Review

New Developments in the Treatment of Metastatic Melanoma: Immune Checkpoint Inhibitors and Targeted Therapies

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Abstract. *The incidence of melanoma has been increasing over the past twenty years. Unfortunately, the prognosis of advanced-stage disease is still poor. Advances have been made in the understanding of melanoma development and progression, resulting in the availability of promising novel therapeutic options. After the approval of ipilimumab, an immune checkpoint inhibitor of cytotoxic T-lymphocyte-associated antigen-4, and vemurafenib, a targeted v-raf murine sarcoma viral oncogene homolog B1 inhibitor, a new era for melanoma has started. Additional compounds, such as dabrafenib and trametinib, also received Food and Drug Administration approval recently and currently several other promising candidates, such as antibodies to programmed death-1, are under clinical development. Even though the novel compounds show impressive results as monotherapy, their efficacy may be enhanced in combination with other agents. In addition, combined treatment may reduce the chance of developing resistance. We review available clinical experience on approved therapies and discuss new developments. Furthermore, promising combination therapies are highlighted.*

Over the past few decades, the incidence of melanoma has increased significantly (1). Each year, more than 132,000 patients are diagnosed with this type of cancer. The incidence has increased by 28% in men and 21% in women (2). The highest incidence rates worldwide are found in Australia and New Zealand, with 60 cases per 100,000 inhabitants being reported annually (3). In men, the predominant anatomical

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location for melanoma lesions is the trunk and in women, lesions are mainly found in the lower limbs.

Both genetic and environmental factors are involved in the development of melanoma, with excess exposure to UV radiation being the most important risk factor. Specific phenotypic characteristics have been shown to correlate with increased risk of melanoma such as green/blue eye color, red/blond hair color and the presence of freckles. Caucasians have a 20-fold increased risk of developing skin melanoma when compared to dark skin populations (4, 5). Primary melanoma can be treated with surgical resection, and prognosis is dependent on the stage of disease (6). Eventually, approximately 20% of all patients with melanoma will develop metastases and in general have a poor prognosis (5).

More than 30 years ago, the first chemotherapeutic, dacarbazine, was approved and is considered as standard treatment for the treatment of advanced melanoma. Unfortunately, response occurs only in 10% to 15% of cases and is in general short in duration. High-dose interleukin-2 (IL2), a cytokine that induces T-cell activation and proliferation, is associated with a response rate of 15% and up to 6% of patients can have complete remissions that can be durable. However, its toxicity and the absence of randomized phase III trials showing a survival benefit, are reasons that this treatment is not considered as standard-of-care (1). New therapies for the treatment of advanced melanoma were, therefore, urgently needed. In 2011, a breakthrough was achieved when the Food and Drug Administration (FDA) approved the novel drugs ipilimumab, an immunostimulatory agent, and vemurafenib, a v-raf murine sarcoma viral oncogene homolog B1 (BRAF) inhibitor. Since then, a great deal of progress has been made in the treatment of melanoma. In this review, we highlight these recent developments and give an overview of the important clinical trials for this type of cancer.

Immunotherapy

Cytotoxic T-lymphocyte-associated antigen-4 (CTL4-A)-directed therapies. The interaction between cancer and the

immune system is complex. While evidence of antitumor immune response is observed in many patients with melanoma, their cancer develops strategies to evade immune detection and death. Therefore, developing therapies to enhance tumor immunity or circumvent immunosuppression is an interesting approach for cancer therapy. Activation of cellular immunity begins when T-cells become activated through Cluster of Differentiation 28 (CD28) after binding to Cluster of Differentiation 80/86 (B7) that is expressed on antigen-presenting cells (APCs) (7). After activation, T-cells express CTLA-4, which competes with a higher affinity for binding to B7, causing a suppressive signal in the T-cells (see Figure 1). Based on successful antibody-mediated CTLA-4 blockade studies showing tumor responses in various murine tumor models, early clinical studies were performed in humans. Ipilimumab and tremelimumab are fully human antibodies against CTLA-4 and in phase I/II studies had potent antitumor activity in patients with various types of advanced solid tumor, in particular advanced melanoma (8-12). In initial studies, small numbers of patients were treated with single fixed doses at 0.3, 1 and 3 mg/kg, followed by repetitive dosing trials. In the first trial, nine patients received a single intravenous dose of 3 mg/kg ipilimumab (9). This trial showed that administration of ipilimumab is well-tolerated; no serious adverse events were observed and the study provided clear evidence of its antitumor activity. In 2005, 56 patients with progressive stage IV melanoma received 3 mg/kg ipilimumab every three weeks, or 3 mg/kg initial dose then 1 mg/kg every three weeks plus a peptide vaccine. This study led to two complete responses and five partial responses (8). Furthermore, Maker *et al.* reported three complete responses and five partial responses in a trial with 36 patients with advanced melanoma receiving 0.1-3 mg/kg ipilimumab every three weeks with IL2 (10). However, this combination therapy increased the incidence of colitis. Dose-dependent responses were observed in patients with metastatic melanoma receiving either 0.3, 3, 10 mg/kg every three weeks for a total of four doses in a phase II study, which enrolled 217 patients with previously-treated stage III (unresectable or stage IV melanoma (13). In this study, the best overall response rate was obtained with 10 mg/kg ipilimumab, with an overall response rate of 11.1%. In a small phase ll trial, 72 patients with previously-untreated melanoma randomly received ipilimumab at 3 mg/kg every four weeks for four doses alone, or in combination with dacarbazine (14). Although not significant, a trend for improved response rate and survival was observed in favor of the chemo-immunotherapy arm. Long-term survival was analyzed in three phase II trials in which patients with melanoma received 10 mg/kg every three weeks. Overall survival ranged from 10.2 to 22.5 months and 18-month survival rates ranged from 34.5% to 39.4% across these phase ll studies (8, 10, 13-15). Prieto *et al.* showed that

ipilimumab given in combination with IL2, had a 17% complete response rate, compared to 7% in patients treated with ipilimumab-alone (16). Phase III trials should be conducted to confirm the safety and improved efficacy of this combined immunotherapy.

