Advances in targeted therapy and immunotherapy for melanoma (Review)

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Abstract. Melanoma is the most aggressive and deadly type of skin cancer and is known for its poor prognosis as soon as metastasis occurs. Since 2011, new and effective therapies for metastatic melanoma have emerged, with US Food and Drug Administration approval of multiple targeted agents, such as V-Raf murine sarcoma viral oncogene homolog B1/mitogen-activated protein kinase kinase inhibitors and multiple immunotherapy agents, such as cytotoxic T lymphocyte-associated protein 4 and anti-programmed cell death protein 1/ligand 1 blockade. Based on insight into the respective advantages of the above two strategies, the present article provided a review of clinical trials of the application of targeted therapy and immunotherapy, as well as novel approaches of their combinations for the treatment of metastatic melanoma in recent years, with a focus on upcoming initiatives to improve the efficacy of these treatment approaches for metastatic melanoma.

Contents

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1. Introduction

Cutaneous melanin pigment production is a unique characteristic of melanocytes, which has a critical role in the protection against the harmful effects of sun exposure and oxidative stress (1). It is produced in melanosomes by melanocytes in a complex process: An enzymatic transformation of L-tyrosine to dopaquinone and subsequent chemical and biochemical reactions resulting in the production of various 5,6-dihydroxyindole (DHI)-2-carboxylic acid and DHI oligomers-main constituents of eumelanin, and the benzothiazine and benzothiazole units of pheomelanin (2).

Increasing evidence indicates that numerous factors, including ultraviolet radiation (UVR), as well as hormones at the tissue, cellular and subcellular levels, may regulate the biosynthesis of melanin, and UVR is known to be the most significant factor (3). UVR, mainly UVB, results in DNA thymidine breaks and generates genotoxic cyclobutane pyrimidine dimers and 6-4 photoproducts in the skin. The intermediates during this process also include reactive oxygen species, which are highly cytotoxic and interact with multiple cellular components and lead to oxidative DNA damage (4). Furthermore, DNA damage also increases the expression of p53 in keratinocytes and activates the transcription of pro-opiomelanocortin, which is cleaved to form α-melanocyte-stimulating hormone and adrenocorticotropic hormone (5), which then bind to their receptor melanocortin 1 receptor on the cell membrane of melanocytes to stimulate cyclic adenosine monophosphate (AMP)-responsive production and activate the transcription of microphthalmic-associated transcription factor (MITF)-induced pigmentation enzymes, including tyrosinase (TYR) and tyrosine-related protein 2 (6). The formation of the multi-enzyme complex in melanocytes control the quantity and quality of melanin pigment production (7). In addition, UVR can also evoke a transient increase in the cellular levels of diacylglycerol, a component of the melanocyte membrane that activates protein kinase C and regulates melanogenesis via TYR phosphorylation (8).

Recently, a 'Yin and Yang' action of melanogenesis was proposed by Slominski *et al* (1). It means that under physiologic conditions, melanogenesis is highly regulated because it takes place within the boundaries of melanosomes, which has a protective role against UVR-induced melanogenesis and is beneficial to the skin (7); however, the presence of melanin may be necessary for the initiation of malignant transformation

of melanocytes: Under pathological conditions, this process is destructive through highly reactive intermediates of melanogenesis leaking out of melanosomes, which affects the behavior of melanoma cells and the outcomes of different types of therapeutic approach. To be specific, the induction of melanogenesis leads not only to stimulated expression of hypoxia-inducible factor 1α (HIF-1α) protein in melanoma cells but also to the robust upregulation of classical HIF-1-dependent target genes involved in angiogenesis and cellular metabolism, including glucose metabolism; furthermore, a highly oxidative environment results in an immunosuppressive effect within the tumor environment and/or systemically (2,9-11), which inhibits the host responses and promotes melanoma progression, and leads to therapeutic resistance. It may therefore be suggested that inhibition of melanogenesis in advanced melanoma may represent a realistic adjuvant strategy to attenuate melanoma growth, as well as improve immuno-, radio- and chemotherapy.

Melanoma is the most aggressive and deadly type of skin cancer (12). In total, 324,635 new cases and 57,043 deaths from melanoma were registered in the GLOBOCAN 2020 database (13). Although early-stage melanoma is considered to be curable with wide local excision (14), due to its potential to invade the dermis within only a few months, melanoma is fatal when metastasis occurs. Alarmingly, approximately one-third of patients with advanced melanoma have already developed lung, liver or brain metastasis by the time they receive a diagnosis (15). Overall, the 5-year survival rate reaches 99% for patients with localized melanoma but decreases to 27.3% for those with distant metastasis (16). Thus, metastatic melanoma is usually associated with poor prognosis. Recently, despite a steady rise in the worldwide incidence of melanoma, novel therapeutic interventions, such as targeted therapy and immunotherapy, have resulted in rapid and extensive changes in mortality rates (13-16). Targeted agents mainly include mitogen-activated protein kinase (MAPK) pathway inhibitors (15,17,18). Immunotherapy includes immune checkpoint inhibitors, tumor vaccinations and adoptive cell therapies (19-21). For targeted therapy, classic v-Raf murine sarcoma viral oncogene homologue B1 (BRAF) and MAPK kinase (MEK) inhibitors are applied specifically for BRAF V600E/K mutation-positive melanoma. Targeted drugs show high efficacy and increase the overall survival (OS) and objective response rate (ORR) of most patients with metastatic melanoma, though these patients may easily acquire drug resistance (22). Immunotherapy, particularly immune checkpoint inhibitors, may improve a patient's duration of response (DOR), despite a slower onset of action (23). Providing insight into the complementary advantages of these two regimens, the present article reviewed clinical trials of current targeted therapies and immunotherapy for the treatment of metastatic melanoma. The current benefits and limitations of monotherapy or combination therapy may encourage researchers to design strategies to allow for the use of these treatments in more patients with metastatic melanoma.

2. Targeted therapy

BRAF and MEK inhibitors. MAPK cascades involve RAF, MEK and ERK kinases. Approximately 50% of patients with metastatic melanoma harbor a BRAF mutation (with >90% being the BRAF V600E mutation), which mediates overactivation of the MAPK signaling pathway and the survival, differentiation and proliferation of melanocytes (24,25). This oncogenic signaling may be blocked by BRAF (vemurafenib, dabrafenib and encorafenib) or MEK (cobimetinib, trametinib and binimetinib) inhibitors (Fig. 1). Several combination therapies comprising BRAF/MEK inhibitors with approved indications are described in detail below and summarized in Table I.

Vemurafenib and Cobimetinib. In 2011, vemurafenib became the first oral inhibitor for BRAF V600E-mutated melanoma. Cobimetinib, a potent MEK inhibitor, was evaluated in combination with vemurafenib in the Phase Ib BRIM 7 study on patients with advanced BRAF V600E-mutated melanoma who had never received a BRAF inhibitor. A confirmed ORR of 87%, including 10% who had a complete response (CR), with a median PFS of 13.7 months, was reported (26). Further evidence of the efficacy of combined vemurafenib and cobimetinib was reported in the international, multicentre, randomized phase III CoBRIM study (27). In this trial, 495 eligible participants were enrolled and randomized 1:1 to receive either dual cobimetinib plus vemurafenib therapy or vemurafenib alone. With at least 5 years of follow-up, the median OS was significantly increased, with 22.5 months in patients on cobimetinib plus vemurafenib treatment compared with 17.4 months in those on vemurafenib alone; OS rates were continuously improved with this dual therapy compared with vemurafenib alone, with 38 vs. 31% at 3 years, 34 vs. 29% at 4 years, and 31 vs. 26% at 5 years. Similar to the OS results, the median progression-free survival (PFS) was 12.6 vs. 7.2 months, and PFS rates were 14 vs. 10% at 5 years (27). Identifying subgroups of patients likely to have a beneficial long-term treatment outcome is of great importance to informing treatment decisions when managing patients with metastatic melanoma. Conventional prognostic factors for survival outcomes in patients with metastatic melanoma include disease stage, baseline lactate dehydrogenase (LDH) serum level, baseline sum of the longest diameters of the target lesion (SLD), baseline Eastern Cooperative Oncology Group performance status (ECOG PS) and presence/absence of liver metastasis (28,29). In this trial, the long-term survival outcomes with dual cobimetinib and vemurafenib were most favourable in patients with normal baseline LDH levels and a low tumor burden (defined as either SLD ≤45 mm or <3 organ sites with metastasis), with 5-year OS rates of 52% in the subgroup defined by normal baseline LDH and SLD ≤45 mm, and 68% in the subgroup defined by normal baseline LDH and <3 organ sites (27). While the safety profile remained consistent with previous data and there existed no new safety signals (30), several protocol-defined adverse events (AEs) of special interest were more common in the cobimetinib plus vemurafenib group compared with the vemurafenib monotherapy group, including retinal detachment or central serous retinopathy, grade ≥3 photosensitivity, grade ≥3 liver laboratory abnormalities, grade ≥2 ejection fraction reduction and grade ≥3 creatine phosphokinase elevation (27). In addition, the long-term OS outcomes were least favourable in those with elevated baseline LDH >2x upper limit of normal (ULN), and almost all patients had died by 3 years. Therefore, it indicated an urgent need to design different treatment strategies to improve long-term survival outcomes for patients in

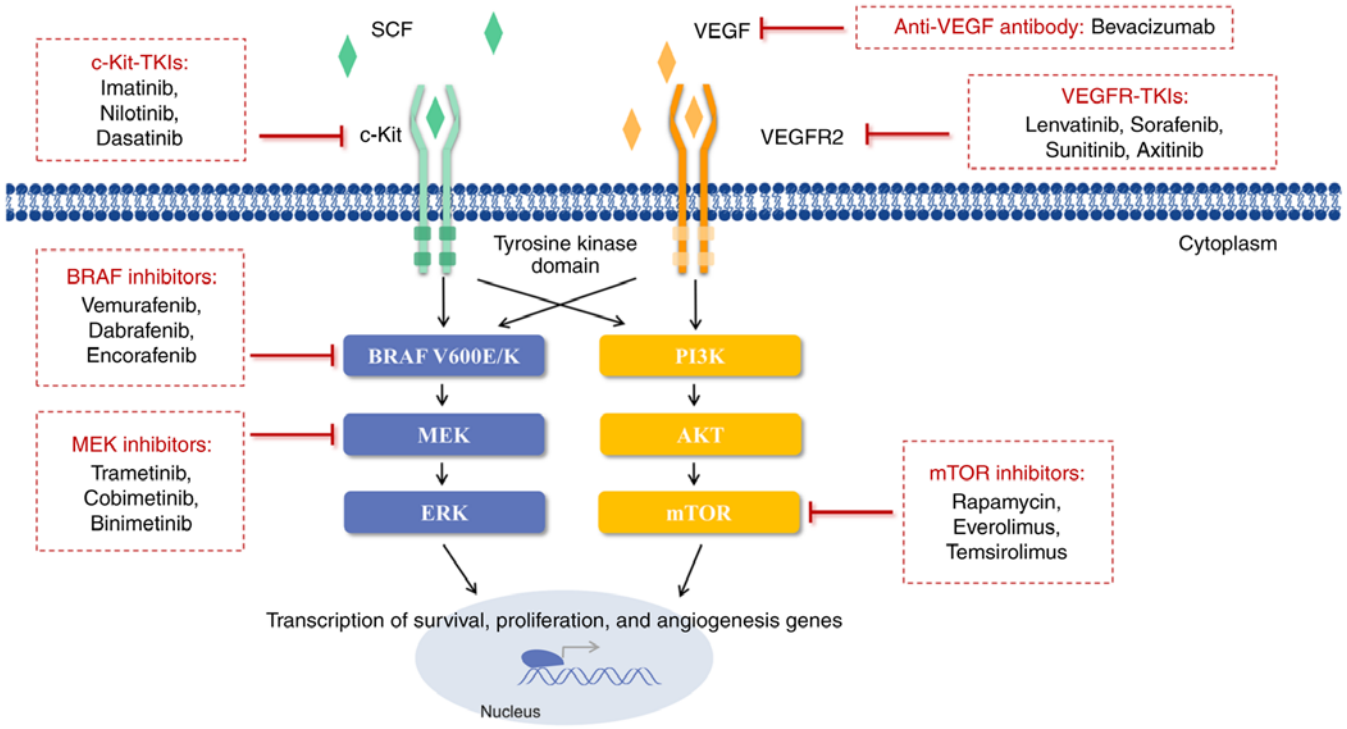

Figure 1. Signal transduction inhibitors for metastatic melanoma. BRAF inhibitors (vemurafenib, dabrafenib and encorafenib) and MEK inhibitors (trametinib, cobimetinib and binimetinib) suppress abnormal MAPK signaling. mTOR inhibitors (rapamycin and rapamycin analogues, everolimus and temsirolimus) downregulate activation of the PI3K/AKT/mTOR pathway, which is related to tumor cell proliferation and angiogenesis. VEGFR-TKIs (lenvatinib, sorafenib, sunitinib and axitinib) inhibit interactions between VEGF and VEGFR2. c-Kit-TKIs (imatinib, nilotinib and dasatinib) inhibit interactions between SCF and c-Kit. Furthermore, bevacizumab directly binds to VEGF, preventing tumor angiogenesis and growth. SCF, stem cell factor; c-Kit, receptor tyrosine kinase; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor; MEK, mitogen-activated protein kinase kinase; ERK, extracellular regulated protein kinase; PI3K, phosphatidylinositol 3 kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; BRAF, v-Raf murine sarcoma viral oncogene homologue B1.

poor prognosis subgroups, particularly those with elevated LDH levels at baseline.

