Biomedical Letters
2017 | Volume 3 | Issue 2|Pages 122-129

REWIEW ARTICLE OPEN ACCESS

A New Era of Immunotherapy in Malignant Melanoma

Anila Kirti, Qiangxiong Wang, Jingjun Zhao*

Department of Dermatology, Shanghai Tongji Hospital, Tongji University School of Medicine, 200065, Shanghai, China

Abstract
Even though melanoma skin cancer is less common than non-melanoma skin cancers (squamous cell carcinoma and basal cell carcinoma), still its mortality rate is relatively higher. Early diagnosis is the mainstay option to improve the disease outcome as early-stage melanoma made a favorable prognosis with surgical intervention. In contrast, advanced stage melanoma which is disseminated to distant sites through the lymphatics is associated with poor prognosis. Earlier traditional treatment modalities like interleukin 2 and non-specific anti-neoplastic agents are ineffective in improving the survival outcome and also the side-effects of these drugs have always been a treatment burden. However, recent understanding of immunotherapeutic approach against melanoma cancer has revolutionized the whole treatment scenario. Various adaptations of immunotherapies like targeted therapy, monoclonal antibodies, Toll-like receptors, T-cell therapy and oncolytic viral therapy has shown significant improvement in prolonging the survival. In this review, we discussed the novel therapeutic agents and summarized the outcomes from recent major clinical trials.
Keywords: Melanoma, MAPK pathway, B-RAF inhibitors, MEK inhibitors, Efficacy.

Received September 21, 2017; **Revised** October 27, 2017; **Accepted** November 15, 2017
***Correspondence:** Jingjun Zhao **Email** zhaomyco@163.com, **Contact**: +8613917696735

***To cite this manuscript*:** Kirti A, Wang Q, Zhao JJ. A New Era of Immunotherapy in Malignant Melanoma. Biomed Lett 2017; 3(2):122-129.

Introduction

Malignant melanoma is the fatal skin neoplasm arising from pigment-containing cells (melanocytes). According to histological features, it is classified into four clinical subtypes; nodular melanoma, lentigo melanoma, acral lentiginous melanoma and the superficial spreading melanoma. The most common one is superficial spreading one that accounts for 50-70% of melanoma cases[1].

Over the past few decades, there has been a step-ladder rise in the incidence rate of melanoma throughout the world [2]. It is more common among fair-skinned Caucasians than Asian populations [3, 4]. In western countries, the risk of occurrence of melanoma is 1 in 50 [5]. In 2017 about 87,110 new cases of melanoma and 9,730 deaths from the disease are estimated to occur in the USA [6]. Several etiological factors exert an important role in melanoma development. Among them, ultraviolet radiation and genetic factor plays a significant role [7, 8]. Melanoma tumor being highly localized in early stage (stage 1 and 2), the prognosis outcome is often positive as surgical excision can easily remove the tumor. In contrast, the prognosis for advanced stage melanoma is extremely poor and five year survival rate varies from 5 to 19%[9]. Advanced stage of melanoma is extremely fatal, so diagnosis at its initial stage is very crucial to reduce mortality. Before immunotherapeutic evolution, Dacarbazine, hydroxyurea, and interleukin-2 (IL-2) were considered as the prominent treatment options for the advanced melanoma, but these drugs have not been able to demonstrate any improvement in the overall survival rate of the patient. Hydroxyurea in conjunction with radiotherapy showed an approximately 20% response rate [10]. Dacarbazine(DTIC) was FDA approved in 1975, and despite being considered as the gold standard for treatment of melanoma for over few decades, it has shown objective response rate only up to 25%[11]. Efficacy of DTIC was not found to be improved when used as a combination therapy with other drugs [12]. In 1998, the FDA approved another drug high-dose recombinant IL-2 for the treatment of malignant melanoma, IL-2 as monotherapy showed low efficacy with only 10% response rate and was associated with severe toxicities[13]. However, several studies reported its concomitant use with other therapies quiet promising [14].

Overview of MAPK pathway

The Mitogen Activated Protein Kinase (MAPK) pathway is normally involved in cell growth and proliferation. Any defect in this pathway leads to uncontrolled cell proliferation and invasion [15]. This tightly regulated pathway consists of signaling molecules RAS, RAF (Rapidly accelerated fibrosarcoma) MEK1/2(Mitogen-activated protein

