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REVIEW ARTICLE

Advanced Melanoma: Current Treatment Options, Biomarkers, and Future Perspectives

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Abstract Malignant melanoma accounts for the highest number of deaths from skin cancer, and the prognosis of patients with stage IV disease has historically been poor. Novel insights into both mutations driving tumorigenesis and immune escape mechanisms of these tumors have led to effective treatment options that have revolutionized the treatment of this disease. Targeting the MAPK kinase pathway (with BRAF and MEK inhibitors), as well as targeting checkpoints, such as cytotoxic T-lymphocyte associated protein 4 (CTLA-4) or programmed death 1 (PD-1), have improved overall survival in patients with late-stage melanoma, and biomarker research for personalized therapy is ongoing for each of these treatment modalities. In this review, we will discuss current first-line treatment options, discuss biomarkers supporting treatment decisions, and give an outlook on (combination) therapies we expect to become relevant in the near future.

Key Points

Since the introduction of immunotherapies and targeted therapies, the historically poor survival of patients with advanced melanoma has dramatically improved.

New treatment combinations are rapidly emerging and much research is focused on biomarkers to select the best treatment options for individual patients.

1 Introduction

1.1 Epidemiology

In the year 2016, as many as 76,380 novel cases of melanoma were diagnosed in the US (making melanoma the fifth and seventh most common malignancy in males and females, respectively), with a total of 10,130 estimated deaths from this disease. Although melanoma accounts for a relatively low number of cases compared with other types of skin cancer, it accounts for the highest number of skin cancer deaths by far [1]. All signs point towards an increasing incidence of this disease [1, 2], including in young adults [3]. Considering the high number of melanomas that are attributable to the seemingly preventable exposure to ultraviolet (UV) radiation [4] and the high number of loss of years per death to melanoma [5], maximum efforts for public education should be made to either prevent this disease or diagnose it in its early stages.

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1.2 Models of Progression

Unfortunately, many patients are diagnosed at a late stage or still experience disease progression despite appropriate locoregional treatment. Melanocytic lesions evolve through several well-defined precursor lesions before these progress to invasive, and eventually metastatic, melanoma [6] (also reviewed by Shain and Bastian [7]). Early (and most likely initiating) mutations occur in genes that regulate proliferation, such as those that belong to the mitogen-activated protein kinase (MAPK) pathway (including *BRAF* and *NRAS*). Mostly known for being the most frequently mutated gene in invasive melanoma [8], mutations in *BRAF* are believed to play a central role in the formation of early and benign melanocytic growths. Additional mutations in genes that regulate genomic integrity (*TP53*), chromatin remodeling (*ARID1A*), telomere length (*TERT*), and the PI3K pathway (*PTEN*) are examples of mutations that are required before invasive and metastatic melanoma can occur [7]. Large-scale analyses have revealed a high number of passenger mutations [8], consistent, on average, with the high mutational rate of this disease, while at the same time uncovering the broad spectrum of consistent gain- and loss-of-function mutations that contribute to disease progression [8].

1.3 Risk Factors

Melanomas occur on both sun-exposed skin and sites that are less commonly exposed to UV radiation (such as the subungual regions, hand palms and soles of the feet). The spectrum of mutations in both types of tumors is quite distinct [9], which suggests differential tumorigenic mechanisms between both types of melanomas. Consequently, severe sunburn, as well as chronic and intermittent exposure to the sun, are associated with the development of melanoma [10]. Although sun exposure is the most common source of UV exposure, artificial sources of UV have also been linked to the development of melanoma [11].

Mutations in the MAPK pathway do not seem to be directly caused by UV, considering the lack of the characteristic C>T and G>T transitions in these genes [8]. Regardless, *BRAF*-mutated melanomas have been linked to sun exposure [12], possibly through alternative mechanisms of UV-mediated mutagenesis (such as oxidative stress). An estimated 46% of driver mutations seem to be directly linked to UV exposure, considering the presence of these distinctive mutational patterns [8]. *TP53* mutations are most commonly associated with UV-induced genetic damage in melanoma [8].

Genetic factors are also commonly accepted risk factors for melanoma. Xeroderma pigmentosum is a hereditary disease that is characterized by a greatly increased susceptibility to UV-mediated DNA damage secondary to deficiencies in nucleotide excision repair (NER). Similarly, familial atypical mole melanoma (FAMM) syndrome, which is associated with *CDKN2A* mutations, vastly increases the risk of melanoma. In addition to these well-described genetic syndromes, a family history of melanoma increases the risk of this disease [13].

1.4 Mutational Load and Immunogenicity

Melanoma cells are characterized by their high number of UV-induced, non-synonymous point mutations that have a propensity for generating neoantigens that the immune system may recognize, and then discriminate melanoma cells from melanocytes [14]. Relatively high mutational rates are found in melanoma compared with other cancer types [8, 14, 15]. The higher the number of mutations, the higher the chance of generation of neoantigens, which in turn could result in the induction of both a CD4$^+$ and CD8$^+$ T-cell response [16]. Unfortunately, it is evident from clinical practice that this immune surveillance is insufficient to prevent the formation and progression of melanomas, which might not only be due, in part, to ineffective presentation of epitopes and reduced expression of potent antigens [16] but also to the expression of immunomodulatory molecules by tumor cells. For instance, the programmed-death ligand 1 (PD-L1) is an important inhibitory signal for cytotoxic T-cell activity [17]. Expression of this ligand for the programmed death 1 (PD-1) receptor is commonly seen on melanoma cancer cells and is associated with the presence of tumor-infiltrating lymphocytes pointing towards adaptive resistance to an endogenous anti-tumor immune response as a mechanism of immune escape [18]. The effect of PD-L1 expression on overall survival (OS) is widely studied but results are controversial [18–20]. These and other mechanisms (broadly captured under the term 'cancer immunoediting' [21]) hinder adequate recognition and cytotoxic activity of the immune system and lead to poor tumor control and patient prognosis.