Two phase III clinical trials were designed to evaluate ipilimumab in metastatic melanoma (Table I). The results of the first phase III trial were published by Hodi *et al.* in August 2010 (15). In this trial, 676 patients with previously treated unresectable stage III or lV melanoma were randomized to receive ipilimumab at 3 mg/kg every three weeks, alone or in combination with GP100 *versus* GP100 peptide vaccine alone. GP100 is a well-characterized immunogenic human melanoma-associated antigen expressed by most melanoma cells and was used in this trial as active control. Ipilimumab demonstrated significantly improved overall survival compared to the arm treated with GP100 peptide vaccine, of 10.1 and 6.4 months, respectively. The 2-year survival rate was 23.5% which was reported in an earlier phase II trial to be 22%, and about 10% higher than for the patients treated with the GP100 vaccine. The most important immune-related adverse effects observed from ipilimumab included the following: dermatitis, colitis, hepatitis, and hypophysitis. Approximately 60% of the 676 patients experienced one of the adverse effects, of which 10%-15% had grade 3 or 4 effects. These adverse effects were treated successfully in most patients. However, Hodi *et al.* reported that 2% died of complications related to ipilimumab treatment (15). In the second phase III trial, 502 patients with previously-untreated metastatic melanoma were randomized 1:1 to ipilimumab (10 mg/kg) in combination with dacarbazine, or dacarbazine alone (17). Patients treated with ipilimumab and dacarbazine had an overall survival of 11.2 months compared with 9.2 months in the dacarbazine-alone arm. Both overall and long-term survival were improved. The combination therapy, ipilimumab and dacarbazine, demonstrated a good safety profile, there were no gastrointestinal perforations and a lower rate of colitis compared to monotherapy. However, approximately 60% of patients receiving ipilimumab plus dacarbazine experienced adverse effects from the therapy. Based on the results of these phase III trials, ipilimumab at a dose of 3 mg/kg was registered for the treatment of metastatic melanoma. The recently closed randomized study CA184-169, investigating ipilimumab at 3 mg/kg *versus* 10 mg/kg, will hopefully determine the optimal dose of ipilimumab in metastatic melanoma. Recently, in a pooled analysis of prospective and retrospective studies with ipilimumab, including about 5,000 patients, Hodi *et al.* showed a 3-year survival rate of 21%. The benefit of ipilimumab appeared to plateau after about three years (18). Beyond seven years, no deaths were reported, and median 7-year overall survival was 17%. The longest recorded survival was almost 10 years. This pooled

analysis clearly demonstrates that ipilimumab can lead to long-lasting tumor control in metastatic melanoma.

Another CTLA-4 antibody in clinical development is tremelimumab. The first phase I trial in 39 patients with solid tumors showed two complete and two partial responses, which were maintained over 25 months. Dose-limiting toxicities were diarrhea, dermatitis, vitiligo, hypopituitarism and hyperthyroidism (12). In a phase I/II trial, in which 28 patients with metastatic melanoma were included, no dose-limiting toxicity was observed in the phase I (up to 15 mg/kg) part. In the phase II part, objective response was observed in 8 of the 84 patients (11). Based on results of this study, a dose of 15 mg/kg every three months was recommended, as it led to tumor response and lower toxicity. However, in a phase III trial, tremelimumab did not cause a statistically-improved advantage in overall survival compared to conventional chemotherapy, 12.6 months and 10.7 months, respectively (19). Although between-trial comparisons are difficult, it is remarkable that toxicity, response rates and survival at three years under ipilimumab and tremilimumab were comparable. The absence of a survival benefit in the tremelimumab trial might also be related to the higher frequency of patients that crossed over to ipilimumab in the chemotherapy-treated control group, while in the ipilimumab trial, cross-over was not allowed (20).

Programmed death-1 receptor (PD1) and programmed death ligand-1 (PDL1). In addition to ipilimumab, other promising immunotherapies are emerging as well. Monoclonal antibodies directed to PD1 and PDL1 are the most interesting currently. Melanoma can evade the immune system through expression of PDL1, which binds to PD1 present on activated lymphocytes. PDL1 interaction with PD1 causes immune tolerance through apoptosis of the activated lymphocytes. Nivolumab (MDX-1106), a monoclonal antibody specific for human PD1, was tested in a phase I clinical trial and showed promising responses in patients with previously-treated, refractory solid tumors including melanoma, colorectal cancer, prostate cancer and non-small cell lung cancer (21). There was one serious adverse event, inflammatory colitis in a patient with melanoma. In another extended phase I trial, in which 296 patients with advanced solid tumors were enrolled, complete or partial responses were observed in 28% of the patients with melanoma. The most common toxicities were fatigue, rash, diarrhea, pruritus, nausea and a decreased appetite. Immune-related grade 3/4 adverse events occurred in 14% of patients. Three patients died due to pneumonitis (22). Immunohistochemical analysis of pre-treatment biopsies showed no response in patients with PDL1-negative tumors and a 36% objective response rate in patients having PDL1-positive tumors. This indicates that PDL1 expression on tumors may serve as a biomarker for selecting patients to treat with anti-PD1 immunotherapy.

Remarkably, responses were long in duration, the median overall survival of melanoma patients treated with nivolumab was over 16 months. Forty percent of patients were still alive after three years of treatment, with a long-term favorable safety profile (23).