kinase kinase1/2) and ERK1/2 (Extracellular signal regulated kinase1/2). Under normal circumstances, when extracellular growth factors or any neurotransmitters bind to receptor tyrosine kinases, activates RAS protein by conversion of GDP to GTP. This activation of RAS stimulates other subsequent proteins RAF, MEK1/2, ERK1/2 which ultimately leads to the activation of MAPK cascade. Activation of MAPK pathway is involved in activation of several transcription factors inside the nucleus that is responsible for gene expression. Regulation of signaling cascades involved in MAPK pathway is crucial for normal cellular growth. However, loss of regulation occurs in melanoma largely due to mutations in the B-RAF and RAS genes. This mutation leads to continuous increased signaling pathway activity promoting uncontrolled proliferation of cellular growth [16]. Extensive studies focusing on the presence of different mutations located at the different level of this pathway which was responsible for the development of melanoma led to the discovery of new therapies[17]. About 60% of melanoma occurs due to a mutation in B-RAF genes [18]. Among all B-RAF genes, V600E and V6OOK mutants are highly frequent [19]. V600E and V600K are mutation of B-RAF genes at position 600 in which amino acid valine (V) is substituted by amino acids glutamic acid (E) and lysine (K) respectively.

Targeted therapies

1. B-RAF Inhibitors

1.1. Vemurafenib

Vemurafenib is a selective BRAF-V600E inhibitor, subsequently approved in 2011 by U.S FDA for the treatment of BRAF V600E mutated metastatic melanoma. In phase I trial, Among 32 patients treated with vemurafenib at a dose of 960 mg, 81% patients showed better response. Complete response was achieved in two patients, while as partial response was achieved in 24 patients [20]. Another phase II trial with melanoma patients showed 53% overall response rate with 6% complete response and 47% partial response rate [21]. Encouraged by the subsequent phases I and II results, phase III randomized clinical trial was held to compare its efficacy with dacarbazine. A total 675 patients with no previous intervention received either vemurafenib or dacarbazine. Overall survival of vemurafenib accounts to 84%. The response rate was up to 48 % for vemurafenib and dacarbazine showed only 5% response rate. Compared to dacarbazine, vemurafenib reduced the risk of death by 63 % while the disease progression was reduced by 74% [22]. Approximately 1% of the patients in clinical trials experienced some adverse effects after treatment with vemurafenib alone or in combination. The most common cutaneous side effects of vemurafenib are rash, skin papilloma, dry skin, squamous cell carcinoma and elevated liver enzymes. With frequent use of vemurafenib, it is evolving as a key treatment approach for BRAF mutated melanoma. However tumor relapse and therapy resistance has been emerging as a major problem which may be addressed by combination with other agents [23]

1.2. Dabrafenib

Dabrafenib is another selective BRAF inhibitor. After encouraging results in phase I and II clinical trials [24, 25], a phase III clinical trial (BREAK 3) was conducted. A total of 250 B-RAF V6000E mutated melanoma patients with no previous surgical intervention were administered either with dabrafenib or DTIC (dacarbazine). Dabrafenib receiving patients showed a 50% response rate while 6% RR was seen in DTIC receiving patients. The median overall survival was 18 months for dabrafenib [26]. Both B-RAF inhibitors dabrafenib and vemurafenib reported an improved efficacy in protein kinase pathway inhibition, either, monotherapy or together with a trametinib (MEK inhibitor) has become a treatment of choice for B-RAF V600E or V600K-mutant melanoma. Despite showing improved clinical efficacy over chemotherapy, acquired resistance is quite common in B-RAF inhibitors monotherapy that limits the duration of response. Recent studies have shown that an addition of MEK inhibitor may overcome the resistance. The addition of a MEK inhibitor with B-RAF inhibitors can improve blockade of the MAPK pathway thereby showing efficacy, as well as reduced cutaneous toxicity [27]. In another phase III trial, a combination of dabrafenib and trametinib were compared in efficacy over vemurafenib monotherapy. Total 704 patients with B-RAF mutated metastatic were assigned to receive either a combination of dabrafenib and trametinib or vemurafenib. The combination-therapy group showed an impressive overall survival rate of 72% and objective response rate of 64% over vemurafenib (OSR-65% and ORR-51%) and median progression-free survival was prolonged to 11.4 months. Vemurafenib monotherapy showed an overall survival rate of 65% with median progression-free survival of7.3 months [28]. The most serious adverse

effects reported in patients receiving dabrafenib were increased risk of cutaneous squamous-cell carcinoma and keratoacanthoma while the combination of dabrafenib and trametinib therapy displayed significant decrease in squamous-cell carcinoma [29].