1.5 Patient Survival

Historically, the survival of patients with advanced melanomas has been very poor, especially patients with metastatic disease [22]. Disease stage is a strong determinant of OS at 5 years. Prior to the era of checkpoint inhibitors and targeted therapy, the 5-year OS was estimated to be 94–100% for stage I disease, 53–92.8% for stage II disease, decreasing to 78, 59 and 40% in stage IIIa, IIIb, IIIc, respectively, and 9–28% for stage IV disease [23–25]. These abysmal survival statistics and increased understanding of the mutations driving tumorigenesis of this

disease have led to novel treatment modalities for patients who previously had almost no viable options for systemic treatment. Ample attempts at combinations of chemotherapy regimens, despite improving response rates, have not translated into survival benefit [26].

In this review, the role of checkpoint inhibitors and targeted therapy in the treatment of metastatic melanoma is presented. We discuss the trials that have shaped our understanding of these treatment modalities and that have led to the approval of agents for the treatment of patients with metastatic disease (Table 1). Current evidence for combination regimens and sequential treatment schemes will be discussed in order to provide an up-to-date picture of the treatment selection for these patients. Certain subgroups of patients and the use of single- and multi-parameter biomarkers that can be used for treatment selection, are discussed separately.

2 Immunotherapy

For decades, interleukin (IL)-2, interferon-α, and a variety of other cytokines have been extensively studied in patients with advanced melanoma. Although a small fraction of patients seemed to achieve durable remission on high-dose IL-2, toxicity was high and all conducted phase III trials failed to show OS benefit [26, 27].

Ipilimumab, a monoclonal antibody that acts as an inhibitor of the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), was the first T-cell checkpoint inhibitor that improved median OS in chemotherapy-refractory patients compared with gp100 vaccination [28]. Ipilimumab in combination with dacarbazine improved OS compared with dacarbazine plus placebo in treatment-naive patients with advanced melanoma [29], but did not seem to be superior to ipilimumab monotherapy. Interestingly, a proportion of patients (22%) achieved long-term survival. This was not seen before and suggests an ongoing immune response, and perhaps even cure [30]. Standard treatment with ipilimumab consists of four cycles of 3 mg/kg every 3 weeks. An atypical treatment response pattern, characterized by an increase in tumor volume before tumor response or disease stabilization, was seen in 10–15% of patients. This phenomenon is also known as pseudo-progression [31]. Response to ipilimumab seems to be dose-dependent as no responses were seen on treatment with 0.3 mg/kg and OS was better in patients treated with ipilimumab 10 mg/kg compared with 3 mg/kg. This increased survival benefit comes at the cost of substantially more toxicity [32]. After the approval of ipilimumab in 2011, research on immunotherapy has exploded, with a special focus on novel checkpoint inhibitors. Targeting the PD-1/PD-L1 axis was one of the main areas of interest. Like CTLA-4, PD-1 is an immune checkpoint molecule involved in maintaining peripheral tolerance and prevention of auto-immunity. Whereas CTLA-4 plays a major role in initial T-cell activation and broadening of the T-cell repertoire [33, 34], PD-1 signaling is thought to mainly alter the T-cell effector phase [35, 36]. Pembrolizumab and nivolumab (both monoclonal antibodies against PD-1), further improved response rates up to 40% and prolonged OS for advanced melanoma patients [37, 38]. The randomized phase III CheckMate 066 study showed that, compared with standard chemotherapy, nivolumab was favorable as first-line treatment in *BRAF* wild-type patients in terms of overall response rate (ORR; 40 vs. 13.9%), progression-free survival (PFS; 5.1 vs. 2.1 months), and OS (not reached vs. 11 months) [39, 40].

Table 1 Approved agents for the treatment of stage IV melanoma

Immunotherapy	Target	Year of approval by FDA
Ipilimumab	Anti-CTLA-4	2011
Nivolumab	Anti-PD-1	2014
Pembrolizumab	Anti-PD-1	2014
Ipilimumab + nivolumab	Anti-CTLA-4 + anti-PD-1	2015 (*BRAF* WT), 2016 (regardless of *BRAF* status)

Targeted therapy	Target	Year of approval by FDA
Vemurafenib	BRAFi	2011 (BRAFV600E)
Dabrafenib	BRAFi	2013 (BRAFV600E)
Vemurafenib + cobimetinib	BRAFi + MEKi	2015 (BRAFV600E or BRAFV600K)
Dabrafenib + trametinib	BRAFi + MEKi	2014 (BRAFV600E or BRAFV600K)
Trametinib	MEKi	2014 (BRAFV600E or BRAFV600K)

BRAFi BRAF inhibitor, *BRAF WT* BRAF wildtype, *CTLA-4* cytotoxic T-lymphocyte-associated protein 4, *MEKi* MEK inhibitor, *PD-1* programmed death-1

Table 2 Objective response rate, PFS and OS on therapies approved for advanced melanoma

