in melanoma (70–90% of all the BRAF V600 mutations). Following these findings on the MAPK/ERK pathway, new drugs targeting the MAPK pathway have generated striking clinical response in melanoma therapy. From the discovery of BRAF mutation in melanoma in 2002 to the approval of the first BRAF inhibitor vemurafenib for melanoma treatment by the USFDA in 2011, therapies targeting the MAPK pathway have been proven effective in less than a decade.

3. Targeted therapies-PART 1: BRAF inhibitors

A. Vemurafenib

Vemurafenib was developed as a first class selective inhibitor of the BRAF serine threonine kinase, which selectively binds to the ATP-binding site of the BRAF-V600E kinase and inhibits its activity [13]. It was the first ever drug approved by the FDA in 2011 for the treatment of unresectable or metastatic BRAF V600E mutation positive melanoma. In a phase I trial, 32 genotype-selected metastatic patients were treated with vemurafenib at the maximum tolerated dose of 960 mg twice daily, and 80 % of them responded to it including two complete responses. A phase II trial with BRAF V600E mutation positive melanoma patients showed a response rate of 53 % and median duration response of 6.7 months. Encouraged by the results of the phases I and II, a phase III randomized clinical trial was held to compare vemurafenib with dacarbazine in terms of efficacy. A 675 patients harboring BRAF mutation with no previous intervention received either vemurafenib or dacarbazine. The results showed the improved OS (OS) rate of 84 % in the vemurafenib group compared with 64 % in the dacarbazine group; moreover, the RR was 48 % for vemurafenib, whereas it was 5 % for dacarbazine. Compared to dacarbazine, vemurafenib reduced the risk of death by 63 % ($P < 0.001$), which validated the clinical efficacy of vemurafenib and led to the approval of this drug by the FDA. Approximately 1% of the

patients in the clinical trials experienced some kind of adverse effects after treatment with vemurafenib alone or in combination. The most common side effects from vemurafenib are cutaneous, and include rash (49%), photosensitivity (31%), alopecia (26%), hyperkeratosis (19%), skin papilloma (15%), dry skin (14%), squamous cell carcinoma (14%) and toxic epidermal necrolysis (TEN) [14]. With frequent and widely use of vemurafenib, it is evolving as a key treatment approach for BRAF mutated melanoma; however, tumor relapse and therapy resistance have been emerging as major problems associated with the use of vemurafenib as a single agent, which may be addressed by its combination with other agents [15].

B. Dabrafenib

Similar to vemurafenib, dabrafenib is another FDA-approved selective BRAF inhibitor for use in patients with melanoma, which tumors express the BRAF V600E gene mutation. After the results of the successful phase I/II clinical trial studies, a phase III clinical trial (BREAK 3) was conducted in patients with previously untreated, metastatic or unresectable BRAF v6000E type melanoma. A total of 250 patients were randomly assigned to receive either dabrafenib or DTIC (decarbazine). Results reported an RR of 50% (6% with DTIC) and a median progression-free survival (PFS) of 5.1 months (2.7 months with DTIC) in patients treated with dabrafenib. Updated survival data reported an improvement of 18 months in the median OS, while it was over 15 months for DTIC [16]. Since dabrafenib and vemurafenib have displayed similar good safety and efficacy in protein kinase (Raf-MAPK) pathway inhibition, these BRAF inhibitors, alone or in combination with a trametinib (MEK inhibitor), have become a standard therapeutic approach in patients with BRAF V600E-mutant or BRAF V600K-mutant advanced melanoma. However, the duration of response is limited in the majority of patients treated with BRAF inhibitor monotherapy because of the development of acquired resistance. The addition of a MEK inhibitor can improve blockade of the MAPK pathway and may help to overcome resistance and thereby prolong efficacy, as well as reduce cutaneous toxicity. Combining dabrafenib and the MEK inhibitor trametinib, as compared with dabrafenib alone, enhanced antitumor activity in this population of patients. In a phase 3 trial, 704 patients with metastatic melanoma with a BRAF V600 mutation received either a