2. MEK inhibitors

2.1. Trametinib

Trametinib, MEK inhibitor is the only known substrate of BRAF, which in turn leads to decreased cell signaling and proliferation that suppresses tumor growth [30]. It is approved as both treatment of choice for monotherapy and in combination with BRAF inhibitor (dabrafenib). In a study, 40 patients with BRAF-mutant melanoma and prior BRAF inhibitor therapy, trametinib were reported being ineffective suggesting that BRAF-inhibitor resistance develops with repeated exposure [31]. The adverse effects associated with trametinib were cutaneous toxicity, fatigue, nausea, peripheral edema, and diarrhea. Squamous cell carcinoma was not observed in other BRAF inhibitors [31]. In phase 3 trial, the activity of trametinib was compared with DTIC in 322 patients with BRAF-mutated melanoma. Median PFS and OS were impressive in the trametinib group (mPFS=4.8 months; OS=81%) than in the chemotherapy group (mPFS=1.5 months; OS=67%) [32]. In an open label study, enrolling 247 patients with BRAF-mutant melanoma reported significant improvement of median PFS in combination therapy with dabrafenib and trametinib compared with those with dabrafenib monotherapy. The median PFS was compared for 9.4 months to monotherapy (5.8 months). The response rate was also significantly higher in the combination group with median OS of 23.8 months [33].

2.2. Cobimetinib

Another potent mitogen-activated protein kinase (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 phase III randomized trial, vemurafenib and cobimetinib as a combination therapy was examined in patients with unresectable stage IIIC or IV BRAFV600 mutation-positive melanoma. The combination therapy not only reported with having significant improvement in PFS in patients but also some serious toxicities. Vemurafenib and cobimetinib were associated with an objective response rate of 68%, and median progression-free survival of 9.9 months. Clinically relevant grade ≥3 adverse events were diarrhea (6%), rash (6%), and photosensitivity (2%), elevated liver function tests (LFTs) (8%-12%), increased creatine kinase (11%), and retinal detachment (3%) [34, 35].

3. Immune Checkpoint Inhibitors

3.1. CTLA-4 inhibitors and PD-1 and PD-L1 inhibitors

CTLA-4 is a protein receptor normally expressed on the surface of T-cells. CDLA-4 protein competes with its homologous T-cell protein CD28 with higher affinity for its binding to B7-1 and B7-2 ligands expressed by antigen presenting cells. Thus,this higher affinity binding transmits inhibitory signals to T-cells and also prevent stimulatory signals transmitted by CD28[36]The understanding of mechanism of action of CDLA-4 protein leads to the development of first CTLA-4 inhibitor ipilimumab in 2011. Similarly, PD-1 is also a protein receptor expressed by T-cells which upon binding to its ligands PD-L1 and PD-L2,inhibits T-cell activation[37]. Both CTLA-4 and PD-1 are considered as the negative regulators of T-cell activation. However, mechanism of signaling pathway, distribution of ligands and timing of inhibition phase is distinct for both pathways. These cell receptors are known to be expressed by melanoma cells. 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 overall survival rate in melanoma. Similarly, PD-1 inhibitors Nivolumab and Pembrolizumab have also been reported to have impressive antitumor responses. PD-L1 is a principal ligand expressed by tumor cells which when binds to PD-1 receptor, inhibits the T-cell activation.PD-L1 inhibitors offers a new approach of targeting PD pathway by binding to ligand PD-L1.The PD-L1inhibitors atezolizumab and durvalumab are currently on extensive preliminary trials but the data from those study is not mature enough to analyze their efficacy[38].

3.2. Ipilimumab

Ipilimumab is an anti-CTLA-4 monoclonal antibody approved by FDA as a mainstay therapy in metastatic melanoma. By targeting the CTLA-4 checkpoint, Ipilimumab activates CTLs to recognize and destroy

cancer cells [39]. Ipilimumab has undergone extensive phase II and phasepatients with progressive melanoma despite treatment were administered ipilimumab and gp100 peptide vaccine, gp100 peptide vaccine alone or ipilimumab alone. The median overall survival rate in the ipilimumab and gp100 peptide vaccine combined group was significantly prolonged to 10.0 months over patient receiving gp100 peptide vaccine alone (6.4 months). The incidence of immune-related adverse events (AEs) was reported in 10-15% of patients treated with ipilimumab including 7 deaths. The immune-related adverse events were mainly fatigue, anorexia, diarrhea and colitis. The cutaneous adverse effects included skin rash, pruritus and vitiligo. However; these adverse events were reversible after corticosteroid therapy. Similarly, in another phase III trial, 502 metastatic melanoma patients with no previous intervention were assigned ipilimumab plus dacarbazine, or dacarbazine alone. Overall survival of 11.2 months was reported in combination therapy over 9.2 months in the dacarbazine monotherapy. Both overall and long-term survival was significantly 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 [40, 41]. Recently, several studies supported the evidence of synergistic activity of ipilimumab with radiotherapy, but systematic studies supporting these evidences are limited. Hence further analysis is needed to clarify the role of radiotherapy with ipilimumab [42].