MK3475, another antibody against PD1, showed antitumor activity both in patients with melanoma who were previously treated with ipilimumab and those who were not. The response rate was 38% and durable for at least 11 months. Frequent toxicities were fatigue, rash, pruritus and diarrhea and most of these adverse events were grade 1 or 2 (24). A randomized phase II trial comparing MK3475 with chemotherapy closed accrual at the end of 2013 and is currently being analyzed (NCT01704287). Furthermore, other antibodies against PD1, AMP-514 and MPDL3280A, are being tested in early clinical trials (clinicaltrials.gov).

In a phase I study, durable tumor regression was observed in patients with advanced cancer, including non-small cell lung cancer, melanoma, and renal cell cancer treated with MDX-1105 (25). A complete or partial response was reported in 9 out of 52 patients with melanoma (17%). The most common drug-related toxicities were fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headache. Most events were of low grade, with treatment-related grade 3 or 4 events observed in 9% of patients (25). Both the response and toxicity rates were lower compared to anti-PD1 antibodies. MEDI4736 is another PDL1 antibody which is currently being investigated in early clinical trials. Although ipilimumab and antibodies against PDL1/PD1 have not been compared in a clinical trial, the toxic effects of these antibodies were less frequent and less severe than those associated with ipilimumab.

Targeted Therapy

BRAF inhibitors. A better understanding of the molecular biology of melanoma has helped the development of new therapies. In 2002, it was discovered that approximately 60% of melanomas harbor a mutation in the gene encoding for the serine/threonine protein kinase BRAF. In 90% of the cases, valine is substituted for glutamate at amino acid 600 (V600E) (26). Other common BRAF mutations in melanoma are V600K (16% of mutations in melanoma) and V600D/R (approximately 3% of all mutations). Mutations in the *BRAF* gene may lead to constitutive activation of the Mitogen-Activated Protein Kinase (MAPK) pathway, causing an increase in proliferation, angiogenesis, preventing apoptosis and therefore enhancing the oncogenic activity of melanoma. Since this discovery, much effort has been made to develop selective BRAF inhibitors. Sorafenib was the first non-selective RAF inhibitor tested in clinical trials, because of its activity observed in *in vitro* studies and in melanoma xenograft models. However, sorafenib does not block BRAF
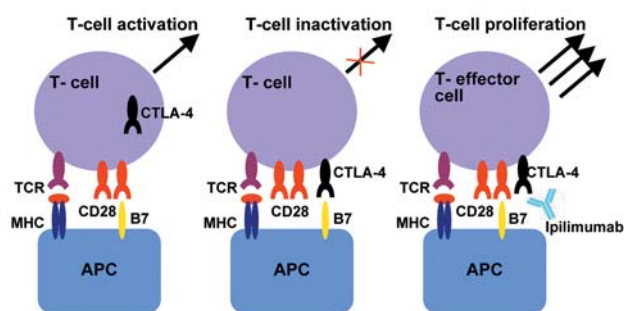
Figure 1. *T-Cell activation and mechanism of action of ipilimumab.adapted from (13). T-Cell activation is initiated by binding of B7 molecules on the antigen-presenting cell (APC) to Cluster of Differentiation 28 (CD28) receptors on the T-cell. Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4), expressed on the T-cell, binds B7 molecules, resulting in T-cell inactivation. Ipilimumab blocks CTLA-4 allowing enhanced T-cell activation by optimal co-stimulation of CD28 receptors.*

Figure 2. *The Mitogen-Activated Protein Kinase (MAPK) pathway is involved in cell proliferation, survival and angiogenesis. Vemurafenib, dabrafenib, and trametinib are compounds that target kinases in this pathway. Vemurafenib and dabrafenib are Food and Drug Administration (FDA)-approved v-raf murine sarcoma viral oncogene homolog B1 (BRAF) inhibitors. Trametinib is a FDA approved Mitogen-activated extracellular signal regulated kinase kinase (MEK) inhibitor.*

harboring the V600E mutation. In addition, it did not show clinical benefit, neither as monotherapy nor in combination with other anticancer compounds, such as dacarbazine, carboplatin and paclitaxel in patients with metastatic melanoma (27-30).

The first selective BRAF inhibitor targeting the mutant V600E form was PLX4720 (vemurafenib) (Figure 2). A structure-guided scaffold-based drug design approach was used to synthesize vemurafenib as BRAF kinase inhibitor. Vemurafenib was co-crystallized with a protein construct containing the kinase domain of BRAF V600E to enable preferential binding to the ATP-binding domain of mutant BRAF (31). Pre-clinical studies showed that vemurafenib inhibits the kinase activity of *BRAF* harboring the V600E mutation with an half maximal inhibitory concentration (IC$_{50}$) of 13 nM, resulting in cell-cycle arrest and the induction of apoptosis in melanoma cells (32). BRAF(V600E)-dependent tumor xenograft models also showed significant tumor growth reduction when treated with vemurafenib, without evidence of toxicity.

