



REVIEW

Looking into a Better Future: Novel Therapies for Metastatic Melanoma

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ABSTRACT

Even though melanoma represents a small percentage of all cutaneous cancers, it is responsible for most deaths from skin neoplasms. In early stages it can be successfully treated with surgery, but as the disease expands the survival rate drops significantly. For many years the mainstay of treatment for metastatic melanoma was chemotherapeutic agents, even though they failed to prove survival prolongation. After the advent of ipilimumab, a survival benefit and better overall response rate could be offered to the patients. Other new therapies, such as immunotherapies, targeted therapies, vaccines, and small molecules, are currently being studied. Also, combination regimens have demonstrated superiority to some monotherapies. Nowadays, ipilimumab should no longer be considered the first-line therapy given its severe

toxicity and lower efficacy, while nivolumab remains efficacious and has a good safety profile. T-VEC as monotherapy has been shown to be an elegant alternative even for the elderly or cases of head and neck melanomas. If the BRAF mutation status is positive, the combination of dabrafenib and trametinib could be an option to consider. Despite the success of the novel treatments, their effectiveness is still limited. New studies have opened up new avenues for future research in melanoma treatment, which is expected to lead to better therapeutic outcomes for our patients. The objective of this review is to discuss the novel therapies for metastatic melanoma that have been tested in humans during the last 3 years to obtain a sharper perspective of the available treatment options for specific patient characteristics.

Alessia Villani and Massimiliano Scalvenzi are contributed equally to the manuscript.

Keywords: Melanoma; Metastatic melanoma; Targeted therapy; Immune checkpoint inhibitors; Vaccines; Small molecules

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Key Summary Points

Even though melanoma represents a small percentage of all cutaneous cancers, it is responsible for most deaths from skin cancers

The survival rate at 5 years for localized melanoma is 98.3% while for metastatic melanoma (MM) is 16%

In the last decade melanoma clinical research has completely changed the scenario of therapeutic approaches for patients with unresectable advanced melanoma

Multiple targets for drug development have been identified to treat advanced melanoma and are currently undergoing development

DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to <https://doi.org/10.6084/m9.figshare.14317787>.

INTRODUCTION

Cutaneous melanoma is responsible for most deaths from cutaneous neoplasms with an increasing incidence worldwide [1, 2]. Survival rates vary depending on tumor stage at the time of diagnosis, which depends on the depth of the tumor (Breslow) as well as lymph node involvement or distant metastasis [3]. Stage I and II involve localized disease, stage III is characterized by metastasis to the local lymph nodes, and stage IV represents distant metastasis [4].

The survival rate at 5 years for localized melanoma is 98.3% while for metastatic melanoma (MM) is 16% [3]. Surgery remains the first

treatment option for resectable melanoma [2]. For many years the mainstay of treatment for MM was chemotherapeutic agents, even though they failed to prove survival prolongation [3, 5]. Finally, in 2010 the advent of ipilimumab changed the overall response rate (ORR) and offered a survival benefit [5]. After ipilimumab, other new therapies, such as immunotherapies, targeted therapies, vaccines, small molecules, and combination therapies, have changed the prognosis for patients with MM. Although nowadays patients can be offered a wider variety of therapies, many characteristics have to be taken into account, such as the presence of melanoma genome mutations, specific features of the tumor, comorbidities and tolerability of the patient, as well as risks associated with treatment [2].

In this study we discuss the novel therapies for metastatic melanoma that have been tested in humans during the last 3 years to obtain a sharper perspective related to the available treatment options for specific patient characteristics.

METHODS

A literature search on PubMed, Medline, EBSCO, Google Scholar, and the Cochrane Library databases regarding treatment of metastatic melanoma from January 2018 to February 2021 was made. Reviews, meta-analyses, clinical trials (CT), real-life studies (RLS), case reports, and series were reviewed. The most relevant articles were included. A revision of the references was also made to include articles that could have been missed. Assessment of treatment efficacy was made through overall survival (OS), progression-free survival (PFS), recurrence-free survival (RFS), disease-free survival (DFS), durable response rate (DRR), and overall response rate (ORR). A summary of available therapies for MM is shown in Fig. 1. All included studies are shown in Table 1. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

Novel treatment for metastatic melanoma

- Chemotherapy: Temozolomide, dacarbazine
- Immuno-Therapy
 - Anti-PD-1: Nivolumab, pembrolizumab
 - Anti-CTLA-4: Ipilimumab, ticilimumab
 - IFN- α
 - IL-2
- Targeted therapy
 - BRAF: vemurafenib, sorafenib, dabrafenib
 - MEK: trametinib, cobimetinib
- Nanotechnology: Carbon NTs, copper NPs, gold NPs, lipid NPs, nanoemulsion
- Vaccines: T-VEC, CAVATAK (CVA21)
- Other inhibitory checkpoints: LAG3, TIM3, KIR, VISTA
- Targeting inhibitory molecules: CSF1R inhibitors, IDO inhibitors
- T-cell agonists: OX40, 41BB, GITR, CD27, ICOS, TLR
- Cytokines
- Adaptive cellular therapy: TILs and CAR T-cell therapies
- Novel immune agonist therapies: anti CD40, CD137, CD27
- VEGF
- Systemic oncolytic virus: ICOVIR-5
- Combination therapy