3.3. Nivolumab

Following Ipilimumab, nivolumab was the first PD-1 inhibitor to be introduced in the field of immunotherapy to treat advanced melanoma without BRAF mutation. This PD-1 inhibitor demonstrated a significant higher survival rate and a favorable safety profile than chemotherapy [43]. In a randomized trial conducted with 418 previously untreated patients with metastatic melanoma with no BRAF mutation, nivolumab showed significant overall survival and median progression survival than dacarbazine. The median progression-free survival was seen up to 5.1 months with an objective response rate of 40% in the nivolumab group and 2.2 months in the dacarbazine group. Fatigue, pruritus, and nausea were common drug-related adverse effects. These adverse effects were observed in relatively fewer patients treated with nivolumab than in patients treated with dacarbazine [44]. Another randomized phase III trial demonstrated an improved progression-free survival (PFS) when nivolumab was combined with ipilimumab. The study included 945 treatment of naive patients with unresectable stage 3 or 4 melanoma. They were categorized into three groups, receiving nivolumab alone, nivolumab plus ipilimumab, or ipilimumab alone. The median progression-free survival was 11.5 months with the group receiving combination therapy, 2.9 months with ipilimumab and 6.9 months with nivolumab. 16.3% of the patients in the nivolumab group reported treatment-related adverse effects, but 55% of patients receiving nivolumab plus ipilimumab reported treatment related adverse events

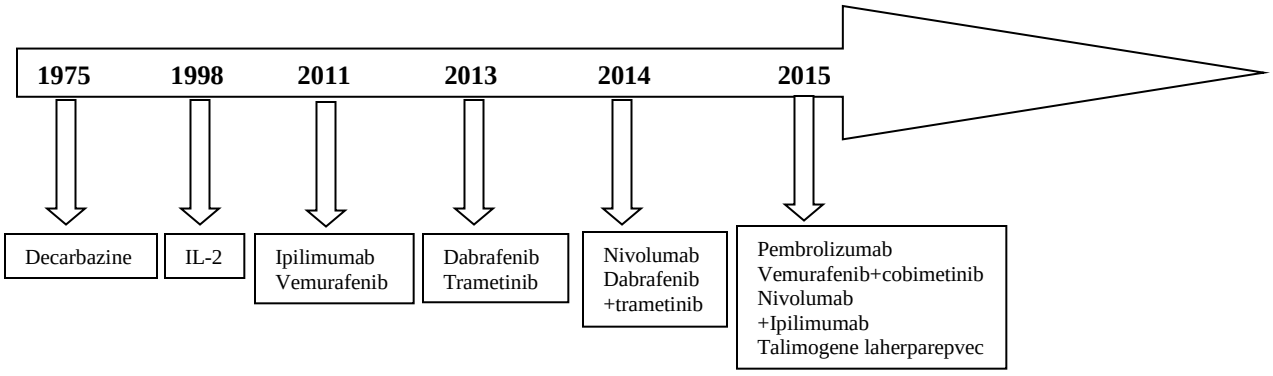

Figure 1: FDA-Approved Drugs for Metastatic Melanoma

3.4. Pembrolizumab

Pembrolizumab is a PD-1 inhibitor which was approved in September 2014 for treatment of BRAF mutant melanoma. This drug is considered as mainstay treatment option in progressive melanoma regardless of previous treatment with other BRAF inhibitors or ipilimumab [46]. In ipilimumab resistant melanoma, pembrolizumab has shown impressive

progression-free survival [47]. A KEYNOTE-006 phase3 trial compared the efficacy between pembrolizumab and ipilimumab. A total 811 patients with advanced melanoma were randomly assigned either infusion of pembrolizumab or ipilimumab. At 24 months, pembrolizumab accounted for the overall survival of 55% whereas overall survival of 43% was for ipilimumab-treated patients [48]. Importantly, the rates of grade 3/4 immune-related AEs were lower in the pembrolizumab group than in the ipilimumab group. These data suggested improved outcomes with the treatment of advanced metastatic melanoma with pembrolizumab over ipilimumab[49]. Despite of proving its effectiveness as a novel therapy for advanced melanoma, several recent reports also suggest evolving incidence of its immune-related toxicities [50].

4. Toll-like receptor Activation

TLRs are signaling receptors which are expressed on the surface of immune cells and helps in recognizing foreign antigens. Upon recognition of foreign antigens, microbes or any pathogens, immune cells stimulate the release of pro-inflammatory cytokines that helps in the induction of innate and adaptive immune response, thereby facilitating elimination of pathogens and tumor cells [51]. Among various TLR agonists being investigated, imiquimod has been successfully demonstrating its efficacy through several preliminary studies. Imiquimod acted as a TLR7/8 agonist with antitumor properties. Drobits et al. demonstrated the mechanism by which imiquimod recruits plasmocytid dendritic cells into tumor and their conversion to cytolytic killer cells capable of eliminating tumor cells independently from adaptive immune system [52]. Several recent published studies have shown imiquimod to have considerable clinical efficacy in the treatment of metastatic melanoma (MM) [53]. Imiquimod has been suggested as a possible synergistic agent with ipilimumab and with cryotherapy however thorough extensive studies is warranted [54, 55].