Based on pre-clinical data, a phase I trial was initiated to evaluate the pharmacokinetics, safety and efficacy of vemurafenib (33). Fifty-five patients were enrolled in the dose-escalation study, of whom 89% had metastatic melanoma and the remaining patients had papillary thyroid cancer that carried the BRAF V600E mutation. The patient groups received 160, 240, 320 or 360, 720 and 1120 mg of a microprecipitated bulk-powder formulation twice daily. Patients who received the highest dose of 1,120 mg twice daily had dose-limiting side-effects including grade 3 rash and fatigue. A dose of 960 mg twice daily was established as the maximum tole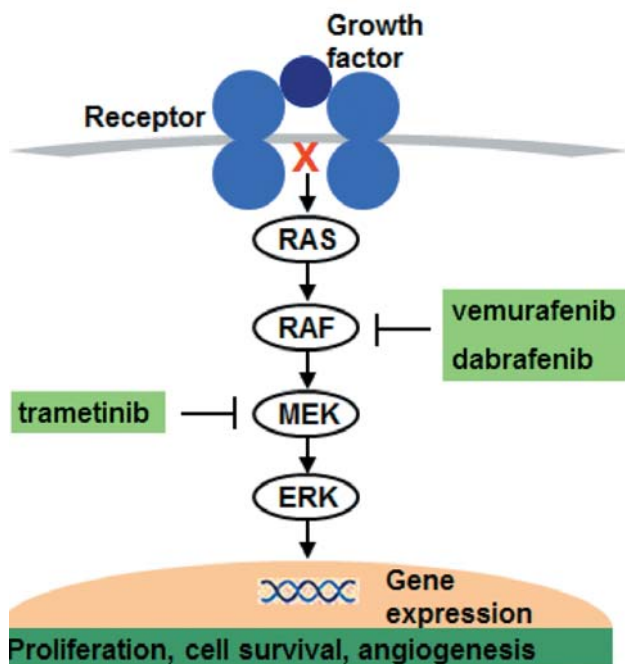rated dose. In the extension cohort, 32 patients with metastatic melanoma carrying the BRAF V600E mutation received the recommended phase II dose. The overall response rate was 80%, including 24 patients who achieved a partial response and two who had a complete response. The progression-free survival among all patients was greater than seven months compared to the historical survival of two months. In the phase II trial, BRIM-2, 132 patients with previously-treated BRAF V600E mutation-positive metastatic melanoma showed positive response to 960 mg twice daily vemurafenib treatment, with a response rate of 53% and a progression-free survival of 6.2 months (34). Dose reductions were required in approximately 45% of patients during treatment due to toxicities. The most common adverse events of vemurafenib were similar to those in the phase I study, including grade 1 and 2 rash, fatigue, hair loss and joint pain. In addition, toxicities of grade 3 rash, arthralgia and liver function abnormalities were observed. An unexpected side-effect, squamous cell carcinoma, grade 3 was seen in 26% of all patients. Squamous cell carcinoma caused by vemurafenib can be explained by the reactivation of the MAPK pathway in non-melanoma BRAF wild-type cells. A long follow-up has been

Table I. *Summary of selected clinical trials with Cytotoxic T-Lymphocyte Antigen 4 (CTL4A)-directed immunotherapies.*

Phase	No. pts	Patient population	Design	RR (%)	PFS (months)	OS (months)	Ref
I/II	56	Progressive stage IV melanoma	Ipilimumab + GP100	13	ND	ND	(8)
I/II	36	Treatment-naive advanced melanoma	Dose escalation of ipilimumab	22	ND	11 to 19	(10)
II	72	Treatment- naive advanced melanoma	Ipilimumab *vs.* ipilimumab + DTIC	5.4 *vs.* 14.3	ND	11.4 *vs.* 14.3	(14)
II	217	Previously treated stage III (unresectable) or stage IV melanoma	Three different doses of ipilimumab	11.1 (10 mg/kg) 4.2 (3 mg/kg) 0 (0.3 mg/kg)	24 months - 18.9% 24 months - 12.9% 24 months - 2.7 %	11.4 (10 mg/kg) 8.7 (3 mg/kg) 8.6 (0.3 mg/kg)	(13)
III	502	Treatment-naive metastatic melanoma	DTIC+ipilimumab vs. ipilimumab	15.2 *vs.* 10.3	ND	11.2 *vs.* 9.2	(16)
III	676	Previously treated advanced melanoma	Ipilimumab *vs.* ipilimumab + GP100 *vs.* GP100	10.9 *vs.* 5.7 *vs.* 1.5	2.86 *vs.* 2.76 *vs.* 2.76	10.1 *vs.* 10.0 *vs.* 6.4	(15)
I/II	117	Unresectable stage III/IV metastatic melanoma	Dose escalation of tremelimumab	9.8 (10 mg/kg)	ND	9.97 (10 mg/kg) 11.53 (15 mg/kg)	(11)
III	655	Treatment-naive unresectable stage IIIc or IV melanoma	Tremelimumab *vs.* DTIC/ temozolomide	9.3 (15 mg/kg) 10.7 *vs.* 9.8	Duration of response 35.8 *vs.* 13.7	12.6 *vs.* 10.7[a]	(19)

DTIC, Dacarbazine; no. pts, number of patients; RR, response rate; PFS, progression-free survival; OS, Overall survival. Note; a, not statistically significant.

conducted in the 132 patients enrolled in the BRIM-2 trial. The median overall survival was 15.9 months (35). In the phase III trial, BRIM-3, vemurafenib was compared to intravenous dacarbazine in previously-untreated patients with *BRAF* V600E mutation-positive metastatic melanoma (36). The companion diagnostic test, cobas 4800 BRAF V600 mutation test, was used for detection of the BRAF V600 mutation in melanoma. Twenty patients in the BRIM-3 study had melanoma with BRAF V600D (one patient) and BRAF V600K (19 patients) mutations. The 675 enrolled patients were randomized to receive; vemurafenib at 960 mg orally twice daily, or dacarbazine chemotherapy of 1,000 mg/m^2 intravenously every three weeks. Patients continued treatment until disease progression or unacceptable toxicity. Only, 38% of the patients treated with vemurafenib required a dose reduction. Sixty-one patients developed the most common grade 3 event, squamous cell carcinoma, which was treated by excision. Other adverse reactions, such as rash, arthralgia, fatigue and photosensitivity, were similar to prior studies. The first analysis in 81% patients found a median value of 5.3 months progression-free survival for vemurafenib treatment *versus* 1.6 months for dacarbazine (Table II). The six-month overall survival was reported to be 84% in the vemurafenib arm and 64% in the dacarbazine arm. The overall response rate was 48% for vemurafenib, including two complete responses compared to 5.5% for dacarbazine. Furthermore, 4 out of 10 patients with BRAF V600K randomized to vemurafenib had partial responses. The promising data of the

BRIM-3 study led to an early termination of this clinical trial and FDA approval on August 17th 2011 of vemurafenib for treatment of metastatic or unresectable melanoma harboring the BRAF mutation V600E.