combination of dabrafenib (150 mg twice daily) and trametinib (2 mg once daily) or vemurafenib (960 mg twice daily) orally as first-line therapy. The OS rate at 12 months was 72% (95% confidence interval, 67 to 77) in the combination-therapy group and 65% (95% CI, 59 to 70) in the vemurafenib group (hazard ratio (HR) for death in the combination-therapy group, 0.69; 95% CI, 0.53 to 0.89; $P=0.005$). Furthermore, the median PFS rate was 11.4 months in the combination-therapy group and 7.3 months in the vemurafenib group (HR, 0.56; 95% CI, 0.46 to 0.69; $P<0.001$). The objective response rate was 64% in the combination-therapy group and 51% in the vemurafenib group ($P<0.001$). The most serious adverse effects reported in patients receiving dabrafenib included an increased risk of cutaneous squamous-cell carcinoma and keratoacanthoma. While dabrafenib and vemurafenib displayed similar efficacy in clinical trials, significant difference exists between them in terms of toxicity. Even though these drugs have not been directly compared in a clinical trial, cutaneous toxicity apparently occurs less frequently with dabrafenib than with vemurafenib. In addition, other side effects such as arthralgia hyperkeratosis, hair loss and fatigue tend to be frequent in patients undergoing the dabrafenib therapy. These findings resulted in the approval of dabrafenib for the first line treatment of unresectable metastatic melanoma; however, its use is limited to the BRAF v600E mutant form of melanoma [17, 18].

PART 2: MEK inhibitors

A. Trametinib

Trametinib was the first MEK inhibitor approved for use in treatment of advanced BRAFV600 mutant melanoma as a single agent and in combination with the BRAF inhibitor, dabrafenib [19]. It is similar to vemurafenib and dabrafenib plus, it also targets the MAPK pathway. Specifically, trametinib acts by inhibiting MEK, the only known substrate of BRAF, which in turn leads to decreased cell signaling and proliferation that suppresses tumor growth. In a study on 40 patients with BRAF-mutant melanoma and prior BRAF inhibitor therapy, trametinib was reported to be ineffective, suggesting that BRAF-inhibitor resistance develops with repeated exposure. The most frequent treatment-related adverse events for all patients were skin-related toxicity, nausea, peripheral edema, diarrhea, pruritus and fatigue. Moreover, no cutaneous squamous cell carcinoma

was observed [20]. Yet in another phase III trial, the activity of trametinib was compared with chemotherapy (DTIC or paclitaxel) in the first line setting of 322 patients with BRAF-mutated melanoma. The median PFS and OS were greater in the trametinib group (mPFS=4.8 months; OS=81%) than in the chemotherapy group (mPFS=1.5 months; OS=67%) [21]. The above excellent outcomes led to the FDA approval in 2013, and most recently, their use in combination with other agents has also received accelerated FDA approval. In an open label study enrolling 247 patients with BRAF-mutant melanoma, significant improvement was reported in the median PFS in combination therapy with dabrafenib and trametinib compared with that with dabrafenib monotherapy. The median PFS in combination therapy was 9.4 months, as compared to that in monotherapy (5.8 months). However, the response rate was significantly higher at 76% and 54% for monotherapy. Moreover, the median OS was 23.8 months for the combination therapy [22].

B. Cobimetinib

Another potent MAPK (MEK) 1/2 inhibitor, cobimetinib, was approved for the treatment of metastatic or unresectable melanoma with serine/threonine-protein kinase (BRAF) V600E or V600K mutations when used in combination with vemurafenib. In a phase III randomized trial, vemurafenib and cobimetinib as a combination therapy were tested in patients with unresectable stage III or IV melanoma with a BRAFV600 mutation. The combination therapy resulted in both significant improvement in PFS and some serious toxicities in patients. Vemurafenib and cobimetinib were associated with an objective response rate of 68% and median PFS of 9.9 months. Clinically relevant grade ≥ 3 adverse events were diarrhea (6%), rash (6%), photosensitivity (2%), elevated liver function tests (LFTs) (8%-12%), increased creatine kinase (11%) and retinal detachment (3%) [23, 24].