Therapy/study	ORR (%)	PFS				OS			
		Median (mo)	1y (%)	2y (%)	3y (%)	Median (mo)	1y (%)	2y (%)	3y (%)
Checkpoint inhibitors									
Ipilimumab [28, 41, 45, 48, 50, 57]	11–19	2.8–3.3	18–19	12–15	10	11.5–19.9	46–58	24–43	34
Pembrolizumab [41, 42, 45]	36–37	8.3	38–39	28–34	NR	32.3	68–74	55	NR
Nivolumab [39, 40, 48, 50, 57]	40–44	5.4–6.9	42–44	37–39	32	37.6	73	58–59	52
Ipilimumab + nivolumab [48–50, 57]	57–58	11.5	49–53	43–51	39	Not reached	73	64	58
Targeted therapy									
Vemurafenib [82–84, 87]	50–51	7.2–7.3	NR	16	10	17.8–18	64	39	31
Dabrafenib [85, 86]	53	8.8	NR	16	12	18.7	68	42	32
Encorafenib [88]	51	9.6	NR	NR	NR	Not reached	NR	NR	NR
Vemurafenib + cobimetinib [83, 87, 97]	70	12.3	52	29	NR	22.3	75	49	37
Dabrafenib + trametinib [82, 84–86]	64–69	11–12	NR	30	22–24	25.1–26.1	73–74	51–52	44–45
Encorafenib + binimetinib [88]	63	14.9	NR	NR	NR	Not reached			

NR not reported, *ORR* overall response rate, *OS* overall survival, *PFS* progression-free survival

The KEYNOTE-006 study demonstrated that pembrolizumab provided significantly more clinical benefit, with an improved toxicity profile compared with ipilimumab in immunotherapy-naive patients (*BRAF* wild-type and V600 mutant); ORR 36–37 vs. 13% [41], PFS 8.3 vs. 3.3 months, and OS 32.3 vs. 15.9 months [42]. Interestingly, the duration of response is identical for patients responding to either pembrolizumab or ipilimumab [41]. Both anti-PD-1 antibodies also proved to be effective in ipilimumab-refractory patients. In this patient population, a response rate of 32% was seen for nivolumab and 21–25% for pembrolizumab compared with 4–11% for chemotherapy (Table 2) [43, 44]. The response to anti-PD-1 antibodies seems to be less dose-dependent as there was no difference in clinical outcome between patients treated with pembrolizumab 2 or 10 mg/kg every 3 weeks [43], and there was also no difference in outcome between two-weekly and three-weekly administration [45]. Interestingly, responses to anti-PD-1 seem to be ongoing, even after cessation of therapy. Of all patients in the KEYNOTE-006 study who completed 2 years of pembrolizumab treatment (*n* = 104), only 9% had progressive disease after a median follow-up of 9.7 months after completion of pembrolizumab [42]. Remarkably, the optimal duration of treatment with anti-PD-1 has not yet been determined. In all but one clinical trial, it was advised to continue treatment until disease progression, unacceptable toxicity, or patient refusal. In the KEYNOTE-006 trial only, the maximum duration of treatment was arbitrarily set at a maximum of 2 years or until disease progression, intolerable toxicity, or complete response (CR). We and others retrospectively analyzed patients who discontinued pembrolizumab in the absence of disease progression and treatment-limiting toxicity. We found that only 2/81

patients (3%) relapsed after a median of 11.0 months post-treatment follow-up. In this cohort, the median time of anti-PD-1 treatment was 14.5 months, and thereby much shorter than the abovementioned clinical trials. We also found that at the time of disease progression, re-induction of PD-1 blockade resulted in tumor control in approximately half of the patients [46].

After the success of the individual checkpoint inhibitors, further research explored the efficacy and toxicity of combining anti-CTLA-4 and anti-PD-1 antibodies. The combination of ipilimumab and nivolumab has indeed improved the response rate even further, up to 58–61% (Table 2) [47, 48], and also demonstrated a superior PFS of 11.5 months compared with 6.9 months for nivolumab (numerically, as the study was not powered for this comparison), and significantly versus the 2.9 months for ipilimumab monotherapy [48]. Significant OS benefit was also seen for the combination regimen compared with single-agent ipilimumab [49]. However, the ominously higher ORR for the combination compared with nivolumab monotherapy appears not to translate into a similar difference in OS. A follow-up report from the CheckMate 067 study showed a 3-year survival of 52% for nivolumab versus 58% for the combination (hazard ratio [HR] 0.85, 95% confidence interval [CI] 0.68–1.07) [50]. Of note, this study was not powered to compare the combination versus nivolumab only.

2.1 Toxicity

With the introduction of immunotherapy, a new range of side effects emerged that are predominantly immune-related. These toxicities are remarkably different from the side effects that are known for traditional chemotherapies

and require special attention from treating physicians. Although these adverse events (AEs) may be life-threatening, treatment with high-dose corticosteroids (or additional immunosuppressive agents) is effective in most cases. Ipilimumab monotherapy induced grade 3 and 4 AEs in approximately 30% of patients, with colitis and hypophysitis occurring most frequently [28, 29]. Anti-PD-1 monotherapy seems to be better tolerable as only 10–16% of patients developed grade 3 or 4 toxicities [40, 45, 48]; however, up to 59% of patients treated with the combination of ipilimumab and nivolumab developed one or more grade 3 or 4 AEs [50]. This led to discontinuation of treatment in approximately one-third of patients. Importantly, patients who stopped due to toxicity did equally well in terms of ORR and PFS compared with patients who continued treatment, and responses seem to be durable [51].

This significant toxicity might hinder the broad application of this regimen. Sequential treatment with CTLA-4 and PD-1 inhibitors might be equally effective and even less toxic. The CheckMate 064 study is the only randomized phase II trial testing sequential administration of CTLA-4 and PD-1 inhibitors, comparing six courses of nivolumab followed by four courses of ipilimumab versus the reverse sequence. Grade 3 and 4 toxicities occurred in 50% of patients in the ipilimumab → nivolumab arm and 63% of patients in the nivolumab → ipilimumab arm, which is comparable to the concurrent combination scheme [52]. However, it is difficult to interpret these results due to the study design of different overlapping amounts of antibodies in both arms.