The second BRAF inhibitor that has been approved for the treatment of BRAF V600-mutated metastatic melanoma by the FDA on May 30th 2013 is dabrafenib (Figure 2). A phase I study was conducted on 184 patients with solid tumors, of whom 156 had melanoma, whereby the safety and tolerability were tested and the recommended phase II dose was selected (37). Notably, the maximum tolerated dose was not reached. The recommended phase II dose, 150 mg twice daily, led to 69% responses in patients with either *BRAF* V600E- or V600K-mutated melanoma. Furthermore, dabrafenib showed activity in melanoma metastases in the brain. The most common adverse events of grade 2 or worse caused by dabrafenib were cutaneous squamous cell carcinoma (11%), fatigue (8%), and pyrexia (11.6%).

In the subsequent single-arm, open-label phase II trial (BREAK-2), 76 patients with melanoma and BRAF V600E and 16 with BRAF V600K mutations were enrolled (38). The response rate was much better in the V600E group than in the V600K group, at 59% (with 7% complete response) *versus* 13%. The progression-free and overall survival were also longer in patients harboring the V600E mutation, at 6.3 months and 13.1 months, respectively (Table II). In patients with BRAF V600K, progression-free and overall survival were 4.5 months and 12.9 months, respectively (38).

Table II. *Summary of selected clinical trials with compounds targeting the Mitogen-Activated Protein Kinase (MAPK) pathway*

Phase	No.pts	Patient population	Design	RR (%)	PFS (months)	OS (months)	REF
I	48	*BRAF*-mutated metastatic melanoma	Vemurafenib, dose escalation	77	>7	ND	(33)
II	132	Previously treated *BRAF*-mutated melanoma	Vemurafenib (BRIM-2)	52	6.2	ND	(34)
III	675	Advanced *BRAF*-mutated melanoma	Vemurfenib *vs.* DTIC (BRIM-3)	48 *vs.* 5.5	5.3 *vs.* 1.6	13.2 *vs.* 9.6	(36)
II	92	Melanoma with *BRAF* (V600E) *vs.* (V600K)	Dabrafenib (BREAK-2)	59 *vs.* 13	6.3 *vs.* 4.5	13.1 *vs.* 12.9	(38)
III	250	Advanced *BRAF*-mutated melanoma	Dabrafenib *vs.* DTIC (BREAK-3)	50 *vs.* 6	5.1 *vs.* 2.7	ND	(40)
I	97	Advanced melanoma B-RAFV600E/K *vs.* WT	Trametinib, dose escalation	33 *vs.* 10	ND	ND	(43)
II	97	Treatment naïve *vs.* previously treated advanced BRAF-mutated melanoma	Trametinib	25 *vs.* 0	4.0 *vs.* 1.8	14.2 *vs.* 5.8	(44)
III	322	Stage IIIc or IV with *B-RAF* V600E/K	Trametinib *vs.* DTIC (METRIC)	22 *vs.* 8	4.8 *vs.* 1.5	ND	(45)

DTIC, Dacarbazine; no. pts, number of patients; RR, response rate; PFS, progression-free survival; OS, overall survival.

Because of the impressive results observed in the phase I trial in patients with brain metastases, a second phase II trial was designed, the BREAK-mb. In this trial, dabrafenib was tested in patients with BRAF V600E-mutant melanoma with untreated, or previously treated but relapsed, brain metastases. Dabrafenib showed activity and acceptable toxicity in both these groups (39).

In a phase III clinical trial (BREAK-3), dabrafenib was compared with dacarbazine in previously untreated patients with advanced melanoma with mutated *BRAF*. A significant reduction of 70% in risk for disease progression was found in patients treated with dabrafenib *versus* those treated with dacarbazine (40). In the BREAK-3 trial, similarly to previous vemurafenib phase III trials, a median of 5.1 months progression-free survival was found for dabrafenib treatment *versus* 2.7 months for dacarbazine (150 mg twice daily). Toxic side-effects included skin lesions, pyrexia, frequent fatigue, nausea and pain. The development of photosensitivity, skin side-effects and squamous cell carcinoma appeared to be less frequently reported in patients treated with dabrafenib than with vemurafenib, although a direct comparison has never been investigated.

Other BRAF inhibitors that are currently being evaluated in clinical trials are LGX818, BMS-908662, XL281 ARQ736, and RAF265 (clinicaltrials.gov). For some BRAF inhibitors, such as GDC0879, PF04880594 and AZ628, pre-clinical studies have been performed, but clinical trials with these compounds have not been initiated yet.

Mitogen-activated extracellular signal regulated kinase kinase (MEK) inhibitors. MEK1/2 are downstream kinases

of RAF and considered to be important targets in BRAF-mutated melanomas. Inhibition of MEK blocks cell proliferation and induces apoptosis (41). The development of MEK inhibitors preceded that of BRAF inhibitors, but they showed limited activity in patients with melanoma. However, the *BRAF* status of the melanoma was not determined in these initial trials. More recent clinical trials in patients with *BRAF*-mutated melanoma showed much better results, leading to FDA approval of the first MEK1/2 inhibitor for *BRAF*-mutated melanoma, trametinib (GSK1120212), on May 29th 2013. Trametinib is a non-competitive MEK1/2 inhibitor that does not compete for the ATP-binding site, but it binds to an allosteric binding site next to the ATP site. The specificity is, therefore, higher than that of the MEK1/2 inhibitors that compete for the ATP-binding site. In a phase I trial in which 206 patients with advanced solid tumors were enrolled, the maximum tolerated dose was 3 mg once daily and the recommended phase II dose was established as 2 mg daily. The most common side-effects were grade 1/2 rash or dermatitis (80%) and diarrhea (42%) (42). A sub-study of this trial included only patients with melanoma (n=97). Patients were divided into different groups, 39 patients had BRAF wild-type status, 36 patients were harbored the *BRAF* V600E/K mutation (30 were not previously treated with a BRAF inhibitor), the *BRAF* status was unknown in six patients and 16 had uveal melanoma. In the *BRAF*-mutated group, a response rate of 33% was reported, and in *BRAF* wild-type patients, the response rate was 10%, indicating that mutational analyses of *BRAF* is also important when selecting patients for trametinib treatment (43). In a phase II trial, trametinib was tested in patients with or without prior