4. Immune checkpoint inhibitors

CTLA-4, PD-1 and PD-L1 inhibitors

Melanoma cells are known to express the cell receptor proteins CTLA-4 and PD-1, which are normally found on the surface of T-cells and function as immune checkpoints, inhibiting further T-cell activation and down-regulating the immune response. Blockade of these checkpoints has emerged as a successful treatment concept. Specifically, inhibition of cytotoxic T lymphocyte

antigen-4 (CTLA-4) with the fully human monoclonal antibody ipilimumab has shown antitumor activity in patients with advanced melanoma, leading to improvement in the OS rate in melanoma. Similarly, PD-1 inhibitors Nivolumab and Pembrolizumab have also been reported to have impressive antitumor responses.

A. Ipilimumab

Ipilimumab is an anti-CTLA-4 monoclonal antibody approved by the FDA as a first line therapy in treatment of metastatic melanoma. By targeting the CTLA-4 checkpoint, ipilimumab activates CTLs to recognize and destroy cancer cells [25]. Ipilimumab has undergone extensive phase II and phase III clinical trials concomitant with a number of other therapies. In a phase III study involving 676 patients with unresectable stage III and IV metastatic melanoma, the disease was reported to progress despite the treatment. Patients administered ipilimumab plus gp100 peptide vaccine, gp100 peptide vaccine monotherapy or ipilimumab monotherapy. The median OS rate in the ipilimumab plus gp100 peptide vaccine combined therapy group was significantly prolonged to 10 months compared to 6.4 months among patients receiving gp100 peptide vaccine monotherapy (HR for death=0.68; $p < 0.001$). The grade 3/4 immune-related adverse events (AEs) occurred in 10-15% of patients treated with ipilimumab, including 7 deaths. Similarly, in another phase 3 trial, 502 patients with previously-untreated metastatic melanoma were randomized 1:1 to ipilimumab (10 mg/kg) in combination with dacarbazine, or to dacarbazine alone. Patients treated with ipilimumab and dacarbazine had an OS rate of 11.2 months compared with 9.2 months in the dacarbazine monotherapy. Both the overall and long-term survival rates were improved. The combination therapy, ipilimumab and dacarbazine, demonstrated a good safety profile. There were no gastrointestinal perforations and a lower rate of colitis compared to monotherapy [26]. Recently, several studies supported the evidence of synergistic activity of ipilimumab with radiotherapy; however, systematic studies supporting these evidences are limited. Hence, further study is needed to clarify the role of radiotherapy with ipilimumab [27].

B. Nivolumab

Following ipilimumab, nivolumab was approved by the FDA on December 22, 2014, for treatment

of patients with BRAFV600 wild-type, unresectable or metastatic melanoma refractory to other therapies [28, 29]. This PD-1 inhibitor demonstrated a significant survival benefit and a favorable safety profile in a randomized trial conducted on 418 previously untreated patients who had metastatic melanoma without a BRAF mutation. They received either nivolumab and dacarbazine-matched placebo or dacarbazine and nivolumab-matched placebo. In the year 1, the overall rate of survival was 72.9% in the nivolumab group, as compared with 42.1% in the dacarbazine group. The median PFS was prolonged to 5.1 months in the nivolumab group and 2.2 months in the dacarbazine group. Moreover, the objective response rate was 40.0% in the nivolumab group. Common adverse events associated with nivolumab included fatigue, pruritus, and nausea. Drug-related adverse events of grade 3 or 4 occurred in 11.7% of the patients treated with nivolumab and 17.6% of those treated with dacarbazine [30]. Another randomized phase 3 trial demonstrated a significantly improved PFS when nivolumab was combined with ipilimumab. The study included 945 treatment naive patients with unresectable stage III or IV melanoma, who received nivolumab alone, nivolumab plus ipilimumab, or ipilimumab alone in 1:1:1 ratio. PFS and OS were primary end points. The median PFS was 11.5 months with nivolumab plus ipilimumab, as compared with 2.9 months with ipilimumab and 6.9 months with nivolumab (HR for the comparison with ipilimumab). In patients with tumors positive for the PD-1 ligand (PD-L1), the median PFS was 14.0 months in the nivolumab-plus-ipilimumab group and in the nivolumab group; however, in patients with PD-L1-negative tumors, PFS was longer with the combination therapy than with nivolumab alone (11.2 months [95% CI, 8.0 to not reached] vs. 5.3 months [95% CI, 2.8 to 7.1]). Treatment-related adverse events of grade 3 or 4 occurred in 16.3% of patients in the nivolumab group, 55.0% of those in the nivolumab-plus-ipilimumab group, and 27.3% of those in the ipilimumab group [31].