Fig. 1 Summary of some of the available therapies for metastatic melanoma

Immune Checkpoint Inhibitors

Programmed Cell Death protein-1 (PD-1) and cytotoxic T-Lymphocyte-associated antigen 4 (CTLA-4) are immune checkpoint molecules that downregulate T cell activation pathways, important in the immune tolerance. PD-1 is expressed on activated T cells, B and NK cells, and monocytes. It binds to its ligand PDL-1 and inhibits the signaling of T cell receptor, preventing T cell activation and the release of proinflammatory cytokines. CTLA-4 is a homolog of CD28 and is expressed on regulatory T cells, inhibiting signals to T cells [6].

Anti-PD1

In 2020, a retrospective, single-center analysis with stage III/IV melanoma patients treated with single-agent anti-PD1 was conducted [7]. Three hundred ninety-six patients with at least 3 months of follow-up after discontinuation of therapy were included in the study. Most of the subjects were treated with pembrolizumab (85.9%) and the rest with nivolumab (14.1%). The median follow-up in patients that survived was 28.9 months, and the median time of treatment was 4.8 months. The most common reasons for treatment discontinuation were disease progression (DP) (49.5%) and toxicity (21.7%). At 5 years after the start of treatment, the treatment failure-free survival was 21.5%.

Five-year OS was 40.9%, and the median OS was 39 months. In 25.8% of the included patients, the oncologist considered a complete response (CR). At 3 years the probability of being alive and of not requiring other therapy was 72.1%. Patients with M1b disease were more likely to have CR compared to other stage IV and stage III combined [7].

Nivolumab In CheckMate 037, a randomized, controlled, open-label phase 3 trial, patients with advanced melanoma (stage IIIC or IV disease) were randomized to receive either intravenous (IV) nivolumab 3 mg/kg every 2 weeks or the investigator's choice of chemotherapy (ICC) (dacarbazine 1000 mg/m² every 3 weeks or carboplatin area under the curve 6 plus paclitaxel 175 mg/m² every 3 weeks) [8]. Patients were treated until progression or unacceptable toxicity, with follow-up of 2 years. In total, 272 patients were assigned to nivolumab and 133 to ICC. More patients treated with nivolumab had brain metastases or increased lactate dehydrogenase levels at baseline. Median OS for nivolumab patients was 15.7 months, compared to 14.4 months in the ICC group. Median PFS was 3.1 months and 3.7 months in the nivolumab and ICC group, respectively. Overall response rate was higher in the nivolumab group (27% versus 10%). Treatment-related AEs were seen in 77% and 82% of patients in the nivolumab and ICC groups. Nivolumab demonstrated a more durable response, but with no difference in survival compared to the ICC [8].

A double-blind, randomized, controlled phase 3 adjuvant trial for patients with resected stage IIIB, IIIC, or IV melanoma was conducted in 2017 (CheckMate 238) [9]. This study provided significant improvement in recurrence-free survival as well as in distant metastasis-free survival in patients treated with nivolumab versus ipilimumab. Later, Ascierto et al. [10] published an updated 4-year report of efficacy, overall survival, and late-emergent safety.

Eligible patients were randomly assigned to receive either nivolumab 3 mg/kg IV every 2 weeks or ipilimumab 10 mg/kg IV every 3 weeks for 4 doses and then every 12 weeks, for up to 1 years or until DR, toxicity, or withdrawal