A novel combined treatment strategy of adding one immune checkpoint inhibitor to BRAF/MEK inhibitor combination therapy emerged at an opportune time. The phase III IMspire150 study, examining how effective approved vemurafenib plus cobimetinib combined with or without atezolizumab (A+V+C or P+V+C) is for patients with BRAF V600 mutation-positive melanoma with elevated LDH levels at baseline, is ongoing (31). Of note, patients with anti-programmed cell death ligand 1 (PD-L1)$^{-}$ melanoma, who have fewer PD-L1-expressing tumor-infiltrating cells and generally benefit less from immunotherapy alone, appeared to derive a clinical benefit from A+V+C similarly to those with PD-L1^{+} tumors. Specifically, with a follow-up of 18 months, the median PFS for A+V+C in the PD-L1^{-} and PD-L1^{+} subgroups was 15.2 and 14.8 months, respectively. In addition, the median PFS at 18 months follow-up for A+V+C in the PD-L1^{-} and high-LDH subgroup was higher than that in the PD-L1^{+} and high-LDH subgroup, at 9.8 and 6.3 months, respectively (32). However, long-term benefits have not yet been reported and it is necessary to identify other subgroups that may benefit from triplet A+C+V therapy. TRICOTEL is another multicentre, single-arm, phase 2 clinical trial evaluating the efficacy and safety of this triplet therapy (atezolizumab combined with vemurafenib plus cobimetinib) in patients with BRAF V600 mutation-positive melanoma who were receiving corticosteroids and had symptomatic central nervous system (CNS) metastasis (33). The results indicated that, at the 9.7-month median follow-up duration, the intracranial ORR was 42%, which is comparable to that reported with other available systemic treatments, with 46% of intracranial ORR with the combination of nivolumab plus ipilimumab (34), and with 55% of extracranial ORR with the combination of dabrafenib plus trametinib (35). Although the incidence of treatment-related grade 3 or worse AEs was 68%, this was similar to the incidences reported with A+V+C (79%) and P+V+C (73%) in patients without CNS metastases in the IMspire150 trial (31,33). Furthermore, the occurrence of serous retinopathy was generally consistent with that observed with vemurafenib plus cobimetinib combination in the coBRIM trial (36). Thus, the triplet combination appears to be recommendable for patients with BRAF V600 mutation-positive melanoma, even with CNS metastases.

Dabrafenib and Trametinib. Dabrafenib is another oral BRAF inhibitor that was approved for use in 2013. In the next year, it was approved in combination with trametinib, an oral MEK inhibitor, for the treatment of unresectable, metastatic BRAF V600E/V600K-mutated melanoma. In the randomized, double-blinded phase 3 study (COMBI d) with a median follow-up of 20 months for the combined therapy arm and 16 months for the dabrafenib monotherapy arm, the combination of dabrafenib and trametinib led to an improved median PFS (11 vs. 8.8 months) and median OS (25.1 vs. 18.7 months),

Table I. Combined BRAF and MEK inhibition with approved indications. Dabrafenib plus trametinib, vemurafenib plus cobimetinib, and encorafenib and binimetinib are specifically intended for a majority of patients with melanoma with the BRAF V600 mutation.

Monotherapy or combined therapy	Indication (year of FDA approval)
Dabrafenib and Trametinib	Metastatic, unresectable melanoma with BRAF V600E/K$^+$ (2014); Adjuvant: Resected, stage III melanoma with BRAF V600E/K$^+$ (2018)
Vemurafenib and Cobimetinib	Metastatic, unresectable melanoma with BRAF V600E/K$^+$ (2015)
Encorafenib and Binimetinib	Metastatic, unresectable melanoma with BRAF V600E/K$^+$ (2018)

MEK, mitogen-activated protein kinase kinase; BRAF, v-Raf murine sarcoma viral oncogene homologue B1; FDA, Food and Drug Administration.

with a 2-year OS of 51 vs. 42% for the dabrafenib monotherapy group (37). These findings were consistent with those reported in another trial (COMBI v), which aimed to compare the combination of dabrafenib and trametinib with vemurafenib monotherapy (38). COMBI I is a phase III trial to evaluate the efficacy of the anti-programmed cell death protein 1 (PD-1) antibody spartalizumab combined with dabrafenib and trametinib (sparta-DabTram) vs. placebo combined with dabrafenib and trametinib (placebo-DabTram), with questionable efficacy. After a minimum follow-up period of 24 months, only 20 and 18% of patients in the sparta-DabTram and placebo-DabTram arms, respectively, achieved complete response (39). In addition, PD-1 blockade seemed to add little to combined BRAF and MEK inhibition when treating BRAF V600-mutated melanoma: The median investigator-assessed PFS was 16.2 and 12.0 months in the sparta-DabTram arm and placebo-DabTram arm, respectively, but with no statistically significant difference (39).

A prospective study on patients with advanced BRAF V600-mutated cutaneous melanoma treated with dabrafenib plus trametinib found that LDH, ECOG PS and a large number of metastatic tumor sites are associated with disease progression. Relevant to real-world practice, the study reported brain metastases as a major prognostic factor (28). Similarly, in the COMBI-MB trial, patients were recruited and divided into 4 cohorts based on LDH levels, ECOG PS, type of mutation and presence/absence of brain metastasis. The results indicated that dabrafenib plus trametinib was efficient, with a manageable safety profile, particularly in the BRAF V600-mutated melanoma subgroup without brain metastasis, with a median PFS ranging from 4.2 to 7.2 months and median DOR from 4.5 to 8.3 months (35). Therefore, it appears necessary to explore a better treatment regimen for patients with melanoma with brain metastasis to improve their survival outcomes.

Efforts have been made to evaluate the role of dabrafenib plus trametinib as an adjuvant treatment for high-risk resected disease. In an adjuvant setting, according to the COMBI-AD trial, patients with completely resected stage III melanoma with BRAF V600 mutations treated with dabrafenib plus trametinib had a significantly lower rate of recurrence (40,41). During the follow-up period of 5 years, 52 and 65% of patients who received adjuvant dabrafenib plus trametinib achieved relapse-free and distant metastasis-free survival (DMFS) vs. 36 and 54% of those receiving adjuvant placebo, respectively. Furthermore, the toxicity was consistent with previous data regarding targeted therapy in the metastatic setting and no new toxic effects were reported (42). Based on the positive results with adjuvant dabrafenib and trametinib in this trial, this combination has become a standard treatment option for adjuvant therapy in patients with surgically resected stage III V600E/K BRAF-mutated melanoma. Of note, in COMBI-AD, pyrexia was the most common adverse event experienced by patients treated with dabrafenib plus trametinib and 9% of all patients discontinued treatment due to pyrexia. The subsequent COMBI-APlus trial, which aims to reduce the burden of serious pyrexia-related events associated with this treatment strategy, is currently being conducted (43). The investigators proposed an adapted pyrexia management algorithm: Both drugs were interrupted if patients developed signs and symptoms of possible treatment-emergent pyrexia syndrome: Fever (body temperature ≥38˚C), chills, rigours, night sweats and influenza-like symptoms. Treatment was restarted at the same dose once patients were symptom-free for ≥24 h. The COMBI-APlus trial has now met its primary endpoint of significant reduction in the incidence of composite pyrexia events compared with a historical control from the COMBI-AD trial (43), with lower rates of grade 3/4 pyrexia (3.8%), hospitalization due to pyrexia (4.3%) and discontinuation due to pyrexia (2.4%), compared with COMBI-AD. It seems helpful for patients to manage pyrexia at home, which may be beneficial for patients' quality of life.

Encorafenib and Binimetinib. In 2018, encorafenib emerged as a second-generation BRAF inhibitor and was approved by the food and drug administration (FDA) in combination with another MEK inhibitor, binimetinib. In the randomized, open-label phase III COLUMBUS trial, comparison of PFS by a blinded independent central review revealed a median PFS of 14.9 months in the encorafenib plus binimetinib arm and 9.6 months in the encorafenib monotherapy arm; furthermore, the ORR was 63% for patients who received the combination therapy, while it was 51% for those who received the encorafenib monotherapy. In general, the modified pharmacological properties of encorafenib are considered crucial to its favourable efficacy and increased tolerability profile for melanoma patients carrying the BRAF V600E mutation, as a result of its enhanced on-target effects and less paradoxical MAPK pathway activation (44). This may be why the rate of grade 3 or 4 AEs was slightly reduced with combined therapy as compared with BRAF inhibitors alone (47 vs. 63%) (45).

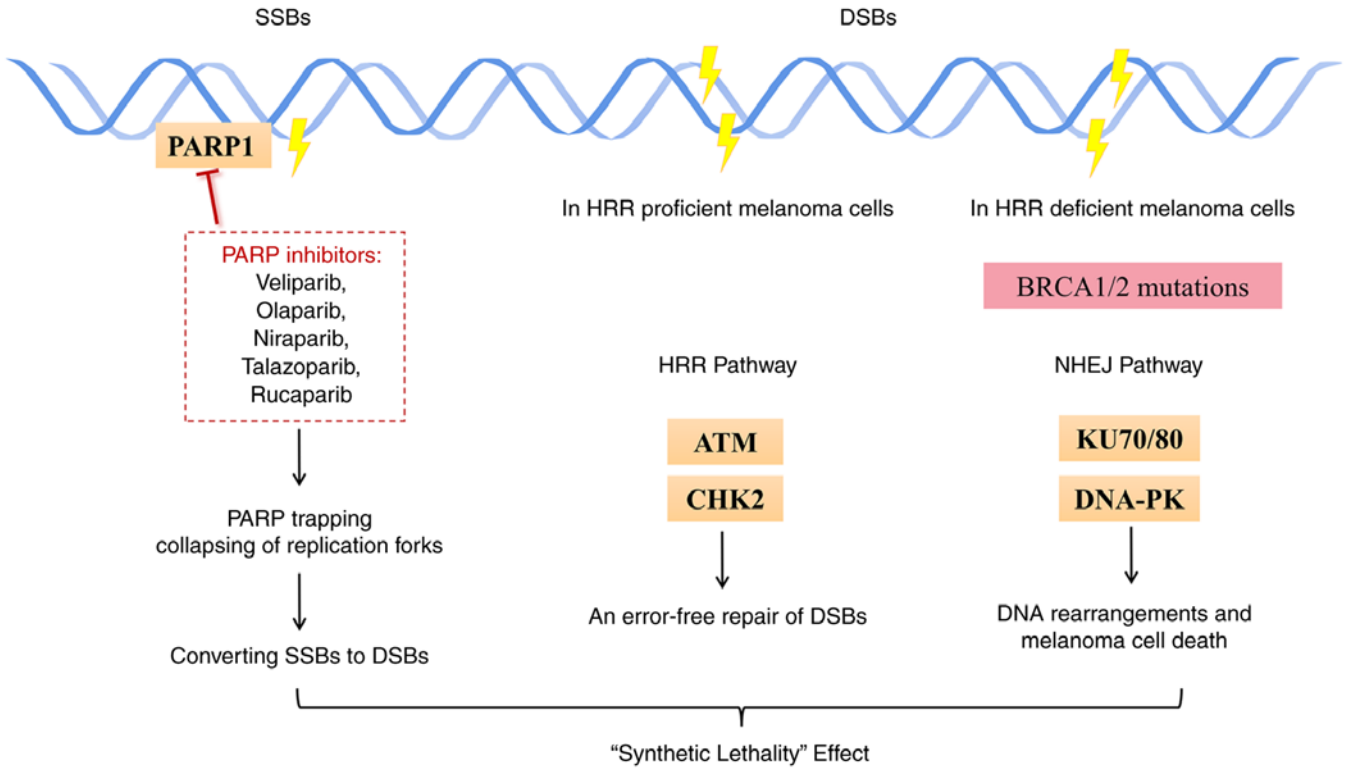

Figure 2. 'Synthetic lethality' effect induced by PARP inhibitors. PARP inhibitors impair the catalytic activity of PARP1, which leads to failure of SSB repair and collapses the progression of replication forks. Deleterious DSBs may accumulate. In melanoma tumor cells with HRR deficiency, such as BRCA1/2 mutations, NHEJ signaling may be activated, which eventually results in tumor cell death. ATM and CHK2, and KU70/80 and DNA-PK are the main components of the HRR and NHEJ pathways, respectively. PARP1, poly(ADP-ribose) polymerase 1; SSBs, single-strand breaks; DSBs, double-strand DNA breaks; HRR, homologous recombination repair; NHEJ, non-homologous end-joining; ATM, ataxia telangiectasia-mutated gene; CHK2, checkpoint kinase 2; DNA-P2, DNA-dependent protein kinase.