Another approach to reduce toxicity is to focus on dose adjustments. The KEYNOTE 029 study tested low-dose ipilimumab 1 mg/kg combined with standard-dose pembrolizumab 2 mg/kg. This combination seems to be less toxic, with a grade 3–4 toxicity rate of 45%, while preserving efficacy (ORR 61%) [53]. A retrospective analysis of patients treated with two courses of ipilimumab 3 mg/kg directly followed by anti-PD-1 (nivolumab 3 mg/kg or pembrolizumab 2 mg/kg), 'The NKI Scheme', revealed a response rate of 55% and a grade 3 or 4 toxicity rate of 38% [54], indicating that this sequenced but overlapping scheme of CTLA-4 and PD-1 blockade might reduce toxicity further, while preserving efficacy. These data should be interpreted with caution as they are generated from a retrospective analysis and a single-arm study, and the patient populations are not identical to the CheckMate 067 study. The CheckMate 511 study (NCT02714218) is the only ongoing randomized trial comparing ipilimumab 3 mg/kg plus nivolumab 1 mg/kg versus ipilimumab 1 mg/kg plus nivolumab 3 mg/kg. We need to wait for the PFS and OS results to conclude which combination is the most favorable option in terms of efficacy and toxicity and might function as a backbone for future (triple) combinations.

2.2 Biomarkers for Clinical Benefit

After the introduction of checkpoint inhibitors, significant efforts have been made to identify markers that are predictive for treatment response and survival. Establishing these biomarkers is especially important if more aggressive treatment combinations arise, where the proportion of patients who develop severe toxicities is almost as high as the proportion who achieve an objective response. Biomarkers predictive for response may optimize patient selection for (combination) immunotherapy and may therefore aid in avoiding unnecessary toxicity and healthcare costs.

Despite immense research efforts for PD-L1 as a marker for response to checkpoint inhibition, it cannot be used as a single biomarker to select melanoma patients for treatment with (combination) immunotherapy. Higher PD-L1 expression is associated with increased clinical activity of pembrolizumab [55], nivolumab, and the combination of ipilimumab and nivolumab [56]. Nevertheless, single PD-L1 expression is not specific enough to identify patients who do not benefit from PD-1 inhibition, as a considerable proportion of patients with PD-L1-negative tumors do respond. As mentioned above, the CheckMate 067 study was not powered to compare the nivolumab arm with the combination of nivolumab and ipilimumab. Although objective response rates are in favor of the combination over nivolumab alone, regardless of PD-L1 expression (at every cut-off: 1, 5, and 10%) [56], there seems to be no difference in PFS and OS for the subset of patients with high tumoral PD-L1 expression [57]. In contrast, for patients with low PD-L1 expression, there was a trend towards better OS for the group treated with the combination (HR 0.74, 95% CI 0.52–1.06) [57]. The use of PD-L1 as a biomarker is not straightforward as the results of immunohistochemistry (IHC) can be influenced by the choice of IHC antibody [58], as well as other variables (ischemic time, type of fixation, age of the tumor sample, intrapatient and intratumor heterogeneity, and scoring systems) [59, 60].

Other factors in the tumor microenvironment also seem to be important in establishing an anti-tumor immune response. Analyses of pretreatment tumor biopsies obtained from melanoma patients treated with anti-PD-1 antibodies showed that responding patients had significantly more CD8 infiltration, as well as PD-1 and PD-L1 expression at the invasive tumor margin [61]. Based on these and other translational research data, a comprehensive, mechanism-driven combination of independent but

interacting biomarkers (including PD-L1 expression mutational load and CD8 infiltration) was proposed [62].

Although these tumor characteristics seem to be important, a plethora of retrospective analyses of randomized trials and real-life patient cohorts has revealed several baseline patient characteristics that are prognostic or predictive for response and survival. Lactate dehydrogenase (LDH, which is also an established prognostic marker for survival of melanoma patients [24]), was the first marker identified as being predictive for response to ipilimumab [63], and also proved to be predictive for response to anti-PD-1 antibodies and the combination regimen [64, 65]. However, there is still a fraction of patients with elevated LDH who do respond to immunotherapy. More importantly, duration of response seems to be independent of LDH level [65, 66]. For patients treated with pembrolizumab, aside from low LDH, the absence of visceral metastasis other than lung or soft tissue and high relative lymphocyte eosinophil count were independent prognostic factors for clinical outcome [67]. Another retrospective analyses of patients treated with pembrolizumab demonstrated that high absolute lymphocyte count, good performance status, low LDH, and also low C-reactive protein (CRP; as a marker for cancer-associated inflammation) were associated with favorable clinical outcome [68].

These observations underscore our idea of the era of single biomarkers for immunotherapy being outdated. Therefore, from a more holistic approach, we have proposed a framework that includes a combination of tumor-specific and patient-specific markers to personalize treatment choice [69]. The cancer immunogram describes seven parameters that are important in the interaction of both the tumor and the immune system: mutational load, T-cell infiltration, expression of immune checkpoints, CRP/IL-6, lymphocyte count, and expression of major histocompatibility class I. Multiple parameter combinations such as these may be superior to single biomarker approaches (such as PD-L1 expression) and tumor-focused signatures (such as inflamed versus non-inflamed tumors).