5. Adoptive T cell therapy

After the successful therapeutic implementation of targeted therapy and immune checkpoint inhibitors, adoptive cellular therapy is rapidly emerging as the promising treatment approach against tumor cells. Adoptive T cell therapy (ACT) is based on the theory that autologous tumor infiltrating lymphocytes (TIL), derived either from a tumor or peripheral blood are administered into the same host either in their natural form or in genetically modified form. These cells then stimulate the immune system to fight against malignant cells [56, 57]. However, the generation of TILs is not possible in all patients, so there has been a limited success [58]. So far, ACT although restricted to small trials, treatment using these TILs together with high-dose interleukin-2 (IL-2) have demonstrated durable clinical response rates near 50% or more.

A pilot trial evaluated the administration of vemurafenib and TIL. After resection of tumor growth for TIL, 11 patients with metastatic melanoma were treated with vemurafenib for two weeks, followed by resection of a second lesion. Prior infusion of autologous TILs, nonmyeloablative preconditioning regimen was performed. TILs were infused with high-dose IL-2. Vemurafenib was restarted at the time of TIL infusion and was continued for 2 years or until disease progression. The treatment was well tolerated and had a safety profile similar to that of TIL or vemurafenib alone. Out of 11, seven patients (64%) experienced an objective clinical response, and 2 patients (18%) had a complete response for 3 years. Administration of Vemurafenib with TILs generated safe and feasible objective clinical responses. The toxicities of treatment reported were largely due to the lymph depleting preparative regimen [59, 60].

6. Oncolytic viral therapy

Oncolytic viral therapy has recently been conceived as the potential therapeutic approach in patients with stage 3 and 4 metastatic melanomas. The concept of talimogene laherparepvec (T-VEC) is the first oncolytic virus to be launched in clinical settings. It is emerging as favorable treatment option owing to its low toxicity profiles and the probability of high synergistic capability with other immune checkpoint inhibitors in advanced melanoma [61]. Other viruses like echovirureoviruses, coxsackieviruses and reoviruses are currently being evaluated in various clinical trials.

Talimogene laherparepvec (T-VEC) is the intralesional oncolytic herpes simplex virus, approved by FDA in 2015 for treatment of advanced melanoma. This virus undergoes genetic variation in the laboratory in order to stimulate its replicating properties so that the virus can attack and replicate within tumor cells causing its lysis without disrupting normal tissues. Additionally, it also induces a systemic immunologic response by releasing proinflammatory mediators like cytokines and tumor-derived antigens. This extensive mechanism of action offers improved durable response benefit [62]. T-VEC has already been assessed in Phase II and III clinical trials and has demonstrated promising efficacy with good safety profile over

Table 1: Summary of clinical trials of immunotherapeutic

Drugs	Study population	Phase	Total patients	OS (%)	ORR (%)	MPFS (months)	AEs (%)
Vemurafenib vs decarbazine	Unresectable previously untreated stage 3or 4 melanoma positive for BRAF V600E	3	675	84 vs 64	48 vs 5	5.3 vs 1.6	38 vs16
Dabrafenib plus trametinib vs vemurafenib	Metastatic melanoma with BRAF V600 mutation positive	3	704	72 vs 65	64 vs 51	11.4 vs 7.3	1 vs 18
Vemurafenib vs cobimetinib	Unresectable previously untreated metastatic BRAF V600 mutation positive	3	495	81 vs 73	68 vs 45	9.9 vs 6.2	65 vs 59
Ipilimumab plus gp100 vaccine vs gp100 vs ipilimumab	Unresectable metastatic melanoma with no previous treatment	3	676	21.6 vs 13.7 vs.23.5	5.7 vs 1.5 vs 10.9	10 vs 6.4 vs 10.9	15 vs 3 vs 10
Ipilimumab plus decarbazine vs decarbazine	Metastatic melanoma with no prior treatment	3	502	47.3 vs 36.3	15.2 vs 10.3	11.2 vs 9.1	56.3 vs 27.5
Nivolumab vs decarbazine	Previously untreated advanced melanoma without BRAF mutation	3	418	72.9 vs 42	40 vs 13.9	5.1 vs 2.2	11.7 vs 17.6
Nivolumab vs nivolumab plus ipilimumab ipilimumab	Previously untreated and unresectable advanced melanoma	3	945	52 vs 58 vs 34	43.7 vs 57.6 vs 19	6.9 vs 11.5 vs 2.9	16.3 vs 55 vs 27.3
Pembrolizumab vs ipilimumab	Previously untreated and unresectable advanced melanoma	3	811	55 vs 43	32.9 vs 11.9	46.4 vs 26.5	30.2 vs 31.64
T-VEC vs GM-CSF	Previously untreated and unresectable advanced melanoma	3	436	23.3 vs 18.9	26 vs 5.7	8.2 vs 2.9	36 vs 21
T-VEC plus ipilimumab vs ipilimumab	Previously untreated and unresectable advanced melanoma	1 B	19	-	-	-	15.8 vs 21.1

Note: Abbreviations; OS=overall survival, ORR=overall response rate, MPFS=median progression free survival, AEs=Adverse events.

granulocyte-macrophage colony stimulating factor.