Combined BRAF and MEK inhibition has become the standard-of-care treatment for BRAF V600E-mutated melanoma. Other clinical trials to further evaluate the best dose and side effects of the combination regimens are ongoing and may provide more information on targeted therapy for melanoma over time (NCT01909453, NCT03543969, NCT01989585, NCT05026983, NCT04741997, NCT04221438, NCT01902173 and NCT02231775).

DNA damage response (DDR) inhibitors. Different forms of DNA damage, including single-strand breaks (SSBs) and double-strand DNA breaks (DSBs), are repaired by different repair mechanisms. SSBs evoke responses by the base excision repair (BER) pathway, whereas DSBs evoke responses by the homologous recombination repair (HRR) and non-homologous end-joining (NHEJ) pathways (46). It is worth noting that the HRR process is based on template-directed DNA repair synthesis to obtain error-free effective repair of DSBs but that NHEJ signaling is an error-prone repair process that causes DNA rearrangements (47,48).

Poly(ADP-ribose) polymerase (PARP) inhibitors. PARP1 is a major factor in the BER process and it is also critical for HRR and NHEJ. PARP inhibitors act through the following two different mechanisms: Inhibition of canonical PARP function and PARP 'trapping'.

On the one hand, PARP inhibitors inhibit the catalytic activity of PARP1, which leads to failure of SSB repair and stalls and/or collapse of replication fork progression; hence, deleterious DSBs may be generated and accumulate. In replicating cells, these DSBs are normally repaired by HRR signaling; in melanoma tumor cells with HRR deficiency, such as BRCA1, BRCA2 and partner and localizer of BRCA2 (PALB2) mutations, NHEJ signaling may be activated, which may result in modulation of DNA replication dynamics, altered gene expression and tumor cell death (49,50). This is called the 'synthetic lethality' effect (Fig. 2). On the other hand, PARP inhibitors are reported to be more cytotoxic than PARP depletion because the former block activated PARP1 on damaged DNA through a poisonous allosteric effect (51). Furthermore, the potency in trapping PARP varies markedly among different inhibitors, in the order of talazoparib » niraparib > olaparib/rucaparib » veliparib (51).

The PARP inhibitors mentioned above have been approved by the FDA for patients with familial breast or ovarian cancer harboring germline BRCA1/2 mutations (52). This rapid translation from a preclinical model to promising clinical data has driven the development of several PARP inhibitors for different types of tumor, including melanoma. Emerging evidence suggested that the efficacy of PARP inhibitors in delaying the PARP-mediated repair of DNA damage was potentiated by the combination of chemo- and/or radiotherapeutic agents (53). A phase I trial assessing the effect and best dose of veliparib when given together with paclitaxel and carboplatin in patients with metastatic melanoma is ongoing (NCT01366144). Another two phase II trials focusing on the therapeutic outcome of temozolomide plus veliparib or rucaparib in patients with metastatic

melanoma demonstrated a trend towards improvement in PFS, yet without reaching statistical significance (54,55). These disappointing data may be because the patients were not stratified based on HR status. Indeed, one case report described the effect of olaparib monotherapy in a patient with advanced metastatic melanoma carrying a PALB2 mutation who had previously progressed on ipilimumab plus nivolumab combined immunotherapy, showing a partial response to the PARP inhibitor olaparib (56). As described by Khaddour *et al* (57) in another recent case report, a patient with unresectable stage IIIC melanoma with a high HRR deficiency score, whose disease progressed on prior nivolumab monotherapy, achieved a near-complete response to olaparib plus nivolumab. Currently, the use of niraparib or olaparib in patients with melanoma known to have mutations in BRCA1/2, PALB2, BRCA1-Associated Protein 1 (BAP1) or other components of the DDR pathway through synthetic lethality is under clinical validation (NCT05169437, NCT03925350, NCT03207347 and NCT05482074), highlighting the importance of testing for genetic HR mutations in patients with melanoma and determining the mutations' impact on treatment decisions.

Inhibitors of other DDR factors are also under development, including those targeting ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia mutated and Rad3-related (ATR) kinases, cell cycle checkpoint kinase 1/2 (CHK1/2), and WEE1 checkpoint kinase. The DDR-DDR inhibitor combination appears to be an emergent approach to treating melanoma, which is able to target, at the same time, modulators involved in the different pathways melanoma relies on (e.g. pro-survival pathways). Preclinical studies demonstrated that the PARP inhibitor veliparib, as well as the PARP inhibitor Olaparib combined with ATR inhibitor ceralasertib, could reduce the viability of melanoma cells sensitive and resistant to BRAF/MEK inhibitors, respectively, *in vitro* (58,59); however, there are no further related clinical trials of them in patients with melanoma refractory to standard targeted therapy or no trials assessing the efficacy of the combination of DDR inhibitor plus standard targeted therapy in patients with melanoma.

Certain evidence pointed to the emerging role of PARP inhibitors in the tumor immune microenvironment and suggested that the addition of PARP inhibitors may potentiate the therapeutic response to checkpoint inhibitors. The immune role of PARP inhibitors is further discussed below and clinical trials of this combined regimen are summarized in section 4 and Table II.

Angiogenesis inhibitors. Angiogenesis is closely associated with the growth and metastasis of solid tumors, including melanoma (60). As the tumor grows, its cells consume a large amount of oxygen and nutrients, leading to hypoxia in the tumor microenvironment, which subsequently induces upregulation of proangiogenic factors, mainly vascular endothelial growth factor (VEGF). The interaction between VEGF and its main receptor, VEGF receptor 2 (VEGFR2), induces several downstream angiogenic signaling pathways, such as Src/vascular endothelial cadherin (VE-cadherin) signaling and phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT)/endothelial nitric oxide (NO) synthase/NO signaling, to become aberrantly activated (61,62). Therefore, VEGF and VEGFR have become predominant targets for the development of anti-angiogenic agents (Fig. 1).

Anti-VEGF antibody. Bevacizumab, a humanized immunoglobulin G1 (IgG1) monoclonal antibody, has been the first approved anti-angiogenic agent since 2004. It is designed to selectively bind to VEGF and block interactions between VEGF and its receptors, thereby preventing tumor angiogenesis and growth. Based on promising previous clinical data, bevacizumab leads to particularly favourable outcomes and has an acceptable safety profile when used in combination with chemotherapy for the treatment of malignant melanoma (63-66). In the most recent randomized controlled phase II trial of bevacizumab in combination with carboplatin plus paclitaxel (a first-line chemotherapeutic regimen) for advanced mucosal melanoma, the addition of bevacizumab markedly increased the median PFS from 3.0 to 4.8 months and the median OS from 9.0 to 13.6 months (66). A phase III study further evaluating the benefits of this new therapeutic protocol carboplatin-paclitaxel-bevacizumab (CPB) is underway (NCT02023710). Overall, the CPB protocol may become an alternative for the treatment of mucosal melanoma in the first-line setting. In addition, Hodi *et al* (19) found that bevacizumab showed significant synergistic efficacy when used in combination with ipilimumab for metastatic melanoma. These authors recently showed that patients with a long-term or delayed increase in soluble PD-L1 had clinically beneficial outcomes, highlighting the cross-talk between tumor immunity and angiogenesis within the tumor microenvironment (67).

VEGFR tyrosine kinase inhibitors (TKIs). Several VEGFR-TKIs have been approved for use as targeted therapy, mainly in metastatic lung cancer, including sorafenib, lenvatinib, sunitinib and axitinib. Recently, the clinical efficacy of VEGFR-TKIs in metastatic melanoma, as a part of combination medications or a single agent, has been evaluated and showed limited but promising outcomes (68-72). High expression of VEGF in was observed in patients with metastatic melanoma, indicating that high vascularity may be a prognostic factor for tumor progression and metastasis. Following the novel idea of dual VEGF and VEGFR signaling blockade, a phase II trial of combined therapy of bevacizumab plus sorafenib evaluated efficacy and safety for advanced melanoma. The results suggested that of 14 patients with malignant melanoma who received treatment, 57% achieved stable disease (SD) lasting ≥16 weeks, including 3 with SD lasting ≥1 year. The median time to progression (TTP) was 32 weeks. Of note, patients with low serum VEGF tended to achieve longer TTP than those with high serum VEGF (50 vs. 15 weeks) (73). In terms of drug safety, hypertension, fatigue and foot syndrome were the most frequently reported drug-related AEs.

To date, the therapeutic strategy of dual VEGF and VEGFR inhibition has not yet been validated, mainly because dual inhibition is likely to generate significant toxicities compared with monotherapy. For instance, despite improved anti-tumor efficacy, the clinical trial of bevacizumab plus sunitinib in melanoma and renal cell carcinoma was suspended due to microangiopathy (74).

Other targeted therapies

Receptor tyrosine kinase (c-Kit)-TKIs. c-Kit, also named cluster of differentiation (CD)117, is a class III transmembrane receptor tyrosine kinase (75). Melanomas harbouring c-Kit

Table II. Ongoing clinical trials combining targeted therapy and immunotherapy. Clinical trials of combined therapy involving BRAF/MEK inhibitors plus immune checkpoint inhibitors or involving angiogenesis inhibitors plus immune checkpoint inhibitors are listed.

NCT number	Trial phase	Targeted therapy	Immunotherapy	Status
NCT04511013	II	Encorafenib, Binimetinib	Ipilimumab, Nivolumab	Recruiting
NCT03235245	II	Encorafenib, Binimetinib	Ipilimumab, Nivolumab	Recruiting
NCT02631447	II	Encorafenib, Binimetinib	Ipilimumab, Nivolumab	Active, not recruiting
NCT02224781	III	Dabrafenib, Trametinib	Ipilimumab, Nivolumab	Active, not recruiting
NCT01940809	I	Dabrafenib, Trametinib	Ipilimumab, Nivolumab	Active, not recruiting
NCT04741997	I	Encorafenib, Binimetinib	Nivolumab	Recruiting
NCT02910700	II	Encorafenib, Binimetinib Dabrafenib, Trametinib	Nivolumab	Recruiting
NCT04375527	II	Binimetinib	Nivolumab	Recruiting
NCT04310397	II	Dabrafenib, Trametinib	Spartalizumab	Active, not recruiting
NCT02967692	III	Dabrafenib, Trametinib	Spartalizumab	Active, not recruiting
NCT01950390	II	Bevacizumab	Ipilimumab	Active, not recruiting
NCT04996823	II	Axitinib	Ipilimumab	Recruiting
NCT03086174	Ib	Axitinib	Toripalimab	Active, not recruiting
NCT03175432	II	Bevacizumab, Cobimetinib	Atezolizumab	Recruiting
NCT03554083	II	Vemurafenib, Cobimetinib	Atezolizumab	Recruiting
NCT04356729	II	Bevacizumab	Atezolizumab	Recruiting
NCT02902042	I/II	Encorafenib, Binimetinib	Pembrolizumab	Completed
NCT04657991	III	Encorafenib, Binimetinib	Pembrolizumab	Recruiting
NCT02858921	II	Dabrafenib, Trametinib	Pembrolizumab	Active, not recruiting
NCT02298959	I	Aflibercept	Pembrolizumab	Recruiting
NCT04633902	II	Olaparib	Pembrolizumab	Recruiting
NCT04187833	II	Talazoparib	Pembrolizumab	Recruiting
NCT03820986	III	Lenvatinib	Pembrolizumab	Active, not recruiting

MEK, mitogen-activated protein kinase kinase; BRAF, v-Raf murine sarcoma viral oncogene homologue B1; NCT, National Clinical Trials registry.

alteration or amplification are mainly found in mucosal, acral and chronically sun-damaged skin (76). c-Kit becomes activated via phosphorylation after stem cell factor binding and is primarily responsible for cell growth, survival and migration. Thus, the c-Kit gene is regarded as an oncogene and small molecule inhibitors targeting c-Kit in metastatic melanoma have been developed and investigated in clinical trials (76). The relevant oncogenic cascades and several targeted agents for melanoma are depicted in Fig. 1.