Table 1 Summary of adjuvant trials for patients with cutaneous melanoma

Author	Study design	Disease stage	Treatment	Number participants	Median follow-up (months)	OS/ORR	PFS/RFS/DFS
Warner et al. 2020 [7]	Retrospective, single center	III/IV	Pembrolizumab or nivolumab	396 (85.9% pembrolizumab, 14.1% nivolumab)	28.9	5 years OS: 40.9%, median OS: 39 months	NA
Larkin et al. 2018 [8]	Randomized, open-labeled phase 3 CT (CheckMate 037)	IIIC or IV	Nivolumab or ICC (dacarbazine or carboplatin + paclitaxel)	405 (272 nivolumab and 133 ICC)	24	Median OS nivolumab: 15.7, ICC: 14.4 months	Median PFS nivolumab: 3.1, ICC: 3.7 months
Ascierto et al. 2020 [10]	Double-blind, phase 3 CT Updated 4-year report of Checkmate 238 (Weber et al. 2017 [9])	IIIB, IIIC or IV	Nivolumab or ipilimumab	906 (453 nivolumab and 453 ipilimumab)	51.1 nivolumab and 50.9 ipilimumab	OS at 4 years nivolumab: 77.9%, ipilimumab: 76.6%	Median RFS nivolumab: 52.4, ipilimumab: 24.1 months ($p = 0.0003$). RFS at 4 years nivolumab: 51.7%, ipilimumab 41.2%
Hamid et al. 2019 [11]	Open-label, phase 1b CT 5-year outcome of KEYNOTE-001 (Kang et al. 2017) [12]	Advanced solid tumors (melanoma and non-small cell lung cancer)	Pembrolizumab	655 (496 previously treated, 151 treatment-naive)	55	5-year OS rate treatment-naive: 41%, all patients: 34%. Median OS treatment-naive: 38.6, all patients: 23.8 months. ORR treatment-naive: 52%, all patients: 41%	NA
Eggermont et al. 2018 [13]	Double-blind, phase 3 CT	III	Pembrolizumab	1019	15.5	NA	12-month rate RFS pembrolizumab: 75.4%, placebo: 61% ($p < 0.001$)
Chanal et al. 2019 [14]	Retrospective, single-center	NA	Pembrolizumab	9 patients over 85 years old	NA	OS for stage IV: 8 months	NA
Lang et al. 2018 [16]	Real-life study	Stage IIIC or IV	Ipilimumab or vemurafenib	80 (40 ipilimumab and 40 vemurafenib)	8 vemurafenib, 10 ipilimumab	Median OS vemurafenib: 8, ipilimumab: 10 months. Survival rate beyond 12 months ipilimumab: 42.5%, vemurafenib: 38%	NA
Drysdale et al. 2019 [15]	Population based retrospective study	NA	Ipilimumab or dacarbazine	464 (298 ipilimumab and 175 dacarbazine)	5.2 dacarbazine, 7 ipilimumab	Median OS dacarbazine: 5.2, ipilimumab: 9.5 months. OS dacarbazine: 1-year 18.9%, 2-year OS of 7.4%, and 3-year OS 4%. Ipilimumab: 1-year 46.5%, 2-year OS 29.8%, and 3-year OS 24.4%	NA

Table 1 continued

Author	Study design	Disease stage	Treatment	Number participants	Median follow-up (months)	OS/ORR	PFS/RFS/DFS
Maio et al. 2018 [17]	Double-blind, placebo-controlled, phase 3 CT	IIC-IIIc	Vemurafenib or placebo	Cohort 2: 184 (93 vemurafenib, 91 placebo); Cohort 1: 314 (157 vemurafenib, 157 placebo)	Cohort 2: 33.5; cohort 1: 30.8	NA	One-year DFS 78.9% and 84.3% in the vemurafenib group versus 58% and 66.2% in placebo group, in cohort 2 and 1, respectively
Perez et al. 2018 [18]	Retrospective	Stage III–IV	T-VEC	27	8.6	ORR of 56.5%	NA
Andtbacka et al. 2019 [20]	Randomized, phase 3 CT (OPTiM)	IIIB-IVM1c	T-VEC or GM-CSF	436 (295T-VEC, 141 GM-CSF)	49	ORR T-VEC: 31.5%, GM-CSF: 6.4%; Median OS T-VEC: 23.3, GM-CSF: 18.9 months	NA
Mohr et al. 2019 [21]	Multicenter, observational, retrospective	IIIB-IVM1a	T-VEC	27	1	NA	NA
Franke et al. 2019 [22]	Real-life study	IIIB/C-IVM1a	T-VEC	26	12.5	ORR 88.5%	NA
Zhou et al. 2019 [23]	Real-life study	NA	T-VEC	40	14	Median OS was not reached	Median PFS: 10.5 months
Louie et al. 2019 [24]	Multicenter, retrospective	IIIB-IV	T-VEC	80	9	NA	Median DFS: 13 months
García et al. 2019 [25]	Accelerated escalating phase I	Uveal and cutaneous melanoma	ICOVIR-5	12 (6 uveal and 6 cutaneous melanoma)	NA	NA	NA
Long et al. 2019 [30]	Randomized phase III double-blind study	III–IV	Epacadostat + pembrolizumab vs. placebo + pembrolizumab	706 (354 epacadostat + pembrolizumab; 352 placebo + pembrolizumab)	12.4	NA	Median PFS: 4.7 epacadostat + pembrolizumab; 4.2 pembrolizumab + placebo
Glisson et al. 2020 [31]	First-in-human study	Solid tumors (including melanoma)	MEDI0562	55 patients	NA	NA	NA
Weide et al. 2019 [37]	Randomized phase II trial	IV	Dacarbazine vs. dacarbazine + L191L2(day 1, 3, 5) vs. dacarbazine + L191L2(day 1, 8, 15)	69 (24 dacarbazine, 23 dacarbazine + L191L2 (day 1, 3, 5), 22 dacarbazine + L191L2(day 1, 8, 15))	NA	ORR: 4.5% dacarbazine vs. 18.4% cumulatively in the other 2 groups	–