BRAF inhibitor treatment. In BRAF inhibitor-naïve patients, the response rate was 25%, while in patients previously treated with a BRAF inhibitor, no response was observed. These data suggest that patients will not benefit from MEK inhibitor monotherapy after resistance to BRAF inhibitor therapy has occurred. The most common toxicities were rash, nausea, peripheral edema, diarrhea, pruritus, and fatigue (44). The phase III trial, METRIC, in patients with *BRAF*-mutated melanoma led to progression-free survival of 4.8 months for trametinib and 1.5 months for dacarbazine or paclitaxel (45). The most frequent trametinib-related toxicities included rash, diarrhea, peripheral edema, which could be managed with dose reduction or interruption. Less frequently, but notably side-effects were cardiac (decreased ejection fraction or ventricular dysfunction) and ocular (blurred vision or reversible retinopathy) toxicities. In contrast to BRAF inhibitors, no cutaneous squamous cell carcinoma was observed in the phase I, II, nor III trials. However, trametinib is not as effective as the BRAF inhibitors, dabrafenib or vemurafenib, but it can be considered for those who cannot tolerate the toxicities of the BRAF inhibitors. Currently, several other MEK inhibitors are under clinical development for the treatment of melanoma. These include selumetinib, PD-0325901, MEK162, refametinib, RO-4987655, TAK-733, and XL518.

Other Targeted Therapies. C-KIT is a receptor tyrosine kinase that when binding to its ligand activates the MAPK, Phosphatidylinositide 3-kinases/ v-akt murine thymoma viral oncogene homolog (PI3K/AKT and Janus Kinase/ Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathways, resulting in proliferative and survival effects. Mutations in the *c-KIT* gene leading to the overactivation of this kinase have been reported in certain melanoma subtypes, namely acral (36%), mucosal (39%) and sun-damaged (28%) melanomas (46). Because *KIT* mutations are also found in imatinib-responsive cancer of other types, phase II trials were conducted with imatinib in patients with melanoma with these subtypes (47, 48). In the first study enrolling 43 patients with metastatic melanoma harboring *c-KIT* aberrations receiving 400 mg imatinib once daily, the overall response rate was 23.3%. Patients who had partial response or stable disease had a progression-free survival of 9.0 months and an overall survival of 15 months. In patients who had disease progression, the overall survival was 9.0 months. For these patients, the dose was allowed to be increased to 800 mg daily (47). In another phase II trial, patients with metastatic mucosal, acral, or chronically sun-damaged melanoma with *KIT* amplifications or mutations also received 400 mg imatinib once-daily or twice-daily if no initial response was observed. Notably, imatinib was only effective in patients with *c-KIT* mutations, with an best overall response rate of 54%, and not in patients with *c-KIT*

amplification. The best overall response rate in this latter group was 0% (48).

Neuroblastoma RAS viral oncogene homolog (*NRAS*) is mutated in approximately 15% to 20% of melanomas (49). Patients with *NRAS* mutations never harbor *BRAF* mutations and therefore represent a distinct subpopulation. Developing drugs targeting mutated *NRAS* is challenging because multiply pathways are unregulated due to this mutation. Instead, current drug development focuses on targeting signaling pathways that the NRAS protein activates, namely the MAPK and PI3K pathways. MEK162 is the most promising MEK inhibitor, showing response in 20% of the patients with *NRAS*-mutated melanoma (50). It is most likely that such patients will benefit much more from combination therapies. One such combination trial, combining MEK162 and LEE011, a cyclin-dependent kinase (CDK)4/6 inhibitor, in patients with *NRAS*-mutated melanoma is currently recruiting (NCT01781572).

Alterations in the PI3K pathway have also been reported in 60% of the melanomas (51, 52). For instance, Phosphatase and tensin homolog (PTEN) is inactivated in 12% of melanomas through mutations or methylation. Loss of function of the *PTEN* gene causes accumulation in phosphatidylinositol(3,4,5)-triphosphate and increases Akt phosphorylation and activity (53). Numerous inhibitors are under development that down-regulate the activation of this survival pathway, including PI3K inhibitors, AKT inhibitors and mammalian target of rapamycin (mTOR) inhibitors (clinicaltrials.gov; NCT01820364; NCT01616199; NCT01337765; NCT01941927; NCT01166126; NCT01014351; NCT00022464).

A genome-wide search of the tyrosine kinome showed mutations in v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4 (ERBB4) (HER4) in approximately 19% of patients with melanoma (54). Mutations in ERBB4 can cause an overactivation of the ERK and AKT pathways. Lapatinib, an Epidermal Growth Factor Receptor/ Human Epidermal growth factor Receptor 2 EGFR/HER2 inhibitor, showed activity in cell lines harboring *ERBB4* mutations and not in *ERBB4* wild-type cells (54). Currently, a phase II trial testing lapatinib in patients with advanced melanoma with *ERBB4* mutations is ongoing (clinicalltrials.gov; NCT012 64081).