Guo *et al* (77) first conducted a phase II trial of the application of imatinib for the treatment of patients with metastatic melanoma harbouring c-Kit mutation and/or of c-Kit gene copy number amplification. The results of this trial indicated that the use of imatinib was associated with a median PFS of 3.5 months and tumor regression in 41.9% of patients, with an overall 6-month PFS rate of 36.6%, an overall 1-year survival rate of 51.0% and an overall disease control rate of 55% in the cohort. It was also reported that imatinib may be preferred for patients with c-Kit genetic aberrations (77). Recently, Jung *et al* (78) performed a pooled analysis (n=130) based on retrospective, 'real-world' experience of imatinib for melanoma. In this study, patients with mucosal melanoma appeared to have a higher response rate (38%) than those with acral melanoma (25%). Patients harboring L576P (exon 11) or K642E (exon 13) mutations displayed the greatest response rates (52 and 42%) and disease control rates (65 and 92%); of note, no patients with mutations in exon 17 had response or disease control. In addition, seemingly longer PFS (median, 4.3 and 4.5 vs. 1.1 months) and OS (median, 19.7 and 15.4 vs. 12.1 months) were observed in patients with exon 11 and 13 vs. exon 17 alterations, but there was no statistical significance. This result indicates that more refined genetic selection strategies for imatinib as a treatment of c-Kit-altered melanoma are needed in subsequent trials.

A new meta-analysis revealed the highest ORR of 20% for nilotinib, another promising c-Kit selective inhibitor, when compared with dasatinib and sunitinib (8 and 8%), though very similar to imatinib, with an ORR of 19% (17). Furthermore, five clinical trials of this small molecule inhibitor have been conducted, in which all eligible participants received nilotinib 400 mg twice daily (79-83). Among these trials, an ORR of 26.2% was observed in the single-arm, phase II trial of nilotinib in 42 patients with c-Kit-mutated advanced or inoperable melanoma (80). The most recent results from the phase II multicentre trial performed by the French Skin Cancer Network reported a durable tumor response in 16% of patients

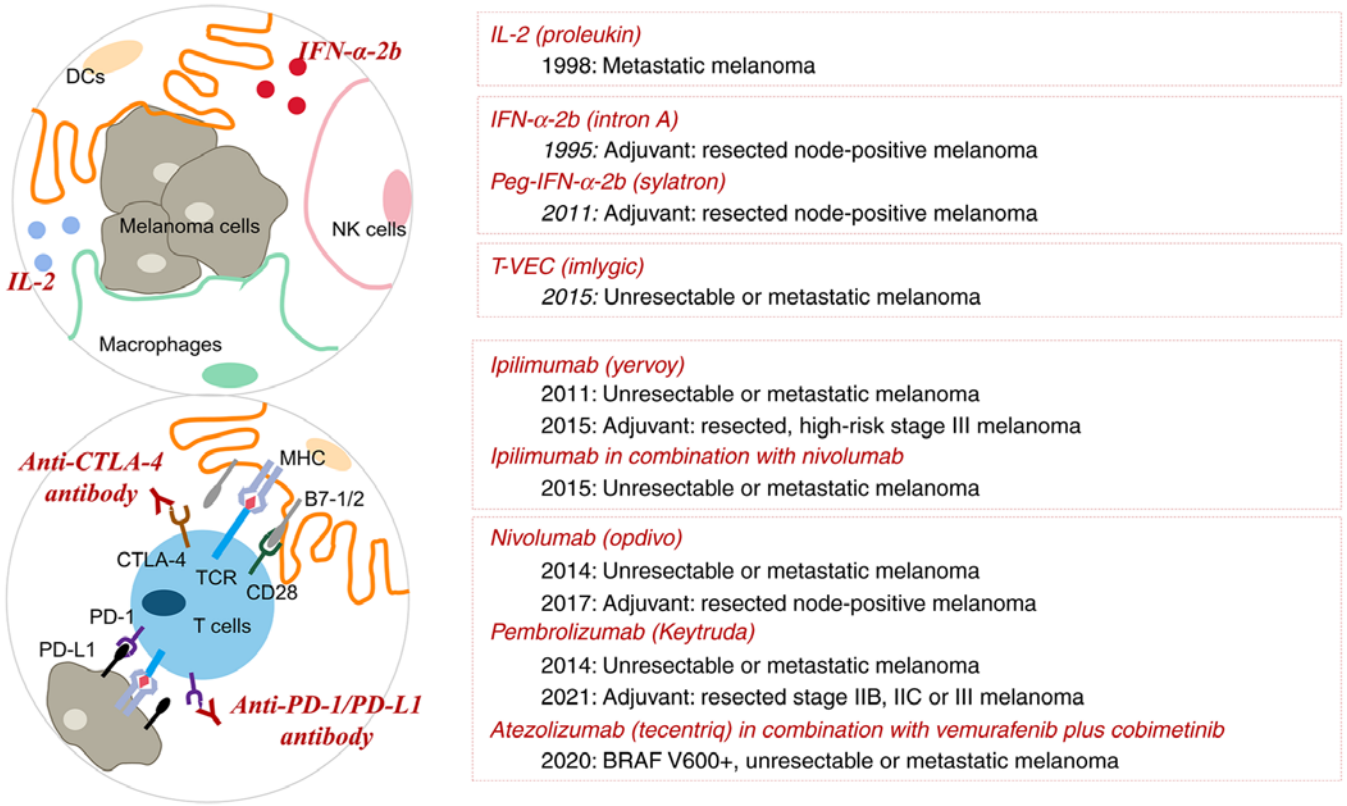

Figure 3. Food and Drug Administration-approved immunotherapy drugs for metastatic melanoma. DCs, dendritic cells; NKs, natural killer cells; IFN-α-2b, interferon-α-2b; IL-2, interleukin 2; GM-CSF, granulocyte macrophage-colony stimulating factor; MHC, major histocompatibility complex; TCR, T-cell receptor; CTLA-4, cytotoxic T lymphocyte-associated protein 4; BRAF, v-Raf murine sarcoma viral oncogene homologue B1.

at 6 months after nilotinib initiation. The best ORR was 20% and the disease control rate was 56%; however, these results were limited to those harbouring exon 11 or 13 mutation (81). These nilotinib studies showed similar ORRs, similar to historical data for imatinib treatment (77,84), indicating that nilotinib may serve as an active agent for patients with disease progression after receiving imatinib.

Mammalian target of rapamycin (mTOR) inhibitors. mTOR is a key kinase downstream of PI3K/AKT signaling and is considered a regulator of cell proliferation, survival, differentiation, apoptosis, angiogenesis and metabolism (85). Aberrant activation of the mTOR pathway is strongly linked to the pathogenesis of melanoma and relevant clinical agents targeting mTOR itself have been developed. *In vitro*, rapamycin and the rapamycin analogues everolimus and temsirolimus show a significant inhibitory effect on melanoma cell proliferation (86). Temsirolimus was reported to be safe when combined with bevacizumab, with enhanced systemic immune function for patients with BRAF-wild-type melanoma (87). In addition, everolimus plus bevacizumab was demonstrated to be well tolerated, despite frequent grade 1 or 2 toxicities, for patients with metastatic melanoma who had previously received chemotherapy and/or immunotherapy (88). Although mTOR inhibitors have an essential role in anti-angiogenesis, a recent clinical trial (NCT02023710) suggested that the addition of everolimus to the CPB therapeutic protocol may not improve PFS among patients with unresectable stage IV melanoma. Overall, the efficacy, safety and scientific validity in metastatic melanoma produced by different treatment options with mTOR inhibition remain unclear and more preclinical and clinical studies are needed to evaluate mTOR-targeted therapeutic approaches.

3. Immunotherapy

Immunotherapy has the ability to prevent tumor growth and recurrence long-term by sensitizing the host's immune system or strengthening the anti-tumor response. Based on decades of research on solid tumor immunology, immunotherapy is considered a promising new modality not only for metastatic melanoma but also for other solid cancers, including kidney cancer, non-small cell lung cancer and head and neck cancer. This approach has definitely led to marked improvements in survival in these patients. To date, several immunotherapy drugs, particularly blockade of negative immune regulatory checkpoints, have been approved by the FDA for metastatic melanoma (Fig. 3).

Immune checkpoint blockade. CD28, which is present on the surface of T cells, acts as a co-stimulatory molecule to induce full activation of T cells by binding to B7-1 (CD80) and B7-2 (CD86) on dendritic cells. Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) is a member of the CD28 family. CTLA-4 is regarded to have a higher affinity to compete with CD28 for binding to its ligands and thereby to mediate T-cell exhaustion, perhaps via inhibition of interleukin 2 (IL-2) accumulation and cell cycle progression (89). PD-1, which is also called PDCD1 or CD279, is another inhibitory molecule

on the surface of various immune cells. Upon binding to the ligands PD-L1 (CD274) and PD-L2 (CD273) on tumor cells or antigen-presenting cells (APCs), PD-1 inhibits the T-cell receptor (TCR) signaling pathway and thus blocks the immune response (90).

The 2018 Nobel Prize in Physiology or Medicine was awarded to Professor James Allison and Professor Tasuku Honjo for their work on CTLA-4 and PD-1, respectively (91,92). From theory to reality, the idea of unleashing the host's immune system to kill cancer cells using CTLA-4 and PD-1/PD-L1 blockade has led to impressive results in cancer therapy.

CTLA-4 blockade. In 2011, ipilimumab, a fully humanized IgG1 monoclonal antibody designed to block the CTLA-4-CD80/86 interaction, was first approved for the treatment of advanced unresectable stage III or IV melanoma, ushering in a new era of immune-based therapies for cancer. In a phase III study among patients with previously treated metastatic melanoma, rates of OS were significantly higher with ipilimumab monotherapy (3 mg/kg) or ipilimumab plus glycoprotein 100 (gp100), a peptide vaccine, than with gp100 monotherapy (45.6 and 43.6 vs. 25.3% at 12 months, 33.2 and 30.0 vs. 16.3% at 18 months, and 23.5 and 21.6 vs. 13.7% at 24 months). However, the removal of self-tolerance induces autoimmune toxicities, which is termed immune-related adverse events (irAEs). The incidence of irAEs was reported to be higher with ipilimumab alone or combined with gp100 (including dermatologic, pruritus, rash, vitiligo, gastrointestinal and diarrhea) compared with gp100 alone (14.5 and 10.2 vs. 3.0%) (93). In an adjuvant setting, approval from the FDA was granted in 2015 based on the results of the EORTC 18071 phase III trial. The trial aimed to evaluate the efficacy of adjuvant ipilimumab at 10 mg/kg in patients with stage III cutaneous melanoma who had undergone a complete regional lymph node dissection. At a mean follow-up of 5.3 years, 5-year rates of recurrence-free survival (RFS), OS, and DMFS were 40.8, 65.4 and 48.3% in patients on adjuvant ipilimumab treatment compared with 30.3, 54.4 and 38.9% in the placebo group, respectively (94). The frequency of grade 3 or 4 irAEs in the ipilimumab group was higher than that in the placebo group (41.6 vs. 2.7%), with the most common ones being gastrointestinal disorders, hepatitis and endocrinopathy. At the cost of substantial toxic effects, this updated analysis was consistent with the previously observed prolongation of RFS in patients on adjuvant ipilimumab treatment (95). While ipilimumab achieved high efficacy and durability of the anti-melanoma response, the clinical use of this drug in tumor immunotherapy is limited because of severe irAEs. Indeed, these irAEs may vary in severity and are occasionally life-threatening. Pancreatitis, nephritis, severe skin reactions including Steven Johnson syndrome, neurologic conditions like inflammatory myopathy, aseptic meningitis, posterior reversible encephalopathy syndrome and myasthenia gravis are also described in the literature, underscoring a wide spectrum of effects of immune activation (96,97).

Tremelimumab is another anti-CTLA-4 monoclonal antibody. At the beginning of phase I and II testing in patients with solid malignancies, promising preliminary anti-tumor activity and safety with single-agent tremelimumab (15 mg/kg) were demonstrated (98,99). The most common AEs were diarrhea, colitis, dermatologic events and fatigue, which were similar to those of ipilimumab treatment. However, in the phase III study, the incidence of AE-related dose discontinuation was 12.5%, compared with 5% in the phase II study. The rates of grade 5 AEs were 2 and 0.8%, respectively. Therefore, the side effect profile of tremelimumab appears comparable to that of ipilimumab (100).