3 Targeted Therapy

Approximately 50% of melanoma patients harbor an activating $BRAF^{V600}$ mutation [70, 71], which results in continuous activation of the MAPK pathway. The discovery of the $BRAF$ mutations in 2002 has received considerable scientific attention. Nowadays, patients harboring this mutation can be successfully treated with small molecules that suppress the MAPK pathway, including selective BRAF inhibitors (BRAFi) and MEK inhibitors (MEKi). Vemurafenib, a selective inhibitor of V600 mutant BRAF,

was the first agent to demonstrate improved clinical outcomes compared with chemotherapy, with an ORR of 48 vs. 5%, PFS of 5.3 vs. 1.6 months, and OS of 13.3 vs. 10 months [72, 73], and was approved by the US FDA in 2011. Treatment with dabrafenib, another selective BRAFi, yielded similar clinical benefit, with an ORR of 50% and a PFS of 5.1 months [74]. As reactivation of the MAPK pathway is one of the most common resistance mechanisms [75, 76], and BRAFi-induced paradoxical activation of the MAPK pathway can result in secondary (skin) tumors [77, 78], preclinical studies tested dual inhibition of BRAF and MEK. This combination enhanced tumor cell apoptosis, delayed resistance and decreased cutaneous hyperproliferative lesions in preclinical models [79, 80]. These preclinical findings were confirmed in three large, phase III, randomized clinical trials—the COMBI-v, COMBI-d and coBRIM studies [81–83]. In the COMBI-v trial, dabrafenib and trametinib were demonstrated to be favorable compared with vemurafenib, with an ORR of 64 vs. 51%, a median PFS of 11.4 vs. 7.3, and median OS of 26.1 vs. 17.8 months [82, 84]. Similar outcomes were seen in the COMBI-d trial, with a favorable ORR of 69 vs. 53%, median PFS of 11 vs. 8.8, and median OS of 25.1 vs. 18.7 for dabrafenib and trametinib compared with dabrafenib plus placebo (Table 2) [85, 86]. In addition, the combination of vemurafenib and cobimetinib has shown similar results, with an ORR of 70%, a median PFS of 12.3 months, and a median OS of 22.3 months (Table 2) [83, 87]. The rates of treatment-related AEs were similar for both the combination and monotherapy, although the frequency of specific AEs was different. In the groups treated with the combination, the rate of cutaneous squamous cell carcinoma was significantly lower, but the frequency of diarrhea, central serous retinopathy, and decreased left ventricular ejection fraction was substantially higher [82, 83, 85].

Recently, results from the phase III study of another combination of the BRAFi encorafenib and the MEKi binimetinib were presented, indicating a promising PFS of almost 15 months for the combination compared with 9.6 months for encorafenib and 7.3 months for vemurafenib in patients who were treatment-naive or who were pretreated with first-line immunotherapy [88].

3.1 Toxicity

In terms of response rate, PFS, and OS, there seems to be no difference between the combinations of dabrafenib plus trametinib and vemurafenib plus cobimetinib, both of which have been approved by the FDA and the European Medicines Agency. The choice for one of these regimens can be made in consultation with the patient based on the different toxicity profiles. Pyrexia, chills, headache,

diarrhea, nausea, and vomiting are the most common toxicities for the combination of dabrafenib and trametinib [89], while the most common side effects of the combination vemurafenib and cobimetinib are photosensitivity reaction, rash, diarrhea, nausea, and elevated liver enzymes [83]. If a patient is not tolerating one of the drug combinations, a switch to the other regimen is a reasonable option.

3.2 Biomarkers for Clinical Benefit

Similar to the research on predictive biomarkers for checkpoint inhibition, research efforts have focused on finding markers that identify the subset of patients who will have long-term benefit from targeted therapies. A pooled analysis of patients treated with dabrafenib and trametinib revealed that LDH concentration, the number of metastasized organ sites, and Eastern Cooperative Oncology Group (ECOG) performance status were independent markers for long-term benefit [90]. Patients with an LDH less than the upper limit of normal (ULN) and less than three disease sites have the best clinical outcome [91]. Additionally, genomic analyses have shown that higher mutational loads are associated with longer OS derived from BRAFi and MEKi [92], suggesting that the immune system might also play a role in the long-term benefit of BRAFi and MEKi. An alternative explanation might be that the OS difference in this scenario results from the benefit of subsequent immunotherapy (after progression on BRAFi and MEKi).

4 First-Line Therapy for Patients with a BRAF^{V600} Mutation

4.1 Current Treatment Guidelines

International guidelines currently recommend treating patients with advanced BRAF^{V600}-mutated melanoma with either checkpoint inhibitors or the combination of a BRAFi and an MEKi as first-line therapy [93, 94]. For patients with symptomatic metastases, the combination of BRAFi plus MEKi is favorable over immunotherapy as it has a higher chance of a fast response (Table 3) [94]. Moreover, patients with symptomatic brain metastases due to brain edema might need corticosteroids, which are immunosuppressive and most likely lower the chance of response to immunotherapy [95].

For patients with asymptomatic disease, the definitive superiority of either targeted therapy or checkpoint inhibition as first-line treatment remains unproven. No studies have directly compared both treatment strategies and the 1- and 2-year OS rates seem to be comparable (Table 2) [93].

Based on the aforementioned biomarkers (LDH, performance status, number of metastasized organs), the group of patients who will benefit most from targeted therapy is the same as the group that is likely to have long-term benefit from immunotherapy [96]. There is an urgent need for additional predictive biomarkers that can guide the choice for first-line therapy for these patients. Although there are no data that directly compare these treatment regimens, many melanoma centers have a preference for immunotherapy as first-line therapy. The PFS curves for checkpoint inhibitors seem to reach a plateau after 2 or 3 years, while the curves for BRAFi plus MEKi are still decreasing, reflecting a higher percentage of patients requiring subsequent therapy. Moreover, the median duration of response to BRAFi plus MEKi is 13–14 months, while the median duration response of patients treated with pembrolizumab in the KEYNOTE-006 study, and nivolumab in the CheckMate 067 study, is still not reached after a median follow-up of 33.9 months in the KEYNOTE-006 study and a minimum follow-up of 36 months in the CheckMate 067 study [42, 50, 84, 97]. Lastly, objective responses to immunotherapy seem to be ongoing after cessation of therapy [42, 46, 98, 99]. The landmark trial directly comparing these first-line treatments in BRAF^{V600}-mutated advanced melanoma is currently recruiting (NCT02224781). In this phase III trial, patients will be randomized between first-line dabrafenib plus trametinib versus ipilimumab plus nivolumab, and, after disease progression, will switch to the other combination treatment.