Combination Therapies to Overcome BRAF Inhibitor Resistance

Although BRAF inhibitors have significant therapeutic effects in patients with melanoma with *BRAF* mutations, some patients are resistant to BRAF inhibitor treatment, so-called intrinsic resistance. In addition, the therapeutic effects of BRAF inhibitors are short in duration and patients develop resistance to these inhibitors after 6-8 months of treatment. Several resistance mechanisms have been reported, these include, mutations in NRAS, overexpression of Cellular

Rapidly Accelerated Fibrosarcoma (CRAF) or Mitogen-Activated Protein Kinase Kinase Kinase 8 (MAP3K8), activation of receptor tyrosine kinases, such as *PDGFR-B* or *IGF-R*, activation of the PI3K/AKT pathway, mutations in *MEK*, the development of RAS-independent *BRAF* V600E isoform splice variants, *BRAF*-mutant amplification, and activation of the Hepatic Growth Factor/ Hepatic Growth Factor Receptor (HGF/MET) axis (55). Remarkably, most of these mechanisms re-activate the MAPK pathway. Combining a BRAF inhibitor with an inhibitor that targets a protein in the MAPK pathway, downstream of BRAF, is therefore an interesting strategy to overcome BRAF inhibitor resistance. In a phase III clinical trial, the combination of dabrafenib and trametinib was compared to dabrafenib-alone. Median progression-free survival in the combination group was significantly improved to 9.4 months compared with 5.8 months in the monotherapy group (56). The most common toxicity in the combination group was fever, at 70%; in the dabrafenib-treated group, this was only 26%. An explanation for this observation is not yet known. Interestingly, skin toxicities, such as cutaneous squamous cell carcinoma, occurred less frequently compared to treatment with dabrafenib alone. This is most likely because trametinib inhibits the dabrafenib-induced reactivation of the MAPK pathway in *BRAF* wild-type cells.

Combinations of other BRAF and MEK inhibitors also hold promise. The combination of vemurafenib and the MEK inhibitor cobimetinib (GDC-0973) was tested in a phase IB trial (BRIM7) of 70 patients with *BRAF*-mutated metastatic melanoma, of which 38 (54%) had disease that failed to respond to prior vemurafenib treatment (57). All the patients who were not treated with a BRAF inhibitor before (n=25) had tumor regression. The response rate in the patients who were previously treated with a BRAF inhibitor (n=32) was just 19%. Similarly to the dabrafenib/trametinib combination, squamous cell carcinoma was much less frequent in the combination group (1.4%) than that observed with vemurafenib-alone (20-25%). The incidence of the other grade 3 toxicities, such as non-acneiform rash (7.1%), arthralgia (4.3%) and fatigue (1.4%), did not differ. A phase III trial, the coBRIM, is evaluating vemurafenib plus the MEK inhibitor cobimetinib, *versus* vemurafenib alone and closed accrual in December 2013; results are awaited with great interest (clinicaltrails.gov; NCT01689519).

The combination of the BRAF inhibitor LGX818 and the MEK inhibitor MEK162 also showed promising preliminary results in a phase I/II trial (58). A complete response was observed in 14% of the patients with BRAF inhibitor-naïve melanoma, and the partial response rate was 71%. In patients with melanoma pre-treated with BRAF inhibitor, the response rate was 22%. The most common treatment-related adverse events (≥20%, all grades) were nausea, abdominal pain, and headache. No events of fever, hand−foot skin reactions, hyperkeratosis, or squamous cell carcinoma were observed. A 3-arm phase III trial comparing LGX818, the combination LGX818/MEK162 and vemurafenib in *BRAF*-mutated melanoma is currently recruiting (NCT01909453).

Furthermore, combining BRAF inhibitors with other agents that target proteins such as PI3K, mTOR, CDK4, c-MET to overcome resistance are also very interesting and several clinical trials have been initiated to evaluate these combinations. An overview of these clinical trials can be found in Table III.

Combining Targeted Therapy and Immunotherapy

The development of ipilimumab, vemurafenib and other novel therapies for metastatic melanoma set the stage for investigating combination therapies that may include immunotherapy and targeted therapy. Both new therapies have a different mechanism of action but each improves the overall survival of patients with metastatic melanoma. Ipilimumab as monotherapy led to slow but long-term durable responses and durable stable disease. Vemurafenib led to transient responses with rapid tumor regression. Evidence exists that BRAF inhibition or inhibition of the MAPK pathway in BRAF-driven tumors is associated with decreased production of the immunosuppressive factor IL10 and enhanced expression of tumor-specific antigens (59). Tumor-specific antigens released as a result of tumor cell death are presented to lymphocytes by APCs. In addition, cytokines and chemokines are then produced to induce the antitumor immune response. In this way, vemurafenib might induce T-cell infiltration before treatment with ipilimumab. In theory, the different approaches to cancer therapy of vemurafenib and ipilimumab may reduce the likelihood that resistance will emerge during combination therapy. It may also be that these agents combined are not synergistic or even less effective than either drug alone. Adverse events, such as hepatitis, rash, and arthralgia, were observed in patients treated with ipilimumab and vemurafenib alone, which could limit the ability to use the optimal dosages of these drugs in combination. In this case, combination of the two drugs may be more tolerable when used sequentially in either order rather than in combination. In November 2011, a phase I/II trial of vemurafenib and ipilimumab in patients with BRAF V600 mutation-positive metastatic melanoma was initiated. The purpose of the phase l study was to investigate the maximum tolerated dose of both drugs that can be administered together, followed by a phase ll part to assess the efficacy of the combination therapy. Patients were enrolled to receive 960 mg vemurafenib twice daily either two or four weeks prior to ipilimumab treatment (3 mg/kg ipilimumab). The dose of ipilimumab was escalated to 10 mg/kg as tolerated. However, this trial was terminated due to hepatoxicity, a side-effect which has been observed for both drugs (60). A phase I trial testing ipilimumab and dabrafenib

Table III. *Selected ongoing combination trials with v-raf murine sarcoma viral oncogene homolog B1 (BRAF) inhibitors for metastatic melanoma.*