PD-1/PD-L1 blockade. In 2014, the FDA granted accelerated approval for two fully humanized anti-PD-1 IgG4 isotype antibodies, nivolumab and pembrolizumab, as first-line treatment of unresectable or metastatic melanoma, thereby markedly advancing treatment. As demonstrated in the CheckMate 037 and KEYNOTE 002 trials, both nivolumab and pembrolizumab had superior efficacy and an improved safety profile compared with investigator-choice chemotherapy for the treatment of ipilimumab-refractory advanced melanoma (101,102). Of note, compared with the ipilimumab trial, a lower rate of high-grade AEs (10-15%) and discontinuations due to AEs (5-7%) was detected in nivolumab and pembrolizumab clinical trials (101,102). In general, anti-PD-1 therapy appears to have fewer severe side effects than ipilimumab. This is probably due to the activation and expansion of a wider variety of T-cell subpopulations from CTLA-4 inhibition (103). In a phase II trial of patients with advanced melanoma, known BRAF V600 mutation status and an ECOG PS of 0 or 1 (CheckMate 069), at a median follow-up of 24 months, the OS rates were 63.8% in the nivolumab plus ipilimumab group and 53.6% in the ipilimumab group. The incidence of grade 3 or 4 AEs in the combination group was 54 vs. 20% in the ipilimumab monotherapy group (104). While this dual therapy has shown impressive efficacy relative to other available therapies, toxicity seems a major barrier. Furthermore, it has demonstrated consistent results in a phase III trial (CheckMate 067). In patients with BRAF-mutated tumors, the patient follow-up data indicated 6.5-year OS rates of 57% in the nivolumab plus ipilimumab group vs. 43% in the nivolumab monotherapy group and 25% in the ipilimumab monotherapy group; in those patients with BRAF-wild-type tumors, the 6.5-year OS rates were 46, 42 and 22%, respectively (105). In terms of the safety profile, almost all patients (82.1-95.5%) experienced a treatment-related AEs (TRAEs), and more than half of the patients in the combination group (55.0%) experienced a grade 3/4 AE, compared with 16.3 and 27.3% in the nivolumab and ipilimumab groups, respectively, with diarrhea, colitis, increased alanine aminotransferase levels, and increased aspartate aminotransferase levels being the most common ones. With the use of immunosuppressive or immunomodulatory agents, most select AEs were also manageable with established treatment guidelines, with no treatment-related death reported in the combination arm (106). Therefore, due to the durable, sustained survival benefits with an acceptable safety profile, nivolumab plus ipilimumab combined therapy was approved as the first dual checkpoint inhibition regimen in patients not only with BRAF-wild type melanoma but also with BRAF-mutated melanoma (21). In addition, nivolumab monotherapy or in combination with ipilimumab was also reported to be effective in immunotherapy-naïve patients with melanoma brain metastases. A high proportion of patients achieved an intracranial response with the combination (107).

Atezolizumab, a monoclonal anti-PD-L1 antibody, was approved for clinical use in combination with vemurafenib and cobimetinib as a first-line treatment in patients with BRAF V600-mutated advanced melanoma. The ongoing phase III IMspire150 study is examining how efficacious the approved BRAF/MEK inhibitor combinations (vemurafenib and cobimetinib) with or without atezolizumab are for patients with unresectable, advanced or metastatic BRAF V600 mutation-positive melanoma (31). Given that the adjuvant roles of nivolumab and pembrolizumab have been verified, a study of neoadjuvant atezolizumab in cutaneous melanoma is currently underway (NCT04020809). In addition, with an increasing understanding of the interactions between the immune system and melanocytes, the efficacy and safety of atezolizumab in combination with other major immune checkpoint blockades may be promising; this is currently under clinical validation (NCT03554083 and NCT03829501).

Furthermore, the adjuvant role of PD-1/PD-L1 blockade in patients with high-risk melanoma is gradually being revealed and data continue to mature. The phase III CheckMate 238 randomized study compared adjuvant nivolumab to ipilimumab in patients with resected stage IIIB/C and stage IV melanoma without evidence of disease and demonstrated a 12-month RFS rate of 70.5% and 18-month RFS rate of 66.4% in the nivolumab group compared to 60.8 and 52.7% in the ipilimumab group, respectively, along with significantly decreased toxicity (108). In addition, longer DMFS was observed in the nivolumab group as compared with the ipilimumab group (108). An update reported a 5-year RFS of 50% with nivolumab vs. 39% with ipilimumab and a 5-year OS of 76 vs. 72%, respectively (109). Furthermore, a recent clinical trial (NCT04099251) of adjuvant nivolumab (CheckMate 76K) has indicated a great reduction in the risk of recurrence or death of 58% in those with completely resected stage IIB/C melanoma. Based on these results, in 2017, nivolumab was approved in the adjuvant setting for resected melanoma. In a phase III trial for resected stage III melanoma (KEYNOTE 054), adjuvant pembrolizumab resulted in a clinically relevant 20% improvement in 3-year RFS compared with placebo (63.7 vs. 41%) (110). At a 42.3-month median follow-up, pembrolizumab improved DMFS (65.3 vs. 45.4%) and recurrence-free survival (59.8 vs. 41.4%) compared with the placebo group, leading to its approval in the USA and Europe for patients with stage IIB or IIC melanoma following complete resection (111). A trial by the Southwest Oncology Group (SWOG 1404) also demonstrated a significant improvement in RFS with adjuvant pembrolizumab in patients with resected stage III melanoma with a high risk of recurrence, compared with high-dose interferon α2b (IFNα2b) or ipilimumab (112). As expected, the incidence of grade 3 or higher toxicity was greater with IFNα2b and ipilimumab compared with pembrolizumab (66 and 43 vs. 17%) (112). Similarly, in the KEYNOTE 716 trial in patients with stage IIB/C melanoma, after a median follow-up of 27 months, adjuvant pembrolizumab markedly improved both 24-month RFS (81.2 vs. 72.8%) and DMFS (88.1 vs. 82.2%) compared with the placebo group (113). Based on these results, the FDA extended its indications to adult and paediatric (12 years of age and older) patients with surgically resected high-risk stage IIB, IIC or III melanoma in December 2021. To date, however, these studies haven't demonstrated any improvement in OS, the reason for which is unclear but it is probably affected by post-relapse treatment, insufficient follow-up or biologic and immune issues not yet fully understood (114). It is also unclear whether it is more efficacious to treat when there is a residual microscopic disease or to wait until patients have disease recurrence to avoid treating those who may have been cured by surgery alone. Integrating biomarkers into adjuvant trials may allow for better selection of those truly benefiting from adjuvant therapy (114). Disease recurrence was still observed in >30% of those patients with high-risk melanoma receiving adjuvant immunotherapies within 2 years after surgery (94,108,114). Administering immunotherapies in a neoadjuvant setting before surgery is a promising strategy. Due to the presence of the tumor at the beginning of the therapy, neoadjuvant therapies may induce a deeper immune response, thereby reducing the tumor burden and facilitating cancer surgery. Recently, a phase II SWOG S1801 trial indicated a 42% reduction in 2-year event-free survival risk with neoadjuvant compared to adjuvant pembrolizumab in patients with resectable stage IIIB-D/IV melanoma (72 vs. 49%), indicating that neoadjuvant single-agent immunotherapy may serve as a new standard of care. In the phase Ib OpACIN trial, 20 participants with macroscopic stage III melanoma were randomized either to receive four cycles of adjuvant ipilimumab plus nivolumab every 3 weeks following therapeutic lymph node dissection or to receive two cycles of neoadjuvant ipilimumab plus nivolumab every 3 weeks before surgery, subsequently followed by total lymph node dissection at week 6 and another two cycles of treatment at week 12. The results in the neoadjuvant arm were promising, with an unexpectedly high pathologic partial response (PPR) of 78% at a median follow-up of 25.6 months, and these responding patients remained free of relapse at 2 years (115). However, this regimen induced similarly high toxicity in both arms, with ~90% of patients experiencing one or more grade 3 or 4 irAEs, resulting in early treatment discontinuation in 18/20 patients (115). To further identify a dosing schedule of the neoadjuvant application of ipilimumab plus nivolumab to make it less toxic but equally effective, the subsequent multicentre phase II OpACIN-neo trial was launched. A total of 86 patients were enrolled and randomly assigned to one of three different dosing groups, and then therapeutic lymph node dissection was planned at week 6 without additional adjuvant therapy. Finally, this trial identified a tolerable neoadjuvant treatment regimen consisting of two cycles of ipilimumab 1 mg/kg plus nivolumab 3 mg/kg every 3 weeks, which may be suitable for broader clinical use, with a high PPR of 77 and 20% grade 3 or 4 irAEs (116). After a median follow-up of 4 years, the OpACIN and OpACIN-neo trials showed that this treatment strategy induced durable RFS in >80% of patients. The investigators also found that those with a high IFN-γ score and high tumor mutational burden (TMB) had a PPR of 100%; For those with a low IFN-γ score/low TMB, the PPR was only 39%, while for those with only a high IFN-γ score or only high TMB, the PPR was 91 and 88%, respectively (117). This indicated that the presence of a pathologic response was possibly a surrogate marker for long-term benefits and showed the predictive potential of TMB and IFN-γ score, which may help discriminate responders from non-responders (117). Recently updated data from the OpACIN and OpACIN-neo

trials showed that at a median follow-up of 69 months for OpACIN, only 14.3% of patients with a pathologic response to neoadjuvant application of combined checkpoint inhibitions had disease recurrence; at a median follow-up of 47 months for OpACIN-neo, the estimated 3-year RFS rate was 95% for those with a pathologic response vs. 37% for those without pathologic response (118).

In order to increase RFS in the non-responders among patients with melanoma, the investigators raised the concept of a pathologic response-driven treatment strategy (119,120). In a multicentre phase 2 PRADO extension cohort of OpACIN-neo, 99 patients with IIIB-D nodal melanoma received 6 weeks of neoadjuvant ipilimumab plus nivolumab. Subsequently, for those who achieved major pathologic response (MPR, ≤10% viable tumor) in their index lymph node (the largest lymph node metastasis at baseline), therapeutic lymph node dissection (TLND) and adjuvant therapy were omitted; those with a PPR (>10 to ≤50% viable tumor) underwent TLND only, while those with a pathologic non-response (PNR, >50% viable tumor) underwent TLND and subsequent adjuvant therapy (nivolumab in BRAF V600E/K wild-type and dabrafenib plus trametinib in BRAF V600E/K-mutant patients). Surprisingly, in contrast to the 4-year RFS rate of 100% for patients with PPR in the OpACIN-neo cohort, these same patients with PPR in the PRADO study had a 2-year RFS rate of only 64%, with a rate of 93 and 71% in patients with MPR and PNR, respectively (119). Thus, the investigators questioned whether MPR, instead of pathologic response, would be a better predictor of outcome. According to the PRADO data, the currently recruiting phase III NADINA trial comparing neoadjuvant to adjuvant therapies in macroscopic stage III melanoma was amended: In arm A, patients with MPR will receive two cycles of ipilimumab plus nivolumab and will undergo TLND at week 6. For those with a PPR or PNR, surgery will be followed by adjuvant nivolumab or BRAF/MEK inhibition (in case of BRAF V600E/K mutation-positivity). Meanwhile, in arm B, patients will undergo upfront TLND followed by adjuvant nivolumab (121). Taken together, the outcomes of the S1801, OpACIN, OpACIN-neo, PRADO, and the awaited results of the NADINA trial not only encouraged neoadjuvant checkpoint immunotherapy to become a new standard of care in patients with high-risk melanoma but also indicated the importance of the concept of personalized treatment strategies based on pathologic response after neoadjuvant therapy.