In a randomized phase III OPTIM trial, out of 436 patients with unresected advanced melanoma, T-VEC showed a significantly higher durable response rate of about 16.3% compared with the GM-CSF (2.1%). The overall response rate (ORR) was also higher in the T-Vec arm (26.4 vs. 5.7%) with median overall survival (OS) of 23.3 months while ORR for GM-CSF was only 5.7% with median overall survival of 18.9 months. Fatigue, chills, pyrexia, and cellulitis were frequently observed as its adverse effects. No fatal treatment-related adverse events were reported. The above trial not only proves T-VEC as a promising oncolytic virus which not only can suppress the growth of injected tumors but also helps in improving overall survival via induction of systemic immunologic response. Based on this observation, several clinical trials of T-Vec in combination with other several systemic administrations with immune checkpoint inhibitors are currently under consideration. Recently in one trial, a combination of T-vec with ipilimumab, shows greater clinical efficacy than either T-vec or ipilimumab mono therapy. In phase Ib trial, T-VEC was intralesionally administered, in 19 patients. Following first T-VEC injection, the second dose was administered after a month and every two weeks after that. Total four infusions of Ipilimumab (3 mg/kg) were administered with 3 weeks interval. Grade 3/4 treatment-related adverse events (AEs) were seen in about 26.3% of patients in which T-VEC attributed to only 15.8% of AEs. On another hand; ipilimumab attributed 21.1% of AEs. 50 and 44% objective response rate were observed with durable response prolonged to 6 months. Progression-free survival at 18 months was 50% and overall survival rate was 67% [63].

Conclusions

The treatment landscape in malignant melanoma has changed considerably owing to the recent emergence of biological therapies like immune checkpoint inhibitors, targeted therapy, T-cell therapy, oncolytic viral therapy and toll-like receptors. While earlier conventional therapies were limited to the use of chemotherapy and radiation therapy. The evolution of different immunotherapeutics and targeted therapies has revolutionized this field. Clinical trials have already proven the potential of these therapies by showing not only improvement in overall response rate but also increasing overall survival rate of the patient. The adverse events associated with these treatment modalities are mild to moderate and are normally tolerable. Nonetheless, resistance development remains a significant challenge and to overcome this problem. Combination therapies may

be of principal value as actually being already demonstrated by a combination of BRAF and MEK inhibitors. Therefore, more clinical studies should be emphasized by focusing on combination therapies that will help in optimizing treatment results further.

References

[1] Singh P, Kim HJ, Schwartz RA. Superficial spreading melanoma: an analysis of 97 702 cases using the SEER database. Melanoma research. 2016;26:395-400.

[2] Nikolaou V, Stratigos AJ. Emerging trends in the epidemiology of melanoma. The British journal of dermatology. 2014;170:11-9.

[3] Lens MB, Dawes M. Global perspectives of contemporary epidemiological trends of cutaneous malignant melanoma. The British journal of dermatology. 2004;150:179-85.

[4] Raimondi S, Sera F, Gandini S, Iodice S, Caini S, Maisonneuve P, et al. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. International journal of cancer. 2008;122:2753-60.

[5] Meyle KD, Guldberg P. Genetic risk factors for melanoma. Human genetics. 2009;126:499-510.

[6] Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA: a cancer journal for clinicians. 2017;67:7-30.

[7] Aase A, Bentham G. Gender, geography and socio-economic status in the diffusion of malignant melanoma risk. Social Science & Medicine. 1996;42:1621-37.

[8] Bowman DM, Lewis RC, Lee MS, Yao CJ. The Growing Public Health Challenges of Exposure to Ultraviolet Radiation From Use of Indoor Tanning Devices in the United States. New solutions : a journal of environmental and occupational health policy : NS. 2015;25:164-71.

[9] Gray-Schopfer V, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. Nature. 2007;445:851-7.

[10] Cassileth PA, Hyman GA. Treatment of malignant melanoma with hydroxyurea. Cancer research. 1967;27:1843-5.

[11] Luke JJ, Schwartz GK. Chemotherapy in the Management of Advanced Cutaneous Malignant Melanoma. Clinics in dermatology. 2013;31:290-7.

[12] Kokoschka EM. [DTIC in malignant melanoma: a perspective (author's transl)]. Wiener klinische Wochenschrift. 1978;90:870-4.