C. Pembrolizumab

Pembrolizumab was approved by the FDA in September 2014 for treatment of patients with advanced melanoma progressive after ipilimumab or BRAF targeted therapy in patients whose melanomas express a BRAF mutation [32]. A recent phase III trial randomized 834 patients with advanced melanoma to pembrolizumab every 2 or 3

weeks and to ipilimumab. The PFS rates were 47.3% and 46.4% for patients treated with pembrolizumab every 2 and 3 weeks, respectively, and 26.5% for patients treated with ipilimumab. Median estimates of PFS were 5.5 months (95% CI=3.4-6.9 months), 4.1 months (95% CI=2.9-6.9 months) and 2.8 months (95% CI=2.8-2.9 months), respectively. The 1-year estimates of survival were 74.1% for patients receiving pembrolizumab every 2 weeks, 68.4% for patients receiving pembrolizumab every 3 weeks and 58.2% for those receiving ipilimumab. Importantly, the rates of grade 3/4 immune-related AEs were lower in the pembrolizumab group (13.3% and 10.1%, respectively) than in the ipilimumab group (19.9%). These data suggested improved outcomes with the treatment of advanced metastatic melanoma with pembrolizumab over ipilimumab [33]. Despite proving effectiveness of pembrolizumab as a novel therapy for advanced melanoma, recent several reports also suggest evolving incidence of its immune related toxicities [34].

5. Adoptive T cell therapy

After the successful therapeutic implication of immune check point antibodies, different forms of adoptive cellular immunotherapies for patients with malignant melanoma have been discovered in the past few years. Among these, adoptive cell therapy (ACT) based on autologous tumor infiltrating lymphocytes (TIL), derived from either tumor or peripheral blood, has been the most frequently applied [35]; however, the generation of TILs is not possible in all patients, and thus, there has been limited success; however, with recent development of novel strategies for redirecting normal T cells to recognize tumor-associated antigens (TAAs) by genetically engineering tumor antigen-specific T cell receptors (TCRs) or chimeric antigen receptor (CAR) genes [36]. ACT treatment, although restricted to small trials so far, using these TILs together with high-dose interleukin 2, has the longest clinical history demonstrating durable clinical response rates near or above 50% .

One of the recent clinical trials evaluated the administration of vemurafenib and TIL.A metastatic tumor resected for growth of TILs, and total 11 patients were treated with vemurafenib for 2 weeks, followed by resection of a second lesion. Then, after nonmyeloablative preconditioning regimen, infusion of autologous TILs and high-dose IL-2 was administered. Vemurafenib was then restarted at the time of the TIL infusion and

continued for 2 years or until disease progression. The treatment was well tolerated and 7 out of the 11 patients (64%) experienced an objective clinical response, and 2 patients (18%) had a complete response for 3 years (one response was ongoing for 46 months). The synergistic activity of vemurafenib and TILs was safe and feasible and generated objective clinical responses. The toxicities of the treatment were reported, largely due to the lymphodepleting preparative regimen [37].