Novel immune checkpoint blockades. Lymphocyte activation gene 3 (LAG-3) is expressed on T cells and is the third immune checkpoint co-inhibitor receptor to be exploited in cancer immunotherapy (122). With its higher affinity for major histocompatibility complex class II than CD4, it mediates the downregulation of T-cell activation and proliferation (122). Preclinical studies have demonstrated enhanced tumor-specific immunity and disruption to melanoma tumor growth not only in dual anti-LAG-3 and anti-PD-1 antibody-treated mice but also in $Lag3^{-/-}Pdcd1^{-/-}$ (Pdcd1 encodes PD-1) mice, suggesting a potentially beneficial combinatorial strategy of dual LAG-3/PD-1 blockade for melanoma (123). In a phase I/IIa cohort expansion study of the fully human LAG-3-specific antibody relatlimab administered alone and in combination with nivolumab in participants with melanoma who progressed during prior anti-PD-1/PD-L1 therapy, escalation to nivolumab plus relatlimab resulted in an ORR of 11.5% in all patients and of 18% in patients with LAG-3 expression ≥1% (124). The randomized, double-blind phase II/III RELATIVITY-047 study of nivolumab with or without relatlimab for treating unresectable melanoma or melanoma that has spread has been underway. Results thus far reported a 12-month PFS rate of 47.7% with a fixed-dose combination of relatlimab and nivolumab compared with 36.0% with single-agent nivolumab. In terms of drug safety, the combination was well tolerated with 21.1% of patients experiencing grade 3 or 4 TRAEs (125). Given its efficacy and favourable toxicity profile in RELATIVITY-047, in March 2022, the FDA approved the use of fixed-dose relatlimab/nivolumab combination in patients with unresectable or metastatic melanoma. Of note, the currently recruiting phase III RELATIVITY-098 (NCT05002569) trial aimed to test this combination vs. nivolumab alone after complete resection of stage III-IV melanoma in the adjuvant setting. In another randomized trial evaluating how well the relatlimab/nivolumab combination worked in treating resectable clinical stage III or oligometastatic stage IV melanoma in the neoadjuvant and adjuvant setting, the eligible 30 participants first received 2 neoadjuvant doses of relatlimab 160 mg and nivolumab 480 mg every 4 weeks followed by surgery and then 10 doses of adjuvant relatlimab/nivolumab combination therapy. These neoadjuvant and adjuvant checkpoint blockades resulted in a high MPR rate of 57% and an improvement in the 1- and 2-year RFS rate (100 and 92%, respectively) in patients achieving any pathologic response compared to those without a pathologic response. Safety during neoadjuvant therapy is favourable, with no grade 3 or 4 irAEs experienced, while 26% grade 3 or 4 toxicities were observed in the adjuvant setting (126). Though the study was limited by its small sample size, these initial results were encouraging and similar to the individual arms in the OpACIN-neo trial, providing further confirmation of the efficacy and safety of this new immunotherapy regimen. Additional follow-up may be necessary to fully assess the clinical impact and the durability of responses.

Adoptive cell therapy. Adoptive cell therapy uses either natural host cells that exhibit anti-tumor reactivity or host cells that have been genetically modified with anti-tumor TCRs or chimeric antigen receptors (CARs). In the mid-1980s, the demonstration from Rosenberg *et al* (127) that adoptive transfer of IL-2-stimulated lymphokine-activated killer (LAK) cells resulted in complete durable tumor regressions provided a stimulus to identify the tumor-specific T cells involved in cancer immunotherapy. Their subsequent *in-vivo* studies suggested that autologous tumor-infiltrating lymphocytes (TILs), a form of T cells originating from tumor tissues with broad-spectrum heterogeneity, in conjunction with IL-2 provide 50 to 100 times more effective anti-tumor activity than LAK cells (128). Currently, TIL therapy has emerged as a promising option to treat patients with solid tumors who were refractory to checkpoint inhibitors and targeted therapies (129-133). Lifileucel, a one-time autologous TIL product, achieved an investigator-assessed ORR of 36% in 66 patients with metastatic melanoma who had progressed on standard checkpoint inhibitors and BRAF ± MEK targeted agents (if BRAF V600

mutation-positive), while only 4-10% of these patients had objective responses to cytotoxic chemotherapy (132). In a recent large multicentre phase II trial (C-144-01) that included 153 patients with advanced metastatic melanoma, combining the 66 previously reported patients, the cryopreserved TIL product lifileucel provided flexibility in treatment scheduling in the real-world clinical setting and demonstrated an ORR of 31.4%, with 8 complete responses and 40 partial responses; the median DOR was not reached at a median follow-up of 27.6 months and nearly half of the patients had responses maintained for ≥18 months. Based on these encouraging results, the investigators supported the use of lifileucel as a novel treatment option for patients with advanced melanoma to address a highly unmet need: The patients with advanced melanoma following failure of checkpoint inhibitors, and targeted agents where appropriate, irrespective of baseline tumor characteristics, should be considered for lifileucel as second-line therapy (third-line if BRAF V600 mutation-positive) if they have an ECOG PS and organ functions adequate for receiving a nonmyeloablative lymphodepletion regimen and a shortened course of IL-2. Of note, the FDA has granted a regenerative medicine advanced therapy designation for lifileucel in advanced melanoma. Furthermore, in two ongoing phase II multicentre, multicohort, prospective, open-label studies (IOV-COM-202 and C-145-04), in PD-1 inhibitor-naïve patients, lifileucel combined with pembrolizumab also produced a high ORR (60%), supporting the potential for improved response rates with earlier TIL cell therapy (134). The recently performed phase III trial of lifileucel plus pembrolizumab in frontline advanced melanoma was expected to be well underway at the time of a potential approval.

Talimogene laherparepvec (T-VEC), an attenuated herpes simplex virus 1 encoding granulocyte-macrophage colony-stimulating factor, has been approved for use as the first oncolytic virus therapy for the local treatment of metastatic melanoma that cannot be surgically removed. Previous findings indicated that T-VEC plus ipilimumab or pembrolizumab combination therapy has great efficacy, with a tolerable safety profile, in treating advanced melanoma (135,136). In a phase II trial, the clinical response rate was doubled from 18% in patients with ipilimumab alone to 38% with T-VEC plus ipilimumab (137). In addition, T-VEC plus pembrolizumab led to a high CRR of 43% in the MASTERKEY-265 phase Ib study (136). According to recent results from the MASTERKEY-265 phase III study, there was no statistically significant difference in median PFS or OS between the combined therapy and pembrolizumab monotherapy groups (138); however, there existed a numerical difference of 5.8 months favouring the T-VEC plus pembrolizumab group (14.3 vs. 8.5 months) and the researchers found that among patients with baseline LDH ≤ ULN, patients with baseline SLD ≤ median and patients enrolled in the United States, the T-VEC-pembrolizumab combination was beneficial for PFS (138). This combination therapy is still under active investigation in those who were refractory to anti-PD-1-based therapy in advanced melanoma (NCT04068181).

Immune-mobilizing monoclonal T-cell receptors against cancer (ImmTACs). ImmTACs are a novel class of T-cell redirector molecules as well as anti-tumor reagents that utilize affinity-enhanced soluble TCRs stabilized by a disulfide bond and fused to an anti-CD3 single-chain variable fragment that engages T cells. This ImmTAC platform allows for highly specific access to the vast pool of intracellular targets (139). The most advanced ImmTAC molecule, tebentafusp (IMCgp100), redirects T cells towards human leukocyte antigen (HLA)-A*02:01-positive melanoma cells expressing a gp100 peptide (HLA-A*02:01 is an important restriction element for peptide presentation to T cells in disease and cancer), inducing the formation of an immune synapse to kill targeted tumor cells (140). In August 2021, tebentafusp was given approval by the FDA to treat HLA-A*02:01-positive uveal melanoma. To date, three clinical trials of tebentafusp have shown promising results in patients with metastatic uveal melanoma: IMCGp-100-01 (NCT01211262), IMCGp-100-102 (NCT02570308) and IMCGp-100-202 (NCT03070392).

The IMCGp-100-01 trial reported that a nearly 40% increased dose of tebentafusp through a 3-week step-up dosing regimen (20-30-68 μg) compared with a fixed weekly dose of 50 μg had a manageable side-effect profile and a signal of efficacy in HLA-A*02- or HLA-A*02:01-positive uveal melanoma (141). This novel treatment regimen of tebentafusp was subsequently used in the IMCGp-100-202 phase III trial, in which patients with metastatic uveal melanoma were assigned in a 2:1 ratio to a tebentafusp monotherapy group or control group (dacarbazine, ipilimumab or pembrolizumab monotherapy). Surprisingly, the data revealed a 1-year OS of 73% with tebentafusp compared with 59% with systemic treatments (142). Indeed, this promising OS result was higher than that recently reported for ipilimumab in combination with nivolumab. Conversely, two single-arm, phase II trials comprising patients with metastatic uveal melanoma treated with ipilimumab plus nivolumab only achieved a 1-year OS rate of 51.9% (143) and 56% (144).

4. Combination of targeted therapy and immunotherapy

MAPK inhibitors plus immune checkpoint inhibitors. For the patient population with highly symptomatic disease and an ECOG PS score of at least 2, and disease affecting crucial anatomical sites, such as symptomatic brain metastasis requiring corticosteroid therapy, which are not amenable to local therapy, BRAF and MEK inhibitors seem to be preferred over immune checkpoint inhibitors. Their duration of response to the first-line targeted agents is often short. However, one limitation of immune checkpoint inhibitors is their slower onset of action. Instead of targeting the tumor cells directly, CTLA-4, PD-1 and PD-L1 blockades first activate the immune system and then let a high number of active immune cells become tumor killers. This same patient population therefore responds poorly to them, although a small percentage may have long-term durable control (145).

Numerous efforts have been made to combine MAPK inhibitors with immune checkpoint inhibitors. In general, understanding the effect of BRAF and MEK inhibition on melanoma tumors and their microenvironment may prove critical in supporting such a combination approach. The scientific rationale for the combination is based on 'immunosensitization', whereby pharmacologic modulation with specific inhibitors of oncogenic events in cancer cells sensitizes cancer cells to immune attack (146). For instance, Koya *et al* (147)

showed that the BRAF V600E-specific inhibitor vemurafenib improved the anti-tumor effects of TCR-engineered ACT in a BRAF V600E-driven murine model of melanoma, with higher immune-stimulating cytokine IFN-γ secretion and better gp100-specific lytic activity. In addition, Wilmott *et al* (148) examined melanoma tumor biopsies and observed an increase in the density of $CD8^+$ and $CD4^+$ TILs following treatment with a BRAF inhibitor. Frederick *et al* (149) showed that BRAF blockade is associated with increased expression of melanoma-associated antigens [gp100, MART-1 (a melanocyte lineage-specific protein; melanoma antigen recognized by T cells 1), and TYRP-1/2 (tyrosinase-related protein-1/2)], increased markers of T-cell cytotoxicity (perforin and granzyme B) and decreased expression of immunosuppressive cytokines (IL-6 and IL-8), all enhancing the tumor microenvironment. Paradoxically, an increase in the T-cell exhaustion markers T-cell immunoglobulin domain and mucin domain 3 (TIM-3), PD-1 and the immunosuppressive ligand PD-L1 was also noted during BRAF inhibition treatment, which may be one reason for the initiation of immune evasion (149). These results support the hypothesis that combining BRAF-targeted therapy with immunotherapy may have superior anti-tumor efficacy in patients with advanced melanoma. With regard to immune evasion, oncogenic BRAF induces decreased expression of melanocyte differentiation antigens (MDAs), indicating defective recognition of melanoma cells by antigen-specific T lymphocytes. Surprisingly, the study by Boni *et al* (150) corroborated that impaired melanocyte antigen expression is reversed by a selective BRAF V600E inhibitor, without compromising T lymphocyte function. As recognition of MDAs is central to immunotherapy for melanoma, it may also provide support for potential synergistic effects of BRAF-targeted therapy plus immunotherapy.

Several clinical trials of such a combinatorial approach that resulted in promising efficacies in patients with advanced melanoma are listed in Table II (NCT02130466, NCT02858921, NCT02967692, NCT02908672, NCT02224781, and NCT03235245).

Dabrafenib and trametinib plus pembrolizumab is a triplet combination therapy assessed as a feasible treatment approach in the KEYNOTE 022 phase I, dose-identification trial (NCT02130466). Ribas *et al* (151) reported that this approach increases the frequency of long-lasting responses in patients with BRAF V600-mutated melanoma and is most suitable for treating those with a poor prognosis on monotherapy. In terms of safety and toxicity, 20% of patients had dose-limiting toxicities and 73% experienced one or more grade 3 or 4 TRAE. Among the AEs, two events (pneumonitis and autoimmune hepatitis) prompted treatment discontinuation. In a parallel phase II study (NCT02130466), Ascierto *et al* (152) found that at a median follow-up of 9.6 months, the primary endpoint of PFS did not show statistically significant improvement in the triplet arm (dabrafenib and trametinib plus pembrolizumab) compared with the doublet arm (dabrafenib and trametinib plus placebo) (16.0 and 10.3 months, respectively). With a longer median follow-up of 36.6 months, the changes of PFS (16.9 and 10.7 months, respectively), DOR (25.1 and 12.1 months, respectively) and OS (not reached and 26.3 months, respectively) were more notable between the two arms (153). Despite clinical advantages, considerable toxicity was the major limitation of this strategy. The incidence of grade 3 through 5 TRAEs was 58% with the triplet regimen (including most commonly fever, increased transaminase levels and rash) but only 25% with the doublet therapy. Of the patients on triplet therapy, 40% discontinued at least one of the agents due to TRAEs, compared with 20% on doublet therapy. There was one death from treatment-related pneumonitis (153). Recently, Maio *et al* (154) identified the maximum tolerated doses (MTDs) for concurrent and intermittent dosing strategies of pembrolizumab plus trametinib, indicating that both were feasible with manageable toxicity and safety profile among participants with BRAF-wild-type melanoma or advanced solid tumors, irrespective of BRAF mutation. A phase II, randomized study is ongoing, with the aim of addressing whether different intermittent or dose-sequencing regimens may be able to reduce the size of tumors prior to surgery in advanced melanoma and prevent recurrence of melanoma after surgery while reducing toxicity (NCT02858921).