Table 1 continued

Author	Study design	Disease stage	Treatment	Number participants	Median follow-up (months)	OS/ORR	PFS/RFS/DFS
Khushalani et al. 2020 [38] (Study design)	Randomized phase III open-label study	IV	Bempegaldesleukin plus nivolumab vs. nivolumab	-	-	-	-
Miura et al. 2019 [39]	Ongoing Phase III open-label trial	IIIB/C	Darumun as neoadjuvant treatment	-	-	-	-
Yan et al. 2021 [40]	Randomized phase II trial	IV	Carboplatin + paclitaxel vs. carboplatin + paclitaxel + bevacizumab	114 patients	-	ORR: 19.7% carboplatin + paclitaxel + bevacizumab vs. 13.2% carboplatin + paclitaxel	OS: 13.6 carboplatin + paclitaxel + bevacizumab vs. 9 carboplatin + paclitaxel PFS: 4.8 carboplatin + paclitaxel + bevacizumab vs. 3.0 carboplatin + paclitaxel

OS overall survival, ORR overall response rate, PFS progression-free survival, RFS recurrence free survival, DFS disease free survival, NA not available, CT controlled trial, ICC investigator's choice of chemotherapy, T-VEC Talimogene laherparepvec

of consent [10]. In total, 906 patients were randomized to nivolumab (453) or ipilimumab (453). Median follow-up was 51.1 months for nivolumab and 50.9 months for ipilimumab. All patients had completed or discontinued treatment by the 18-month analysis. After the database lock 316 patients in the nivolumab group and 298 patients in the ipilimumab group continued in the study. At a minimum of 4 years of follow-up, 212 recurrence events were found in the nivolumab group and 253 in the ipilimumab group. Median RFS in the patients treated with nivolumab was 52.4 months and 24.1 months for the ipilimumab group ($p = 0.0003$). RFS at 4 years was 51.7% and 41.2% in the nivolumab and ipilimumab group, respectively, and an absolute risk difference of 10.5% was found. The OS at 4 years was 77.9% and 76.6% with nivolumab and ipilimumab. Late-emergent treatment-related adverse effects (AEs) were found in 4% of the nivolumab and 6% of the ipilimumab group, while grade 3–4 AEs were reported in 1% of the nivolumab and 2% of the ipilimumab group. No patients in the nivolumab group died because of toxicity, while two patients in the ipilimumab group died because of marrow aplasia. Nivolumab seems to have a more tolerable safety profile than ipilimumab, with good efficacy [10].

Pembrolizumab In 2019 Hamid et al. [11] reported the 5-year outcomes of the KEYNOTE-001 study [12], an open-label, phase 1b CT that included patients with advanced solid tumors, such as melanoma and non-small cell lung cancer [12]. Six hundred fifty-five patients with a diagnosis of advanced or metastatic melanoma were included; 496 were previously treated and 151 were treatment naïve. They received pembrolizumab 2 mg/kg every 3 weeks, 10 mg/kg every 3 weeks, or 10 mg/kg every 2 weeks until progression of disease, toxicity, or patient or investigator decision to retract. Median follow-up was 55 months and median duration of exposure to pembrolizumab was 5.6 months (1 day to 67 months). At time of data cut-off, 33 patients (5%) were still on treatment; 569 patients (87%) discontinued treatment, 275 (42%) because of DP, 166 (25%) adverse events, 80 (12%) physician choice, 36

(5%) patient withdrawal, and 8 (1%) violation of protocol, and 4 were lost to follow-up.

At data cutoff, 81 patients (54%) of treatment-naïve patients and 412 (63%) of all patients had died. The 5-year OS rate was 41% in treatment-naïve patients and 34% in all patients. Median OS was 38.6 months in treatment-naïve and 23.8 months in all patients. Among all treated patients ($n = 655$), 104 (16%) achieved CR, and 163 (25%) partial response (PR); 156 (24%) had stable disease (SD), and the disease control rate (DCR) was 65%. Median time to response was 2.8 (0.5–49.6) months. AEs related to treatment occurred in 562 patients (86%). Sixty-five (10%) discontinued therapy because of treatment-related AEs, 114 (17%) experienced grade 3–4 AEs, and none had treatment-related death. This study represents the longest pembrolizumab follow-up in patients with MM [11].

A phase 3 double-blind trial evaluated pembrolizumab as adjuvant treatment in patients with high-risk stage III melanoma [13]. A total of 1019 patients were randomized to receive either pembrolizumab 200 mg IV ($n = 514$) or placebo ($n = 505$) every 3 weeks for a total of 18 doses (approximately 1 year). Of the patients treated with pembrolizumab, 13.8% (70) discontinued treatment for AEs and of the placebo group 13% (66). As for discontinuation due to disease recurrence, 109 patients (21.4%) in the pembrolizumab and 179 (35.7%) in the placebo group discontinued the regimen. Overall median duration of follow-up was 15.1 months. The 12-month rate of RFS was 75.4% and 61% in the pembrolizumab and placebo group, respectively. A significant difference was found in the RFS between pembrolizumab and placebo ($p < 0.001$). In total, 351 patients had a first recurrence of disease or died: 135 for the pembrolizumab and 216 for the placebo group. AEs occurred in 396 patients (77.8%) in the pembrolizumab and 332 patients (66.1%) in the placebo group. AEs of grade 3–5 occurred in 14.7% vs. 3.4% in the pembrolizumab and placebo group. One pembrolizumab-related death due to myositis occurred [13].