6. Oncolytic viral therapy

Oncolytic viral therapy has recently been recognized as the most promising therapeutic approach in patients with metastatic melanoma owing to its ease of administration, low toxicity profiles and probability of high synergistic capability with other immunotherapeutic agents such as immune checkpoint inhibitors in advanced melanoma [38]. Various trials are currently ongoing in oncolytic viruses, including HSVs (such as T-VEC), coxsackieviruses (such as CVA13), reoviruses (such as Reolysin) and echoviruses.

Talimogene laherparepvec (T-VEC; Imlygic™) is a genetically modified herpes simplex virus, type 1, and the first oncolytic virus therapy to be approved for the treatment of advanced melanoma by the USFDA in October 2015. This therapy was subsequently approved in Europe in January 2016 and in Australia in May 2016. T-VEC selectively targets tumor cells, causing regression in injected lesions and inducing immunologic responses that mediate regression at uninjected/distant sites [39]. T-VEC has now been assessed in Phase II and III clinical trials, and has demonstrated a tolerable side-effect profile and promising efficacy, showing an improved durable response rate and a trend toward superior OS, as compared to the granulocyte-macrophage colony-stimulating factor.

A randomized phase III OPTiM trial was performed in patients with unresected stage IIIB–IV melanoma. A total of 436 patients were randomly assigned to T-Vec intralesionally or GM-CSF subcutaneously. The durable response rate was significantly higher in the T-Vec (16.3%) compared with the GM-CSF (2.1%). The overall response rate was also higher in the T-Vec arm (26.4 vs 5.7%) and the median OS was 23.3 months for the T-Vec and 18.9 months for the GM-CSF. With regard to safety, the most common adverse events observed with T-Vec were fatigue, chills, pyrexia and cellulitis. No fatal treatment-related adverse events were reported. The results from this trial not only

proved that local intralesional injections with an oncolytic virus can suppress the growth of injected tumors, but also prolonged the OS, supposedly via induction of a systemic antitumor. Based on this observation, several clinical trials of T-Vec in combination with other several systemic administrations with immune check point inhibitors are currently under consideration. Recently, in one trial, combination of T-vec with ipilimumab showed greater clinical efficacy than either T-vec or ipilimumab mono therapy. In the phase Ib trial of T-VEC in combination with ipilimumab, 19 patients were administered to T-VEC intratumorally in week 1, and then, in week 4 and every 2 weeks thereafter. Ipilimumab (3 mg/kg) was administered intravenously every 3 weeks for four infusions. Grade 3/4 treatment-related adverse events (AEs) were observed in 26.3% of patients; 15.8% had AEs attributed to T-VEC, and 21.1% had AEs attributed to ipilimumab. The objective response rate was 50%, and 44% of patients had a durable response lasting more than 6 months. Eighteen-month PFS was 50%; 18-month OS was 67% [40].

7. Conclusion

The treatment perspective in malignant melanoma has changed considerably due to the recent emergence of biological therapies. While initial therapies were limited to the use of chemotherapies, the evolution of different immunotherapies and targeted therapies has revolutionized this field. Clinical trials have already proven the potential of these therapies for long-term improvement in patients' care and survival. The adverse events associated with these treatment modalities are mild to moderate and generally tolerable. Nonetheless, resistance development remains a significant challenge and to overcome this, combination therapies may be of principle value, as already demonstrated by the combination of BRAF and MEK inhibitors. Therefore, more clinical studies should be emphasized focusing on combination therapies that further optimize treatment results.

Abbreviations

RR: Response rate; PFS: Progression free survival; MPFS: Mean progression free survival OS: overall survival; CI: confidence interval; CTL: cytotoxic T lymphocyte; CTL-4: cytotoxic T lymphocyte associated protein; PD-1: Programmed cell death protein; PD-1: Programmed death-ligand 1; FDA: Food and Drug Administration; GM-CSF:

Granulocyte macrophage-colony stimulation factor gp100:Glcoprotein 1

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