4.2 Combination Regimens

Perhaps the combination of targeted therapy and immunotherapy is an interesting new option for first-line therapy, theoretically coupling the high response rate of targeted therapy and the long duration of response mediated by immunotherapy. Preclinical data indicate that targeted therapy can induce tumoral T-cell infiltration, enhance antigen presentation, and reduce immunosuppressive cytokines [100, 101]. Preliminary data derived from the KEYNOTE 022 study (investigating pembrolizumab in combination with dabrafenib and trametinib) showed a high response rate of 67%, paired with a high percentage of grade 3 and 4 treatment-related AEs (73%) [102]. In addition, the combination of atezolizumab, vemurafenib, and cobimetinib demonstrated effectiveness, with an ORR of 83% [103]. This combination is currently being tested in the ongoing phase III TRILOGY study (NCT02908672). Another randomized study is testing the combination of dabrafenib, trametinib, and PDR001 (anti-PD-1 antibody) compared with dabrafenib, trametinib and placebo (NCT02967692). The first data of these triple

Table 3 Favorable and unfavorable features of immunotherapy and targeted therapy

Immunotherapy
+ Highest chance of durable response
+ Response can be ongoing even after therapy has been stopped
− Time to response might be longer
− Substantial fraction of patients do not respond
Targeted therapy
+ High objective response rate
+ Fast and deep tumor response
+ Therapy not hampered by consecutive corticosteroids or other immunosuppressive agents
− Majority of patients will develop resistance

combination studies are very promising, although it was only tested in a small group of patients and follow-up is still very short. The high response rates might still only reflect the response of targeted therapy, and it remains to be seen if these responses will be durable.

Our group is currently performing a small phase II study (NCT02625337) testing short-term intermittent BRAF and MEK inhibition combined with pembrolizumab. The rationale for this design lies in the fact that an increase in tumor T-cell infiltration mediated by targeted therapy seems to be transient and can return to below baseline levels after several weeks of treatment [104, 105]. Therefore, boosting the efficacy of checkpoint inhibitors does not necessarily require long-term treatment with targeted therapy. In addition, combining only short-term targeted therapy with immunotherapy might provide a superior toxicity profile, while at the same time reducing the chance of developing resistance to targeted therapy.

Another interesting approach, especially for patients with elevated LDH levels, is induction with short-term targeted therapy prior to the start of checkpoint inhibition. Although LDH is a strong prognostic marker for long-term benefit of targeted therapy, the response rate of targeted therapy is still relatively high in patients with LDH > 2 ULN (51%), albeit substantially lower than in patients with a normal LDH (70%) [90]. By reducing tumor load and, subsequently, LDH levels, short-time induction with targeted therapy may increase the likelihood of responding to immunotherapy. The COWBOY study (NCT02968303) will test this hypothesis by comparing induction therapy with vemurafenib plus cobimetinib, followed by ipilimumab plus nivolumab, to upfront ipilimumab and nivolumab, in patients with elevated baseline LDH levels. Another interesting trial investigating the best sequence strategy, as well as the value of short-term targeted therapy pretreatment, is the three-arm randomized SECOMBIT trial (NCT02631447). This trial will compare first-line ipilimumab plus nivolumab followed by encorafenib plus binimetinib at the time of disease progression, versus first-line encorafenib plus binimetinib followed by ipilimumab

plus nivolumab at the time of disease progression, versus induction therapy for 8 weeks with encorafenib plus binimetinib followed by a combination of ipilimumab and nivolumab. The ImmunoCobiVem trial (NTC02902029) has a similar approach in which patients with $BRAF^{V600}$-mutated melanoma will be randomized, after 3 months of treatment with vemurafenib and cobimetinib, between arm A (further treatment with this combination therapy until disease progression) and arm B (switch to atezolizumab). If patients progress, they can crossover to atezolizumab (arm A) or cross back to vemurafenib and cobimentib (arm B).

5 Cerebral Metastases

Approximately 20% of patients with melanoma have brain metastases at the time of diagnosis of stage IV disease [106]. Furthermore, up to 45% of stage IV patients will develop brain metastases during the course of their disease [107]. Historically, patients with brain metastases have a poor median OS of 4–5 months [107–109]. The large phase II and III registration trials testing targeted therapy and immunotherapy excluded patients with brain metastases from participating in the trials. The BREAK-MB study was the first study that tested BRAFi (dabrafenib) in patients with brain metastases, irrespective of prior local treatment. The intracranial response (ICR) rate in $BRAF^{V600E}$-mutated patients was 31 and 39% in pretreated and non-pretreated patients, respectively. Median PFS of both groups was 17 weeks, and median OS was 31 weeks for pretreated patients compared with 33 weeks for non-pretreated patients [110].

Vemurafenib showed an ICR rate of 18% in patients with either pretreated or non-pretreated brain metastases. Median PFS and OS were comparable with dabrafenib, with a median PFS of 3.6–4.0 months and a median OS of 8.9–9.6 months [111], almost double the OS when compared with historic data. Although these studies showed clinically meaningful OS benefit, median OS remained lower compared with patients without cerebral metastases.

The Combi-MB study revealed that the ICR rate of the combination dabrafenib and trametinib was 58% in patients with non-pretreated, asymptomatic brain metastases, 44% in pretreated, asymptomatic brain metastases, and 59% in patients with symptomatic metastases [112]. These response rates are promising and much higher than what was seen for BRAFi monotherapy. The duration of response, with a median of 4.5–6.5 months, was unfortunately substantially lower than the median of 12.9 months that was seen in patients without brain metastasis [85, 112].