A monocentric retrospective case series included nine patients with MM over 85 years old [14]. All patients were treated with

pembrolizumab IV (2 mg/kg every 3 weeks). One patient had been previously treated with dacarbazine; the rest received pembrolizumab as first-line treatment. Mean number of infusions was 4; however, the five patients with stage IV disease were able to receive only three or fewer infusions. Seven patients had treatment-related AEs of grade 3 or 4. One patient died suddenly at home 4 days after infusion so a fatal AE could not be ruled out. The results in patients that received at least two infusions (6 patients) were one CR, two PRs, and 3 DPs. Two patients received just one infusion; one died 1.5 months from DP, and the other had partial regression. At the end of the study, 8 patients had died. The OS was 8 months for stage IV patients. Pembrolizumab treatment in patients older than 85 years, even when a reduced number of infusions are delivered, may induce a response, but it may also be associated with a higher risk of toxicity [14].

Anti-CTLA-4

Ipilimumab Ipilimumab is an anti-CTLA-4 monoclonal antibody; it was the first checkpoint inhibitor for treating metastatic and unresectable melanoma that showed efficacy [15]. Lang et al. [16] published a real-life study in which they included 40 patients treated with vemurafenib 960 mg twice daily (BID) and 40 patients treated with ipilimumab IV 3 mg/kg body weight every 3 weeks for four infusions. Patients in the vemurafenib group had BRAF^{V600E} mutation, while 87.5% of the ipilimumab group were wild type. Both groups presented with metastasis in the lymph nodes, lung, liver, and skin; 90% of the patients were in stage M1c before beginning the therapy. Vemurafenib was applied as first-line therapy in most of the patients (85%), while ipilimumab was second line in most patients (85%). Mean duration of treatment for vemurafenib was 11.4 months; 32.5% required a reduction of the dose, and 12.5% had an early termination due to adverse events (AEs). Of the patients treated with ipilimumab 47.5% received the four infusions; 32.5% had a reduced number of infusions due to side effects and 20% due to massive tumor progression. Median OS in the vemurafenib group was 8 months (± 1.25 ; 95% CI:

5.55–10.45) and 10 months (\pm 3.16; 95% CI: 3.81–16.19) in the ipilimumab group. No significant difference between groups was found ($p = 0.689$). Survival rate beyond 12 months was 42.5% in the ipilimumab group and 38% in the vemurafenib group, and after 24 months 35.5 and 25.5%, respectively. Among the patients treated with vemurafenib, the survival without brain metastasis was significantly longer compared to those with brain metastasis (log-rank p -value = 0.013). Long-term survival (\geq 24 months) was reached by 32.5% of patients (57.7% ipilimumab and 42.3% vemurafenib) [16].

A population-based study of patients treated for metastatic or unresectable melanoma with ipilimumab or dacarbazine was published in 2019 [15]. The control group included 175 patients treated with dacarbazine and 289 patients treated with ipilimumab. Among the control group, 41 patients received dacarbazine combined with a platinum agent and/or cytotoxic agents. Median follow-up was 5.2 months for dacarbazine and 7 months for the ipilimumab group. Median survival was 5.2 months for dacarbazine and 9.5 months for ipilimumab. One-year OS was 18.9%, 2-year OS 7.4%, and 3-year OS 4% for dacarbazine, while for ipilimumab they were 46.5%, 29.8%, and 24.4%, respectively. The improved OS was greater in patients treated with ipilimumab who were younger than 65 years old, male sex, and with no comorbidities [15].

Targeted Therapy

BRAF

Approximately half of cutaneous melanomas have an activating mutation in the BRAF oncogene, which leads to a constitutive activation of the mitogen-activated protein kinase (MAPK) signaling pathway. Several drugs that target BRAF mutant cells have been proposed as treatment for metastatic melanomas, including vemurafenib and dabrafenib [1].