COMBI I (NCT02967692) is a global, randomized, phase III study of spartalizumab plus dabrafenib and trametinib (sparta-DabTram) for patients with BRAF V600-mutated metastatic melanoma, though it did not meet its primary endpoint of PFS (155). In that study, at the data cut-off, median PFS was 16.2 months in the sparta-DabTram arm compared with 12.0 months in the placebo-DabTram arm and the median OS was not reached in either arm, with ongoing analyses. Similar to the KEYNOTE 022 study, this triple combination was associated with greater toxicity than the doublet regimen. The incidence of grade 3 through 5 TRAEs was 55% with the triplet regimen and 33% with the doublet therapy.

Positive results were also reported from the phase III IMspire150 trial (NCT02908672): Adding atezolizumab to vemurafenib and cobimetinib prolonged the primary endpoint of investigator-assessed PFS from 10.6 to 15.1 months in previously untreated patients with BRAF mutation-positive advanced melanoma. From this, a delayed separation of PFS curves (started after 7 months) was evident, maintaining a benefit in favour of the triplet arm. The DOR was also improved with the addition of atezolizumab (21.0 vs. 12.6 months) with, importantly, little change in the grade 3/4 toxicity rate (the main grade 3/4 TRAEs were presented similarly in the atezolizumab and control groups, including increased creatine phosphokinase, increased liver enzymes/lipase/amylase and rash). It is also notable that the OS curves in the KEYNOTE 022 trial separated later. Taken together, the addition of PD-1/PD-L1 checkpoint inhibitors to BRAF/MEK inhibitors may provide response rates that are similar to those observed with BRAF/MEK-targeted therapy alone but with prolonged durable responses, which may be a critical factor in the design of immunotherapy combination evaluation.

Overall, according to the data from KEYNOTE 022, COMBI I and IMspire150, the role of BRAF and MEK targeted agents plus immune checkpoint inhibitors was highlighted in patients with treatment-naïve BRAF V600-mutated melanoma. However, combination therapies appear to increase toxicity. On the other hand, the differences among these three published studies in terms of study design, patient population and agents investigated preclude direct comparison (153). A full evaluation of the long-term benefit of these treatments

is needed from ongoing clinical trials to determine their feasibility. In particular, these triplet combinations may be applicable for treating urgent cases in which the patient has limited time to wait for a response to a checkpoint inhibitor.

Although the results do not support the routine use of first-line immunotherapy plus targeted therapy, certain prognostic biomarkers may be helpful for informing treatment selection in patient subpopulations (156). In the COMBI I study, a baseline intra-tumoral and blood $CD4^+/CD8^+$ T-cell ratio above the median was a useful non-invasive biomarker and associated with the response to sparta-DabTram therapy. In addition, patients with a clinically higher tumor burden and detectable baseline circulating tumor DNA shedding appeared to derive a PFS benefit with sparta-DabTram vs. placebo-DabTram. Similarly, a small proportion of this subgroup of patients carrying the BRAF V600K mutation tended to have greater survival benefits with sparta-DabTram treatment. Thus, stringent patient selection based on biomarkers is of utmost importance for delineating patients who require this triplet therapy.

However, there is still no definitive answer regarding which treatment should be given first or which treatment sequence is preferable. Recently, a retrospective study of 114 patients with melanoma showed that those who had progressed on anti-PD-1 agents experienced inferior survival outcomes after starting subsequent BRAF-targeted therapy compared with those who were not previously treated with anti-PD-1 agents. Similarly, pre-treatment with BRAF-targeted therapy was seemingly associated with worse outcomes after receiving anti-PD-1 (157). Other data also suggested that anti-PD-1 exposure may influence the frequency and magnitude of AEs, including rapid onset of pyrexia or a sepsis-like syndrome with or without hypotension, which was associated with subsequent BRAF and MEK inhibition (158).

Currently, initial treatment with ipilimumab/nivolumab followed by dabrafenib/trametinib compared to the same sequence in reverse is being evaluated in the randomized phase III DREAMseq trial (NCT02224781) (159). In this trial, 265 participants with treatment-naïve BRAF V600-mutated metastatic melanoma were randomly assigned (1:1) to receive ipilimumab/nivolumab (arm A) or dabrafenib/trametinib (arm B). Upon disease progression, patients were enrolled to receive the alternative combination therapy: Dabrafenib/trametinib (arm C) or ipilimumab/nivolumab (arm D). The data indicated that after a median follow-up duration of 27.7 months, the sequence starting with dual immune checkpoint blockade followed by dual BRAF/MEK inhibitor resulted in a 20% absolute improvement in 2-year OS compared to the inverse sequence (71.8 vs. 51.5%). Other efficacy endpoints, such as median PFS duration (11.8 months in arm A vs. 8.5 months in arm B) and median DOR (not reached in arm A vs. 12.7 months in arm B), also indicated a benefit of prior first-line immunotherapy. Of note, the ORR was similar between arms A and B, at 46.0 and 43.0%, respectively, but rates were inferior in arm D vs. arm C (29.6 vs. 47.8%). In terms of safety, grade ≥3 toxicities occurred with similar frequency between arms, with more grade 4 toxicities seen in arm A. TRAEs in arms A and D were primarily immune-related and for arms B and C, they were primarily fevers, leukopenia and hyponatremia. Furthermore, prior ipilimumab plus nivolumab was associated with higher mortality, with 24 patients (18%) dying within 10 months, with notable rapid disease progression and poor prognostic features (159). Further relevant clinical trials are needed to identify whether this subgroup with the aggressive disease may benefit from at least a brief preceding course of BRAF/MEK-targeted therapy. Ascierto *et al* (160) reported the results of the randomized phase II SECOMBIT study (NCT02631447). In this trial, 209 patients were randomly assigned to three treatment arms (arm A: Immunotherapy with ipilimumab plus nivolumab followed by targeted therapy with encorafenib plus binimetinib at disease progression; arm B: The reverse order of arm A; arm C: A sandwich arm with a short course of targeted therapy followed by a switch to immunotherapy followed by targeted therapy at disease progression). Similar to the phase III DREAMseq trial, the survival results of 2-year OS rates for patients receiving initial immunotherapy were 65% in arm A, 73% in arm B and 69% in arm C, and no new safety signals emerged (160). The phase II EBIN EORTC trial (NCT03235245) is further exploring the effect of ipilimumab plus nivolumab preceded or not by encorafenib plus binimetinib in patients with BRAF V600 mutation-positive unresectable or metastatic melanoma. A key difference between the DREAMseq and SECOMBIT trials is inclusion of the sandwich approach, which also showed clinical benefits. This warrants further investigation, as there is no one-size-fits-all strategy for treating patients with BRAF V600-mutated melanoma.

Such a sequencing trial is clinically critical to defining optimal sequential approaches and the sequencing trials are already underway in patients with melanoma to identify efficacious treatment approaches in PD-(L)1 refractory disease in the adjuvant setting (NCT03991130, NCT03385486, NCT05061134 and NCT04250246) (161).

Anti-angiogenesis agents plus immune checkpoint inhibitors. Using a murine model of melanoma, Schmittnaegel *et al* (162) demonstrated that dual VEGF and angiopoietin 2 blockade increase the proportion and cross-presentation capability of intra-tumoral APCs. Normalized tumor blood vessels and increased extravasation of IFNγ-expressing $CD8^+$ CTLs were also observed. Schoenfeld *et al* (163) found that CTLA-4 blockade elicited a humoral reaction that broadly targeted multiple angiogenic cytokines. According to another study, anti-VEGF targeted therapy has the ability to downregulate the expression of exhaustion markers such as PD-1, LAG-3 and TIM-3 on tumor-educated T cells upon resistance to immune checkpoint inhibitors (164). Such reduced VEGF levels were also shown to be associated with improved response to PD-1 blockade in patients with melanoma (165). These results indicate that antiangiogenic treatments may be effectively combined with immune checkpoint inhibitors to improve patient outcomes.

Currently, several clinical trials of such a combinatorial approach are ongoing (Table II) (NCT03820986, NCT01950390, NCT04356729, NCT03175432, NCT04996823, and NCT02298959).

Lenvatinib can shift the tumor microenvironment to an immune-stimulatory state by targeting VEGF and fibroblast growth factor signaling (166), and the combination

of lenvatinib and pembrolizumab activates IFNγ-positive $CD8^+$ T cells through a reduction in tumor-associated macrophages (166,167). Clinically, lenvatinib monotherapy in previously treated participants yielded an ORR of only 9.7% (168); however, the ORR of 48%, median DOR of 12.5 months and median PFS of 5.5 months in a phase IB/II trial of lenvatinib plus pembrolizumab indicated an encouraging improvement (169). The single-arm LEAP-004 trial was designed to identify whether this combination therapy is able to improve survival in the population of patients with unresectable stage III/IV melanoma experiencing rapid clinical progression on anti-PD-1/PD-L1 monoclonal antibody treatment alone, anti-PD-1/PD-L1 plus anti-CTLA-4 therapy and, if applicable, BRAF/MEK-targeted therapy. The eligible 103 patients received lenvatinib 20 mg orally once a day plus up to 35 cycles of pembrolizumab 200 mg by intravenous infusion once every 3 weeks. At the 15.3-month follow-up, an ORR of 21.4%, 12-month PFS rate of 18.5% and 12-month OS rate of 54.5% were observed in the total population; an ORR of 33.3% was also observed in the subgroup (30 patients) refractory to prior anti-PD-1 plus anti-CTLA-4 therapy (170). Of note, lenvatinib plus pembrolizumab induced antitumor activity and tolerability irrespective of baseline tumor characteristics (55.3% of patients had elevated LDH) or number of previous therapies [58.3% of patients were treated with ≥2 prior lines of therapy (170)], and it may thus be a treatment option for this growing population with a highly unmet medical need. Another phase III LEAP-003 trial assessing the safety and efficacy of this combined therapy as a first-line intervention in adults with previously untreated stage III/IV melanoma is ongoing (NCT03820986).

In addition, a randomized phase II trial (NCT01950390) to compare OS among patients with advanced melanoma who receive ipilimumab plus bevacizumab or ipilimumab monotherapy is currently underway. Another phase II study (NCT04356729) is evaluating the safety and effectiveness of a combination of atezolizumab and bevacizumab in unresectable, metastatic melanoma, which is currently in the patient recruitment phase. In particular, for patients with active melanoma brain metastasis, the safety, tolerability and preliminary efficacy of atezolizumab plus bevacizumab with or without cobimetinib is also being investigated in a phase II trial (NCT03175432). Another phase II study of axitinib plus ipilimumab combined therapy in patients with advanced melanoma refractory to anti-PD-1 therapy is also ongoing (NCT04996823). Aflibercept is a soluble decoy VEGF receptor with anti-angiogenic effects, and given that it has been confirmed to have potent preclinical anti-melanoma activity (171), a phase I study (NCT02298959) testing whether aflibercept plus pembrolizumab is an appropriate combinatorial approach in patients with metastatic melanoma and other solid tumors is ongoing.

PARP inhibitors plus immune checkpoint inhibitors. An emerging body of evidence supports that the addition of PARP inhibitors can potentiate the therapeutic response to checkpoint inhibitors. The rationale for PARP inhibitor plus checkpoint inhibitor combined therapy mainly involves the following three aspects: i) The accumulated DSBs induced by PARP inhibitors that are not repaired may release tumor neo-antigens and eventually induce a profound anti-tumor immune response. In addition, there is evidence of a close association between melanoma with innate deficiencies in DDR genes and a durable benefit from immunotherapy (172). ii) PARP inhibition promotes the accumulation of toxic DNA DSBs in tumor cells with DNA repair deficiency, such as BRCA1/2 mutation, and PARP inhibition also triggers the DNA-sensing cyclic GMP-AMP synthase-stimulator of interferon genes pathway and upregulates type I interferons to induce an anti-tumor immune response that is independent of DNA repair deficiency (173). iii) PARP inhibitor treatment can upregulate the expression of PD-L1 in cancer cells and attenuate its efficacy due to immunosuppression, which is reversible by blocking the PD-1/PD-L1 interaction (174).