In a phase II study of pembrolizumab in 18 patients with untreated brain metastases, 22% achieved an objective ICR and another two patients had stable disease (SD) [113]. The first results of the CheckMate 038 study demonstrated an ORR of 40% for the combination of ipilimumab plus nivolumab compared with 30% for nivolumab monotherapy in patients with previously untreated brain metastases. In a trial of the ABC (Anti-PD-1 Brain Collaboration) group, patients with asymptomatic brain metastasis (who had not been treated with radiotherapy) were randomized between the combination ipilimumab and nivolumab, and nivolumab monotherapy. The ICR rate was 42% for the combination and 20% for nivolumab monotherapy [114]. A third trial, CheckMate 204, also examined the efficacy of the combination of ipilimumab and nivolumab as first-line treatment in patients with asymptomatic brain metastasis. The ICR rate was 54% and, more interestingly, the median duration of response was not reached after a median follow-up of 9 months [115]. One must be aware that only patients with asymptomatic brain metastases (who did not require high-dose corticosteroids) were included in these studies.

Additionally, in the trial of the ABC group, patients with either symptomatic, locally pretreated, or leptomeningeal brain metastases were treated with nivolumab. In this cohort, the intracranial response rate was very low, being only 6%. A retrospective analysis of patients with brain metastases treated with pembrolizumab revealed that patients who use corticosteroids and/or had symptomatic brain metastases have a significantly lower median PFS and OS [95]. Moreover, a phase II study of ipilimumab demonstrated a disease control rate of 18% at week 12 in patients with asymptomatic brain metastases compared with only 5% for patients who had stable brain metastases but required corticosteroids [116].

These data indicate that targeted therapy and immunotherapy are both effective treatment options for patients with brain metastases. In patients with asymptomatic brain metastases, the combination of ipilimumab plus nivolumab seems to achieve the same response rates as in the periphery, while in patients who need corticosteroids, targeted therapy might be preferable.

6 Non-Cutaneous Melanoma

6.1 Mucosal and Acral Melanoma

The lessons learned from the treatment of cutaneous melanoma do not automatically translate to (metastatic) non-cutaneous melanoma. Since these diseases are commonly diagnosed at a later stage and are associated with a relatively poor prognosis [117], the optimal treatment of non-cutaneous melanoma is of utmost importance.

Based on distinct differences in pathogenesis and prognosis of these diseases, mucosal and acral melanomas cannot be considered interchangeable with cutaneous disease. While these types of melanomas have a relatively low mutational rate [117, 118], structural mutations occur more frequently [118]. On the other hand, BRAF mutations occur at a much lower rate than in cutaneous melanomas [119], and evidence of the clinical benefit of BRAF inhibition is lacking for these tumors.

Randomized trials investigating the efficacy of checkpoint inhibitors have rarely included patients with non-cutaneous melanoma; however, several retrospective studies have investigated the efficacy of these agents. Response to ipilimumab in mostly pretreated patients with predominantly metastatic mucosal disease has been shown to be between 7 and 12%, with a 1-year survival and median survival of approximately 36% and 6.4 months, respectively [120, 121]. Response rates to PD-1 inhibitors of 23 and 32% were seen in patients with mucosal and acral melanoma, respectively [121]; these were also mostly pretreated patients who showed a median survival of 12.4 months (mucosal melanoma) and 31.7 months (acral melanoma) [121]. Although these data are hindered by the retrospective study design and the inclusion of a heterogeneous and relatively small patient population, they do support the use of PD-1 inhibitors as first-line therapy for these patients.

6.2 Uveal Melanoma

Uveal melanomas have been shown to be even more biologically distinct from cutaneous melanoma, with their frequent reliance on GNAQ or GNA11 mutations [122]. The prognosis of uveal melanomas is poor [123] and both conventional and experimental treatments have shown very little effect on the OS of patients with metastatic uveal melanoma [124]. However, since the eye is relatively devoid of immune infiltration, these tumors might be highly immunogenic as they were less subjected to immune editing during tumor progression. However, uveal melanomas have much fewer somatic mutations than sun-exposed cutaneous melanomas [125], and may therefore have

less expression of neoantigens and may be less well-recognized by T cells [16].

As is the case with mucosal and acral melanomas, the evidence for checkpoint inhibition stems mostly from retrospective cases series. In two studies that investigated the efficacy of ipilimumab in patients with metastatic uveal melanoma, the response rate was estimated to be approximately 5% [126, 127]. Additionally, one study identified a subset of patients (23%) who had durable SD in response to ipilimumab [126].

Treatment with PD-1 and PD-L1 inhibitors has shown disappointing results in uveal melanoma. One retrospective study of 58 patients with metastatic disease treated with one of these treatment modalities found an objective response rate of 3% [128], but the origin of uveal melanoma was not confirmed in the responders by *GNAQ/GNA11* mutation analysis. In our analysis of genetically confirmed uveal melanomas, none of the patients had a response to pembrolizumab [129]. Recently, a subgroup analysis of 34 patients with uveal melanoma who progressed on ipilimumab and who were treated with nivolumab in the CheckMate 172 study revealed a response rate of 6% (2/34 patients) [130].

Thus, treatment of these patients outside of clinical trials is not recommended. Promising signals have been observed in an interim analyses of a phase II study testing adoptive T-cell therapy in patients with uveal melanoma, where a promising response rate of 35% was seen [131]. Somewhat less encouraging results were seen for the bispecific biologic IMCgp100. This antibody induces objective responses in only 2 (11%) patients and SD in another 12 (63%) patients, although responses do not seem to be durable as the disease control rate (DCR) of 53% (> 16 weeks CR, partial response [PR], SD) dropped to 32% (> 24 weeks CR, PR, SD) [132].