BRAF inhibitor	Combination drug	Phase	Primary outcome	Trial	Estimated N of patients
Vemurafenib	GDC-0973; MEK inhibitor	III	PFS (RECIST)	NCT01689519	500
Vemurafenib	PLX3397; KIT, CSF1R and FLT3 inhibitor	IB	Safety, ORR, response duration, PFS	NCT01826448	90
Vemurafenib	BKM120; PI3K inhibitor	I/II	Safety, recommended phase II dose, PFS	NCT01512251	46
Vemurafenib	P1446A-05; CDK inhibitor	I	Safety, MTD, DLT	NCT01841463	100
Vemurafenib	XL184; c-MET inhibitor	I	MTD, DLT	NCT01835184	34
Vemurafenib	PX-866; PI3K inhibitor	I/II	Toxicity, PFS	NCT01616199	146
Vemurafenib	XL888, HSP90 inhibitor	I	MTD, recommended phase II dose	NCT01657591	36
Vemurafenib	SAR260301, PI3Kβ inhibitor	I/Ib	MTD	NCT01673737	75
Vemurafenib	Everolimus/temsirolimus, mTOR inhibitors	I	MTD	NCT01596140	114
Dabrafenib	Trametinib, MEK inhibitor	III	PFS	NCT01584648	340
Dabrafenib	GSK2141795, AKT inhibitor	I/II	MTD, objective Response Rate (RECIST)	NCT01902173	66
LGX818	MEK162, MEK inhibitor	III	PFS (RECIST)	NCT01909453	900
LGX818	LEE011, CDK4/6 inhibitor	Ib/II	Toxicity (dose limiting), PFS, ORR	NCT01777776	150
LGX818	BKM120; PI3K inhibitor	II	ORR	NCT01820364	100
LGX818	BGJ398, FGFR inhibitor	II	ORR	NCT01820365	101
LGX818	INC280, c-MET inhibitor	II	ORR	NCT01820366	102

ORR, Overall response rate; PFS, progression-free survival; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; no. pts, number of patients,

with/without trametinib is currently ongoing (Clinicaltrial.gov; NCT01767454), and hopefully this combination will be better tolerable.

As mentioned earlier, antibodies against PD1 led to less frequent and less severe toxicities than ipilimimab. Combining vemurafenib with an antibody to PD1 could therefore be a more attractive approach to developing a less toxic and potent treatment. A phase Ib, open-label study has been initiated investigating the safety and pharmacology of MPDL3280A, an antibody to PD1, in combination with vemurafenib in patients with previously untreated *BRAF* V600-mutated metastatic melanoma (NCT01656642).

If patients with BRAF-mutated melanoma have a good performance status and a relatively low tumor load, first-line treatment with an immunotherapy could be considered because immunotherapy is most effective in patients with low tumor burden and can achieve long-term remission. In cases of progressive disease, treatment with a BRAF/MEK inhibitor can always be considered as salvage treatment because these targeted-therapies can also induce rapidly tumor responses in patients with a high tumor burden (61, 62).

Combination of Immunotherapies

CTLA-4 and PD1 have complementary roles in regulating adaptive immunity. Targeting both these immune checkpoints could help the immune system to better fight cancer much better. In a murine B16 melanoma model, the combination of anti-PD1 and anti-CTLA-4 antibodies was more effective at inducing tumor regression compared to either treatment alone (63). Based on these pre-clinical data, a phase I clinical trial was initiated to compare overall survival with nivolumab and ipilimumab as monotherapy or combination therapy (64). Concurrent administration of nivolumab (1 mg/kg) and ipilimumab (3 mg/kg) resulted in a tumor regression of 80% or more in 53% of the patients with advanced melanoma. Notably, drug-related adverse events were not more frequent or more severe than those with administration of either drug alone. A long follow-up survival remains to be determined for this combination. Currently phase II and III trials are ongoing to evaluate the nivolumab/ipilimumab combination regime and the first data are expected in 2015 (clinicaltrials.gov).

Concluding Remarks

Melanoma diagnosed at an early stage and radically resected has the best chance for cure. However, later stages have poor prognosis due to lack of responsiveness to traditional chemotherapeutics. Multiple new treatment options are being evaluated in the clinic and the results with immunotherapy and targeted therapy are very exciting. Ipilimumab and vemurafenib lead to improved overall survival. Ipilimumab, monoclonal antibody against CTLA-4, is now FDA-approved and was the first drug leading to an improvement in overall survival of patients with unresectable melanoma. New advances in targeting the MAPK pathway also showed impressive responses in patients with melanoma harboring activating mutations in *BRAF*. The BRAF inhibitors vemurafenib and dabrafenib, and the MEK inhibitor trametinib, are also FDA-approved as personalized medicine for patients with BRAF V600E mutation-positive metastatic melanoma. A diagnostic test can identify patients with *BRAF-*

mutant melanoma for whom treatment will more likely improve progression-free survival and overall survival outcomes. These drugs are well-tolerated and the BRAF inhibitors have a rapid time to initial treatment response, with sustained responses of 6-8 months. Unfortunately, melanomas seem to develop resistance with prolonged treatment. Immunotherapy, on the other hand, leads to slow but long-term durable responses. With the availability of both targeted kinase inhibitors (vemurafenib, dabrafenib and trametinib) and the immune checkpoint inhibitor, ipilimumab, treatment options are increasing in metastatic melanoma. Other novel immunotherapies, antibodies against PD1 and PDL1 also showed promising results in clinical trials. Based on successes of ipilimumab, vemurafenib, dabrafenib and trametinib, future improvements of these drugs in combination with other approaches are expected to provide long-term benefits to more patients with metastatic melanoma. The ideal combination will be one that has a very high rate of long clinical response and no severe toxicities. The introduction of new targeted-therapies and immunotherapies will most likely markedly improve the prognosis of patients with melanoma.

Disclosure

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