Vemurafenib Maio et al. [17] performed a phase 3, double-blind, randomized, placebo-controlled study with BRAF^{V600} mutation-

positive melanoma stage IIC to IIIC patients (BRIM8). Subjects were randomly assigned to receive either vemurafenib 960 mg BID or matching placebo for 52 weeks (13×28 -day cycles). Patients with stage IIC-IIIB were included in cohort 1 and patients with stage IIIC in cohort 2. In total, 184 patients (93 vemurafenib and 91 placebo) were enrolled in cohort 2 and 314 patients (157 vemurafenib and 157 placebo) in cohort 1. At the time of data cutoff, median follow-up was 33.5 months in cohort 2 and 30.8 months in cohort 1. In cohort 2, 30% of the vemurafenib group and 34% of the placebo discontinued the study. In cohort 1, 23% of the vemurafenib group and 26% of the placebo withdrew [17]. In cohort 2, median disease-free survival (DFS) was 23.1 months in the vemurafenib group and 15.4 months in the placebo group. In cohort 1, median DFS was not reached in the vemurafenib group. One-year DFS was 78.9% and 84.3% in the vemurafenib group versus 58% and 66.2% in the placebo group in cohort 2 and 1, respectively. At data cutoff, 83 deaths had occurred in the overall population. Thirty-five deaths occurred in the vemurafenib group (16 in cohort 1 and 19 in cohort 2) and 47 in the placebo group (28 in cohort 1 and 19 in cohort 2). Regarding the safety profile, the most frequently reported AEs were skin-related events (rash and alopecia), arthralgia, nausea, and fatigue. Grade 3 or 4 events were found in 141 patients (57%) and 37 (15%) of the vemurafenib and control group, respectively. Serious AEs occurred in 40 patients (16%) and 25 patients (10%) of the vemurafenib and control group, the most common being basal cell carcinoma. One fatal event, which was not considered drug related, was reported in the vemurafenib group [17].

Vaccines

Talimogene Laherparepvec (T-VEC)

Talimogene laherparepvec (T-VEC) is a modified herpes simplex virus 1 (HSV-1). It recruits antigen-presenting cells and expresses granulocyte macrophage-colony-stimulating factor (GM-CSF) [18]. This virus infects and replicates inside tumor cells, causing lysis of the infected

tumoral cells. T-VEC is administered intraleitionally in cutaneous, subcutaneous, and nodal lesions in stage IIIB, IIIC, and IVM1a melanomas after initial surgery [19].

A retrospective study including 27 patients with melanoma, 81.5% with stage III disease, and 18.5% with stage IV was made in 2018 [18]. Thirteen patients were previously treated with regional or systemic therapies. Patients were injected with an initial dose of 10^6 plaque-forming units (PFU) per milliliter; 3 weeks later they received a second injection at a dose of 10^8 PFU per milliliter, which was continuously repeated every 2 weeks until DP, CR, or intolerance to therapy. Median follow-up was 8.6 months (2.8–20.5). Three patients did not complete the 8-week follow-up and were excluded from analysis; 43.5% experienced CR and 13% PR, and 21.7% had stable disease (SD). An overall response rate of 56.5% was found and a disease control rate of 78.3%. Five patients presented DP. Median time to response was 2.1 months. A significant association between disease stage and treatment response was found ($p = 0.041$), with 100%, 39%, and 50% of patients responding to treatment with stage IIIB, IIIC, and IV, respectively. Most AEs were limited to mild constitutional symptoms within 48 h of injection [18].

In OPTiM, a randomized phase III CT for patients with unresectable stage IIIB-IVM1c melanoma, participants were randomized 2:1 to receive intratumoral T-VEC or subcutaneous recombinant GM-CSF [20]. Of the 436 included patients, 295 were treated with T-VEC and 141 with GM-CSF. Treatment was given for 6 months. After this time, therapy was continued until DP, consent withdrawal, intolerance, lack of response, CR, or lack of response by 12 months. Median duration of treatment was 10 weeks in the GM-CSF and 23.1 weeks in the T-VEC group. Median follow-up was 49 months. DRR was higher in the population treated with T-VEC vs. GM-CSF (19.3% vs. 1.4%). ORR was also higher in the T-VEC arm (31.5% vs. 6.4%). Fifty (16.9%) and 1 patient in the T-VEC and GM-CSF group, respectively, achieved a CR; 43 (14.6%) and 8 (5.7%) achieved a PR. Median time to CR in the arm treated with T-VEC was 8.6 months. CR achievement with T-VEC was

significantly associated with a baseline tumor burden of $< 14.5\text{cm}^2$ and an early melanoma stage. Median OS with T-VEC was 23.3 months and 18.9 months with GM-CSF. Most common AEs were chills, pyrexia, fatigue, influenza-like symptoms, and nausea. Grade 3/4 treatment-related AEs were found in 33 patients (11.3%) in the T-VEC patients. The only AE that occurred in more than two patients was cellulitis [20].