[13] Marabondo S, Kaufman HL. High-dose interleukin-2 (IL-2) for the treatment of melanoma: safety considerations and future directions. Expert opinion on drug safety. 2017:1-11.

[14] Weide B, Martens A, Wistuba-Hamprecht K, Zelba H, Maier L, Lipp HP, et al. Combined treatment with ipilimumab and intratumoral interleukin-2 in pretreated patients with stage IV melanoma-safety and efficacy in a phase II study. Cancer immunology, immunotherapy : CII. 2016.

[15] Amaral T, Sinnberg T, Meier F, Krepler C, Levesque M, Niessner H, et al. The mitogen-activated protein kinase pathway in melanoma part I - Activation and primary resistance mechanisms to BRAF inhibition. European journal of cancer (Oxford, England : 1990). 2017;73:85-92.

[16] Wellbrock C, Arozarena I. The Complexity of the ERK/MAP-Kinase Pathway and the Treatment of Melanoma Skin Cancer. Frontiers in Cell and Developmental Biology. 2016;4:33.

[17] McCain J. The MAPK (ERK) Pathway: Investigational Combinations for the Treatment Of BRAF-Mutated Metastatic Melanoma. Pharmacy and Therapeutics. 2013;38:96-108.

[18] Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417:949-54.

[19] Long GV, Menzies AM, Nagrial AM, Haydu LE, Hamilton AL, Mann GJ, et al. Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2011;29:1239-46.

[20] Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. The New England journal of medicine. 2010;363:809-19.

[21] Sosman JA, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. The New England journal of medicine. 2012;366:707-14.

[22] Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. The New England journal of medicine. 2011;364:2507-16.

[23] Bauer D, Werth F, Nguyen HA, Kiecker F, Eberle J. Critical role of reactive oxygen species (ROS) for synergistic enhancement of apoptosis by vemurafenib and the potassium channel inhibitor TRAM-34 in melanoma cells. Cell death & disease. 2017;8:e2594.

[24] Falchook GS, Long GV, Kurzrock R, Kim KB, Arkenau TH, Brown MP, et al. Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase 1 dose-escalation trial. Lancet (London, England). 2012;379:1893-901.

[25] Long GV, Trefzer U, Davies MA, Kefford RF, Ascierto PA, Chapman PB, et al. Dabrafenib in patients with Val600Glu or Val600Lys BRAF-mutant melanoma metastatic to the brain (BREAK-MB): a multicentre, open-label, phase 2 trial. The Lancet Oncology. 2012;13:1087-95.

[26] Menzies AM, Long GV, Murali R. Dabrafenib and its potential for the treatment of metastatic melanoma. Drug Design, Development and Therapy. 2012;6:391-405.

[27] Spain L, Julve M, Larkin J. Combination dabrafenib and trametinib in the management of advanced melanoma with BRAFV600 mutations. Expert opinion on pharmacotherapy. 2016;17:1031-8.

[28] Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. The New England journal of medicine. 2015;372:30-9.

[29] Carlos G, Anforth R, Clements A, Menzies AM, Carlino MS, Chou S, et al. Cutaneous Toxic Effects of BRAF Inhibitors Alone and in Combination With MEK Inhibitors for Metastatic Melanoma. JAMA dermatology. 2015;151:1103-9.

[30] Gilmartin AG, Bleam MR, Groy A, Moss KG, Minthorn EA, Kulkarni SG, et al. GSK1120212 (JTP-74057) Is an Inhibitor of MEK Activity and Activation with Favorable Pharmacokinetic Properties for Sustained In Vivo Pathway Inhibition. Clinical Cancer Research. 2011;17:989.

[31] Kim KB, Kefford R, Pavlick AC, Infante JR, Ribas A, Sosman JA, et al. Phase II study of the MEK1/MEK2 inhibitor Trametinib in patients with metastatic BRAF-mutant cutaneous melanoma previously treated with or without a BRAF inhibitor. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31:482-9.

[32] King JW, Nathan PD. Role of the MEK inhibitor trametinib in the treatment of metastatic melanoma. Future oncology (London, England). 2014;10:1559-70.

[33] Menzies AM, Long GV. Dabrafenib and trametinib, alone and in combination for BRAF-mutant metastatic melanoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20:2035-43.

[34] Ascierto PA, McArthur GA, Dreno B, Atkinson V, Liszkay G, Di Giacomo AM, et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. The Lancet Oncology. 2016;17:1248-60.

[35] Signorelli J, Shah Gandhi A. Cobimetinib. The Annals of pharmacotherapy. 2017;51:146-53.

[36] Buchbinder EI, Desai A. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. American Journal of Clinical Oncology. 2016;39:98-106.