Targeted agents have also been tested in patients with uveal melanoma. The MEK inhibitor selumetinib was demonstrated to be clinically potent, with an improvement of objective response rate and PFS compared with chemotherapy. This modest efficacy comes at the cost of significant toxicity and did not result in OS benefit over chemotherapy [124]. Preclinical research has shown that the combination of protein kinase C (PKC) inhibitors with mammalian target of rapamycin (MTOR) or murine double minute 2 (MDM2) inhibitors may be promising [133, 134]. Phase I trials with combinations of these targeted agents are ongoing.

7 Future Perspectives

The fact that patients with low LDH, low disease burden, and good performance status have the highest chance of long-term benefit from both targeted therapy and immunotherapy, advocates the start of systemic therapies in an earlier stage of the disease. It was demonstrated that adjuvant high-dose ipilimumab 10 mg/kg improved relapse-free survival (RFS) and OS in stage III melanoma patients, but at the cost of significant toxicity [135]. It was recently shown that adjuvant nivolumab resulted in significantly longer RFS and a lower grade 3/4 toxicity rate compared with adjuvant ipilimumab [136]. Promising results were also seen for adjuvant targeted therapy with dabrafenib and trametinib in patients with BRAF-mutated stage III melanoma. At a median follow-up of 2.8 years, the 3-year RFS rate was 58% in the group treated with the combination versus 39% in the group treated with placebo [137].

Numerous neoadjuvant and adjuvant studies with targeted therapy and (combination) immunotherapy are currently ongoing (NCT02362594, NCT01972347, NCT02231775, NCT02519322, NCT02977052). In our neoadjuvant arm of the OpACIN trial, we observed impressive deep responses in 8 of 10 patients [138]. In the subsequent phase II international OpACIN-neo trial (NCT02977052), we are currently testing different combination schemes of neoadjuvant ipilimumab plus nivolumab to improve tolerability, while preserving the high efficacy.

Although considerable improvements have been achieved with these new effective treatments, a substantial proportion of patients is still not having a durable response to the currently approved treatment combinations. As described above, the combination of targeted agents with checkpoint inhibitors seems to be promising for patients with $BRAF^{V600}$ mutations as targeted therapy may enhance the effect of immunotherapy. For patients with *BRAF* wild-type melanoma, an MEK inhibitor in combination with checkpoint inhibition is perhaps an option. Although the first results showed grade 3–4 AEs in 59% of patients, the combination seems to be effective, with an ORR of 50% and a DCR of 80% in *BRAF* wild-type patients [139]. For patients harboring an *NRAS* mutation, single-agent targeted therapy may also be an option after progression on checkpoint inhibition. In NRAS-mutant melanoma patients, the selective MEK inhibitor binimetinib has shown prolonged PFS (2.8 months) compared with dacarbazine (1.5 months) [140]. Remarkably, the median PFS on treatment with binimetinib seems to be higher in patients pretreated with immunotherapy (median of 5.5 vs. 2.8 months).

Moreover, a plethora of new combinations of immunotherapeutic agents are currently being tested. The combination of epacadostat (an inhibitor of indoleamine-pyrrole 2, 3-dioxygenase [IDO]) and pembrolizumab showed promising clinical activity, with an ORR of 56% and a median PFS of 12.4 months. This combination was

well tolerated, with a grade 3/4 toxicity rate of only 20% [141], and is currently being investigated in a phase III trial (NCT02752074). Phase I/II trials exploring the effect of new checkpoint inhibitors such as anti-LAG3 and anti-TIM3, either alone or in combination with anti-PD-1, are ongoing. The first results of the combination of anti-LAG-3 (relatlimab) and nivolumab showed promising results, with a response rate of 11.5% in patients who progressed on prior anti-PD-1 therapy. The response rate was higher (18%) in patients with an LAG-3 expression of $\geq 1\%$ on immune cells in the tumor. This combination regimen has very good tolerability as only 10% of patients had one or more treatment-related grade 3 or 4 AEs [142].

Furthermore, the clinical activity of co-stimulatory antibodies anti-GITR, anti-CD137, and anti-OX-40 is currently being explored in phase I and II trials, either as single-agent therapy or in combination with anti-PD-(L)1. We need to be aware that when testing so many (promising) combinations, a lot of patients will need to be included in phase III trials. Translational studies exploring biomarkers for patients who will benefit most from these new (triple) combinations are urgently needed. Biomarkers, as described in our cancer immunogram [69], might help to identify populations of melanoma patients in need of triple therapies, which would allow the design of trials with a smaller number of patients.

8 Conclusions

Treatment options for patients with advanced melanoma have improved dramatically over the past decade. Fifteen years ago, the median survival of patients with metastatic melanoma was 7–10 months when treated with chemotherapy [143], and only 5–10% of patients achieved long-term survival. The introduction of targeted therapy and immunotherapy has led to a significant improvement in OS, with 1- and 2-year survival rates of approximately 70 and 50–60%, respectively (Table 2). The chance of a durable response on either of these therapies seems to be highest in patients with a low LDH, low disease burden, and a good performance status. For *BRAF*-mutated patients, the choice of either targeted therapy or immunotherapy as first-line treatment should be based on shared decision making, taking baseline characteristics (e.g. presence of brain metastases [symptomatic vs. asymptomatic], baseline LDH, performance status, and disease burden), AEs, and comorbidities into account. A large array of new combinations and sequences are being tested, which will likely enhance clinical activity, thus necessitating further biomarker exploration for the optimal treatment per individual patient. We need to be aware that more research is needed to improve outcomes for patients

with unfavorable characteristics (such as high LDH levels or poor performance status) or brain metastases, which are both underrepresented in the treated population in clinical trials [106]. Current study survival data might now show an overestimation of real-world patient survival because patients with a poor prognosis are excluded from most of the phase III registration trials and therefore these data cannot easily be extrapolated to the everyday patient population.

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