A multicenter, observational, retrospective review that included patients with stage IIIB-IVM1a melanoma treated with T-VEC was made in Germany [21]. Twenty-seven patients were included; 56% had stage IIIB/C and the remaining 44% stage IVM1a. All the included patients had a history of prior surgery, and more than half had a sentinel lymph node biopsy (SLNB) performed. Also, about half of the patients received a prior local treatment before T-VEC (radiation, electrochemotherapy, IL-2 or interferon-alfa, or local ablation). At the end of the study, 13 patients had ongoing and 14 had discontinued therapy. Three patients had no remaining injectable lesions and seven PD; three discontinued because of patients' decisions and one because of side effects (nausea). The median duration of treatment was 22.1 weeks, and the median duration of follow-up was 30.6 weeks [21]. Another real-life study in patients with stage IIIB/C-IVM1a melanoma treated with T-VEC was made by Franke et al. [22]. Twenty-six patients were included, with a median follow-up time of 12.5 months. Best ORR was 88.5%, CR was 61.5%, and DCR 92.3%. Eight patients (30.8%) had PD. Two patients were re-treated with T-VEC because of locoregional recurrence after a prior CR; both reached a CR again. AEs occurred in 100% of patients, but serious AEs only occurred in one patient (pancolitis). This study had a higher ORR than most clinical trials, which could be explained by the fact that no stage IV patients were treated [22]. Later, a real-life study by Zhou et al. [23] that included 40 patients with MM treated with T-VEC was made. Of the included patients, 85% had \leq stage IV M1a and 15% had stage \geq IV M1b-d; 47.5% had any tumor ≥ 2 cm. Eighty percent of the patients had received prior therapies (35% conventional or targeted therapy and 45% immune checkpoint inhibitors). As

results, 17 patients (42.5%) had CR and 2 (5%) PR, and the remaining patients had either DP or were non-responders. Median follow-up was 14 months (1.9–26.7 months). Median PFS was 10.5 months. Patients with poorer performance status defined by the Eastern Cooperative Oncology Group (ECOG) had significantly decreased OS ($p = 0.0049$) and PFS ($p = 0.03$). Also, larger tumors (≥ 2 cm) had significantly decreased OS ($p = 0.046$), PFS ($p = 0.0085$), and DRR ($p = 0.029$). Age ≥ 75 years did not affect OS compared to patients aged < 75 years. AEs were mild (fevers and fatigue), with three patients (7.5%) discontinuing treatment because of AEs. No treatment-related deaths were seen [23]. The largest published series on the efficacy and safety of T-VEC in locoregional advanced melanoma patients included 80 patients with a median follow-up time of 9 months [24]. Twenty-one patients (36%) had head and neck as the primary site of disease. Most patients underwent systemic or regional therapy before receiving T-VEC (57.5%). Six patients did not have a previous resection. Thirty-six patients (46%) had clinical stage IIIB disease, 25 had stage IIIC (31%), 1 had clinical stage IIID, and 16 (20%) had distant metastasis at the time of treatment. The included patients received a median of five cycles of T-VEC. Twenty-nine patients (37%) had CR, 6 (8%) PR, 15 patients (19%) SD, and 25 (30%) DP. Fifty-eight percent of patients did not have any AE, 28% had constitutional, flu-like symptoms, and 6% had an early termination of treatment because of AEs (1 cold sore, 4 infections). Patients with stage IIIB were more likely to have CR (68%) compared to stage IIIC, IIID, and IV (26%, 0%, and 6% respectively). As the authors showed, T-VEC can be used in locally advanced head and neck melanomas with a good response [24].

Intravenous Oncolytic Virus

ICOVIR-5

The first-in-human phase I trial of IV administration of ICOVIR-5 that included patients with uveal and cutaneous metastatic malignant melanoma was conducted by García et al. [25].

ICOVIR-5 is derived from an adenovirus type 5 that is responsive to the pRB pathway, a pathway that is deregulated in many tumors. This was an accelerated escalating phase I design with a cohort of 1 patient in case no more than grade 2 toxicity was found and later with a cohort of 3–6 patients after the first grade 3 toxicity was found. A dose-limiting-toxicity (DLT) observation period of 4 weeks was established before starting the next dose level for the first patient [25].

Thirteen patients were included in the study: six had uveal and six cutaneous melanomas. Six patients (50%) had received at least one regimen of chemotherapy, and four (33%) had received previous immunotherapy. No partial or complete responses were observed. At the lower dose level two patients had SD, while at the highest level five out of six patients presented SD. A longer survival of uveal melanoma patients compared to cutaneous melanoma was found. Acute toxicity involved flu-like syndrome with chills, fever, headache, myalgias, nausea, vomiting, and diarrhea 4–6 h after infusion for 2–4 days. This phase I study shows IV administration of adenovirus ICOVIR-5 is well tolerated [25].

Targeting Inhibitory Molecules: CSF1Ri and IDOi

CSF1R Inhibitors

Immune modulation of macrophages has been found to represent a novel approach to anti-cancer therapy. Colony-stimulating factor 1 receptor (CSF1R) is a transmembrane protein receptor for colony stimulating factor 1, altering macrophage production and function, which is normally overexpressed on tumor-infiltrating macrophages [26]. Binding of CSF1 promotes the activation of the CSF1R-mediated pathway, thus inducing differentiation and macrophage survival, which are linked to worse survival outcomes. According to this principle, new molecules targeting CSF1 (pexidartinib, PLX7486, ARRY-382, JNJ-40346527, BLZ945, emactuzumab, AMG820, IMC-CS4) have been developed and entered early phase clinical trials

for the treatment of solid tumors, including melanoma [27].