[37] Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annual review of immunology. 2008;26:677-704.
[38] Gordon MS, Hamid O, Powderly J, Anderson M, Fine G, Mokatrin A, et al. Abstract LB-288: A phase I study of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic tumors. Cancer Research. 2013;73:LB-288-LB-.
[39] Letendre P, Monga V, Milhem M, Zakharia Y. Ipilimumab: from preclinical development to future clinical perspectives in melanoma. Future oncology (London, England). 2017;13:625-36.
[40] Tang T, Eldabaje R, Yang L. Current Status of Biological Therapies for the Treatment of Metastatic Melanoma. Anticancer research. 2016;36:3229-41.
[41] Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. The New England journal of medicine. 2011;364:2517-26.
[42] Koller KM, Mackley HB, Liu J, Wagner H, Talamo G, Schell TD, et al. Improved survival and complete response rates in patients with advanced melanoma treated with concurrent ipilimumab and radiotherapy versus ipilimumab alone. Cancer biology & therapy. 2017;18:36-42.
[43] Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. The Lancet Oncology. 2015;16:375-84.
[44] Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. The New England journal of medicine. 2015;372:320-30.
[45] Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. The New England journal of medicine. 2015;373:23-34.
[46] Chuk MK, Chang JT, Theoret MR, Sampene E, He K, Weis SL, et al. FDA Approval Summary: Accelerated Approval of Pembrolizumab for Second-Line Treatment of Metastatic Melanoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017.
[47] Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. The Lancet Oncology. 2015;16:908-18.
[48] Schachter J, Ribas A, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). Lancet (London, England). 2017.
[49] Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. The New England journal of medicine. 2015;372:2521-32.
[50] Hsieh AH, Faithfull S, Brown MP. Risk of cumulative toxicity after complete melanoma response with pembrolizumab. BMJ case reports. 2017;2017.
[51] Akira S, Takeda K. Toll-like receptor signalling. Nature reviews Immunology. 2004;4:499-511.
[52] Drobits B, Holcmann M, Amberg N, Swiecki M, Grundtner R, Hammer M, et al. Imiquimod clears tumors in mice independent of adaptive immunity by converting pDCs into tumor-killing effector cells. The Journal of Clinical Investigation. 2012;122:575-85.
[53] Coati I, Miotto S, Zanetti I, Alaibac M. Toll-like receptors and cutaneous melanoma. Oncology Letters. 2016;12:3655-61.
[54] Joseph RW, Cappel M, Tzou K, Bagaria S, Gilstrap C, Swaika A, et al. Treatment of in-transit and metastatic melanoma in two patients treated with ipilimumab and topical imiquimod. Melanoma research. 2016;26:409-12.
[55] Sisti A, Sisti G, Oranges CM. Topical treatment of melanoma skin metastases with imiquimod: a review. Dermatology online journal. 2014;21.
[56] Besser MJ, Shapira-Frommer R, Treves AJ, Zippel D, Itzhaki O, Hershkovitz L, et al. Clinical responses in a phase II study using adoptive transfer of short-term cultured tumor infiltration lymphocytes in metastatic melanoma patients. Clinical cancer research : an official journal of the American Association for Cancer Research. 2010;16:2646-55.
[57] Wu R, Forget MA, Chacon J, Bernatchez C, Haymaker C, Chen JQ, et al. Adoptive T-cell therapy using autologous tumor-infiltrating lymphocytes for metastatic melanoma: current status and future outlook. Cancer journal (Sudbury, Mass). 2012;18:160-75.
[58] Shi H, Liu L, Wang Z. Improving the efficacy and safety of engineered T cell therapy for cancer. Cancer letters. 2013;328:191-7.
[59] Deniger DC, Kwong ML, Pasetto A, Dudley ME, Wunderlich JR, Langhan MM, et al. A Pilot Trial of the Combination of Vemurafenib with Adoptive Cell Therapy in Patients with Metastatic Melanoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017;23:351-62.
[60] Lee S, Margolin K. Tumor-infiltrating lymphocytes in melanoma. Current oncology reports. 2012;14:468-74.
[61] Babiker HM, Riaz IB, Husnain M, Borad MJ. Oncolytic virotherapy including Rigvir and standard therapies in malignant melanoma. Oncolytic Virother. 2017;6:11-8.
[62] Harrington KJ, Puzanov I, Hecht JR, Hodi FS, Szabo Z, Murugappan S, et al. Clinical development of talimogene laherparepvec (T-VEC): a modified herpes simplex virus type-1-derived oncolytic immunotherapy. Expert review of anticancer therapy. 2015;15:1389-403.
[63] Rehman H, Silk AW, Kane MP, Kaufman HL. Into the clinic: Talimogene laherparepvec (T-VEC), a first-in-class intratumoral oncolytic viral therapy. Journal for immunotherapy of cancer. 2016;4:53.