At present, there are no FDA-approved standard PARP inhibitors for the treatment of metastatic melanoma. However, given the immunological role of PARP inhibitors, combination therapy with immune checkpoint inhibitors may become an option. Two clinical studies of this combined regimen for treating melanoma are currently in the patient recruitment phase (Table II). NCT04633902 is an open-label phase II trial aiming to explore the benefit of olaparib plus pembrolizumab in patients with advanced melanoma with genetic HR alterations, particularly BAP1, ATM, AT-rich interactive domain-containing protein 1A/B, CHK2, and BRCA1/2, whose disease progressed on prior immunotherapy and/or BRAF-targeting therapy. Similarly, the efficacy of talazoparib plus nivolumab in patients with BRCA1/2 or other DDR mutations is also being assessed (NCT04187833).

5. New directions to overcome primary or acquired resistance to targeted therapy and immunotherapy

In spite of the numerous targeted therapy- and immunotherapy-based treatments that are now available for clinical use in melanoma, a large proportion of patients with melanoma do not show any durable benefit, with either no clinical response or disease progression. There remains the question of how to identify better treatment strategies to overcome resistance in order to confer clinical benefits for these patients.

Under targeted therapy and/or immunotherapy, certain tumor cells are still durable by showing resistance to cell death and escaping from immunological surveillance in cancer patients (including melanoma and other cancer cells), impairing the immune system and enabling cancer recurrence. Thus, the vital immune cells and the regulatory signaling networks involved in the interaction between tumor cells and the immune system are main factors to modulate the susceptibility of cancer cells to death (175). Immunogenic cell death (ICD) is a new key factor in the treatment of metastatic melanoma, which is featured by the release or expression of molecules with danger-associated molecular patterns, such as calreticulin, ATP, high mobility group box 1, heat-shock proteins, Annexin A1 and type 1 IFN. By binding to their receptors, these molecules result in the recruitment and stimulation of immune cells and finally cause damage to tumor cells through the maturation of dendritic cells, the activation of CTLs, the enhancement of the cytotoxic activity of natural killer cells, as well as the production of proinflammatory cytokines and chemokines (176). The ICD inducer

SD-101 is a synthetic CpG oligonucleotide that stimulates Toll-like receptor 9 (TLR9). Early data from a phase Ib study demonstrated that by concomitantly releasing PD-1-mediated inhibition with pembrolizumab, the injection of SD-101 into peripheral or visceral lesions was able to induce broad immune activation in the tumor microenvironment at that site. Among the 13 patients with melanoma who had prior anti-PD-1 therapy, the ORR was 15%. Of note, one patient refractory to prior anti-PD-1 therapy receiving 1 mg of SD-101 had a partial response ongoing at 10.5 months of follow-up (177). Similarly, intratumoral vidutolimod, a virus-like particle-encapsulated CpG-A TLR9 agonist, combined with pembrolizumab also improved cancer immunotherapy outcomes in patients with advanced anti-PD-1-refractory melanoma through triggering a strong IFN response to induce and attract antitumor T cells. Durable responses were observed in 25% of patients, with tumor regression in both vidutolimod-injected and noninjected target lesions, including visceral metastases (178). Among the intralesional agents, oncolytic viruses would also be anticipated to work by stimulating host anti-tumor immunity through preferential replication in tumor cells and production of cytokines and other immunomodulatory molecules. However, according to the results from the MASTERKEY-265 phase III study of T-VEC plus pembrolizumab, there was no statistically significant difference in median PFS or OS between the combined therapy and pembrolizumab monotherapy groups (138). This combination therapy is still under active investigation (NCT04068181) in those patients with advanced melanoma who were refractory to anti-PD-1-based therapy.

Microbiota and their metabolites have been demonstrated to have a significant impact on potentiating immune checkpoint inhibitor therapy (179). Fecal microbiota transplantation (FMT), as an effective strategy for manipulating the gut microbiota, may transfer the fecal material isolated from a healthy donor to a recipient via colonoscopy, nasogastric tube or prepared capsules (180). In preclinical mouse models, reconstitution of germ-free mice with fecal material from anti-PD-1-responding patients resulted in augmented T-cell responses and greater efficacy of PD-1 blockade. On the contrary, if the mice were reconstituted with non-responder fecal material, the corresponding mice were also non-responders to PD-1 blockade (181). Based on this strong correlation between commensal microbial composition and clinical response to immunotherapy, several clinical trials in patients with metastatic melanoma are underway to test the potential for FMT to enhance the efficacy of immune checkpoint blockade therapy. Whether reprogramming the gut microbiota can overcome resistance to anti-PD-1 in patients with advanced melanoma remains to be evaluated. To address this question, Davar *et al* (182) performed a phase II study (NCT03341143) of concurrent FMT together with pembrolizumab in patients with PD-1-resistant melanoma. This combination was reported to have favourable safety results and provided a clinical benefit in 6 of 15 patients, along with a rapid and durable microbiota perturbation. A response to this combination was associated with an increased number of $CD8^+$ T cells in the tumor microenvironment. Among the responding patients, several circulating cytokines and chemokines were also detected to be decreased after FMT, including C-C motif chemokine ligand 2, IL-8 and IL-18, which have been reported to be associated with adverse prognosis to PD-1 blockade in multiple cancer types, including melanoma (183), indicating that, beyond the immunostimulatory potential, the microbiota may also be employed to decrease tumor-associated immunosuppression. The other recent clinical trial (NCT03353402) also confirmed the safety and feasibility of FMT and anti-PD-1 therapy in patients with anti-PD-1-refractory metastatic melanoma, and treatment with FMT was also associated with favourable changes in intra-tumoral $CD8^+$ T-cell infiltration, IFN-γ mediated signalling pathway, dendritic cell differentiation and T helper type 1 immune response in the tumor microenvironment (184).

Furthermore, tumor cell plasticity also contributes to targeted therapy and immunotherapy resistance. An expression profiling of melanoma cell lines has established the existence of two major transcription programs. The two programmes are expressed in distinct cell populations, defined as either of 'proliferative cellular phenotypes' or 'invasive cellular phenotypes' (185). The former contributes to high rates of proliferation and low motility, while the latter is characterized by lower rates of proliferation and high metastatic motility (186). As replicated *in vivo*, melanoma cells may perform proliferative-to-invasive phenotype switching in response to the tumor microenvironment, which is thought to drive melanoma progression and influence the ability to adjust to drug exposure during treatment (186). MITF is a marker of gene expression signatures linked to the proliferative phenotype, and the tyrosine-protein kinase receptor, AXL, is linked to the invasive phenotype. In the initial response phase, >78% of patients with melanoma on BRAF and MEK inhibitors treatment displayed high MITF expression in $MITF^{high}$ cells, which may lead to a drug-tolerant state and overcome the cytotoxic effects of drugs, as MITF-mediated survival signalling may counteract BRAF or MEK inhibitor-induced melanoma cell death (187). MITF knockdown may enhance the overall cytotoxicity of BRAF inhibitors (188) and this molecule is therefore an important therapeutic target for melanoma. The compound TT-012 has been identified to destroy the growth of $MITF^{high}$ B16F10 and GAK melanoma cell lines, and potently suppress the tumor growth and metastasis with tolerable toxicity to liver and immune cells in animal models, indicating that the inhibition of MITF through TT-012 may present a novel approach to benefit melanoma treatment (188). Smith *et al* (189) also identified that in BRAF V600E-melanoma allografts and in patients with BRAF-mutant melanoma, BRAF and MEK inhibitors increase the number of tumor-associated macrophages and lead to high expression of MITF and TNFα, a crucial melanoma growth factor. They indicated that targeting the NF-κB pathway inhibited the MITF- and TNFα-mediated resistance to the MAPK-targeted agent. Of note, Müller *et al* (190) detected an inverse correlation between the loss of MITF and gain of expression of AXL in the context of acquired resistance. A current phase Ib/II randomised open-label trial of the AXL inhibitor BGB324 is being undertaken in patients with advanced non-resectable or metastatic melanoma to assess the efficacy of BGB324 given together with standard treatment, pembrolizumab or dabrafenib and trametinib, compared to standard treatment alone (NCT02872259) (191). However, this study did not include any participants with primary or acquired resistance to targeted therapy or immunotherapy.

Overall, it may be considered that continuously analyzing the role of ICD and gut microbiota in the tumor microenvironment is necessary, as both factors influence immune response and outcome of treatment with immune checkpoint inhibitors. According to preclinical and clinical studies, two strategies, including TLR9 agonist-mediated inhibition with pembrolizumab as well as FMT together with pembrolizumab, appear promising in patients with advanced anti-PD-1-refractory melanoma. On the other hand, BRAF and MEK inhibitors induce TNFα production, NF-κB pathway activation and higher MITF expression, and thus, focusing on inhibiting TNFα/MITF-mediated survival signals that protect melanoma cells from MAPK pathway-targeted therapies may be helpful to overcome the resistance. As $MITF^{low}/AXL^{high}$ aggressive phenotype in melanoma cells also contributes to resistance to both MAPK pathway and PD-1 inhibitors (192,193), AXL inhibitors combined with pembrolizumab or dabrafenib/trametinib may also provide a new direction expected to enhance the efficacy of these therapies in metastatic melanoma.

6. Conclusion

Melanoma develops due to sun exposure-induced DNA damage, which triggers the malignant transformation of melanocytes. Since 2011, with several new drugs having been approved for clinical use, the treatment of patients with metastatic melanoma has seen drastic changes and therapeutic options are now available. In the present review, several promising dual BRAF and MEK inhibition and PARP inhibitors, VEGF/VEGFR inhibitors and other molecularly targeted therapies, such as c-Kit inhibitors and mTOR inhibitors, were extensively discussed. In the field of immunotherapy, in addition to cancer vaccination and adoptive cell therapy, monoclonal antibodies blocking CTLA-4 and PD-1/PD-L1 have been successfully developed.

Currently, there is no one-size-fits-all strategy for treating patients affected by metastatic melanoma. The decision-making in the first-line setting for BRAF-mutant metastatic melanoma is still guided by clinical parameters and the biological aspects of melanoma, including LDH level, organs involved, performance status, tumor burden and disease progression kinetics. On one hand, considering patients with BRAF-mutant and aggressive diseases and those needing an immediate benefit in the reduction of tumor burden, targeted therapy remains the backbone of any first-line approach with a rapid onset of action. On the other hand, for patients with less extensive BRAF-mutant and non-rapidly progressive disease and more favourable parameters, first-line therapy with immunotherapy should generally be preferred.

BRAF and MEK-targeted therapies show high efficacy and increase the OS and ORR of a relatively high proportion of patients with melanoma with somatic activating BRAF mutations in a very short time, although a main characteristic of acquired treatment resistance remains. Meanwhile, immunotherapy, particularly immune checkpoint inhibitors, demonstrates durable antitumor activity in a subset of patients with both BRAF-mutant and wild-type melanoma, despite a slower onset of action. Thus, the identification of a novel treatment strategy of combining immune checkpoint inhibitors with BRAF and MEK-targeted agents seems a promising perspective for this setting. Several clinical and real-life studies have confirmed the role of this combination in improving the prognosis of patients with metastatic melanoma; however, this benefit is at the cost of substantial toxic effects compared with monotherapy, which also indicates a 'Yin and Yang' action of combination therapy. Making the treatment more effective with fewer side effects remains a critical objective in patients on combination therapy.

According to most but not all results from clinical studies of different sequencing strategies, frontline ipilimumab plus nivolumab followed by targeted therapy seems to produce more excellent response rates in patients with melanoma containing BRAF mutations, compared with the inverse sequence. More and larger studies are still required in order to confirm whether immunotherapies are less effective if given after targeted therapy, and confirm whether patients exactly benefit more from prior immune checkpoint blockade. The need to answer the above questions has led investigators to design clinical trials of novel combinatory strategies. Because the safety profile of encorafenib plus binimetinib is advantageous compared with other combinations of BRAF and MEK inhibitors, two promising clinical trials (NCT04657991 and NCT04655157) were designed to investigate the efficacy of triple therapy, in particular encorafenib plus binimetinib plus pembrolizumab and encorafenib with or without binimetinib plus nivolumab and low-dose ipilimumab, respectively.

Of note, a large proportion of patients with melanoma remains who do not experience any durable benefits under targeted therapy- and immunotherapy-based treatment, with either no clinical response or disease progression. More evidence is needed to clarify the optimal combinations between recent treatments and new therapeutics to manage patients with recurrent melanoma while taking into account patient-related characteristics, such as currently targetable BRAF mutations, frontline treatment regimens (single-agent or combination), type of resistance pattern (primary or acquired) and CNS metastasis (presence or absence).

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ZQ and MZ both made a significant contribution to the study, including establishing the background, analysis or interpretation, or all of these areas. Both drafted, wrote, substantially revised and critically reviewed the manuscript. All authors read and approved the final version to be published, agreed on

the journal to which the article has been submitted and agreed to be accountable for all aspects of the work. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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