IDO Inhibitors

Indoleamine 2,3-dioxygenase 1 (IDO1), which regulates tryptophan catabolism, plays a pivotal role in the cancer immune surveillance mechanism. It is associated with the progression of many types of tumors, including melanoma [28]. However, there are few data regarding IDO modulation of tumor cells and the effect of BRAF inhibitor (BRAFi) treatment and resistance. Although several clinical trials using IDO inhibitor as monotherapy have already been conducted, not reporting promising results, combinations of the IDOi epacadostat with pembrolizumab or nivolumab showed response rates in untreated melanoma cohorts of approximately 60% [29]. In 2019, Long et al. [30] conducted a randomized, placebo-controlled, double-blind phase 3 trial of pembrolizumab with or without epacadostat for patients with stage III or IV melanoma (ECHO-301/KEYNOTE-252). The combination treatment did not improve PFS or OS compared with placebo plus pembrolizumab, and the trial was terminated prematurely.

T Cell Agonists

T cell agonists have been identified as new targets for anti-cancer therapy, acting through the upregulation of cytotoxic T cell activity and having a key role in the activation of regulatory T cells. These targets, including OX40, GITR, 41BB, and CD27, are co-stimulatory receptors expressed on CD4 and CD8 cells that act by activating nuclear factor kappa B and c-Jun-N-terminal kinase signaling cascade signaling transcription factors thus facilitating CD4 and CD8 T cell differentiation.

OX40

The antitumor activity of OX40 antibody is associated with the infiltration of T cells and the proliferation of effector T cells at tumor sites. Currently, there are several clinical trials evaluating the role of anti-OX40 agents in patients with melanoma and other solid tumors. The

phase I study evaluating PF-04518600, a novel fully human IgG2 agonistic mAb against human OX40 in patients with advanced solid tumors, led to anti-tumor responses and was well tolerated [31]. Correspondingly, the most recent first-in-human study evaluating the agonistic antibody MEDI0562 also showed good tolerability with the majority of AEs being of grade 1 or 2 [31].

GITR

Glucocorticoid-induced tumor necrosis factor receptor family-related protein (GITR) is expressed in both effector and regulatory T cells. Several trials have studied the efficacy and safety profile of anti-GITR monoclonal antibodies in patients with advanced melanoma. In 2019, a phase I clinical trial evaluating the fully humanized GITR monoclonal antibody TRX518 (NCT01239134) reported no substantial clinical responses with tumor progression observed in 29 patients during treatment. Similar results were also seen in another phase I study that evaluated the GITR monoclonal antibody (mAb) AMG228. To date, other trials are currently assessing the efficacy of the mAb INGAGN1876, and results are not yet available [32].

4-1BB

4-1BB, also known as CD137 and TNFSFR9, is a stimulatory coreceptor expressed on T, NK, and dendritic cells; binding to its corresponding ligand, it causes proliferation and survival of effector T cells and cytokine release. Urelumab (BMS-663513, clone 10C7) and utomilumab (PF-05082566) are two CD137/4-1BB agonistic mAbs under clinical evaluation. In a phase I trial of utomilumab including melanoma and non-melanoma patients, clinical objective responses in tumor regression were reported. Nevertheless, treatment-related adverse events, especially immune-related toxicities, were also reported; further preclinical studies demonstrated utomilumab to be better tolerated than urelumab [33, 34]. Currently, there are ongoing studies of CD137 agonists for the treatment in patients with late-stage melanoma.

CD27

CD27 (also known as TNFRSF7) is mainly expressed on thymocytes, naive T cells, memory T cells, and a subset of NK cells. CD27 engagement with its ligand CD70 delivers a co-stimulatory signal to further promote the differentiation and expansion of T cells and their activation. In a phase I trial evaluating the use of varilumab, a novel agonist anti-CD27 mAb, in melanoma patients, modest clinical responses were achieved [35]. Further investigations in larger cohorts of patients are still needed.

TLR

Toll-like receptors (TLRs) are mammalian homologs of *Drosophila* Toll protein, and ten have been identified in humans. TLRs of the cells of the innate immune system recognize pathogen-associated molecular patterns and activate the immune system through the activation of nuclear factor- κ B and interferon regulatory factor signaling pathways. In the last decade TLR agonists have been studied for their anti-tumoral activity. In 2018, a phase I trial demonstrated evidence of efficacy in patients with PD-1 refractory metastatic melanoma. Three patients were treated with IMO-2125 (tilsotolimod), a TLR9 agonist, with one patient reporting complete remission and two reporting partial remission of their tumor [36].

Adoptive T Cell Therapy

The use of selected in vitro tumor-infiltrating lymphocytes (TIL), recognizing melanoma antigens, has also been studied to treat highly selected patients with unresectable stage IV melanoma. Initially, this treatment showed promising results with ORR ranging between 38 and 55%, with significant durable responses. Nevertheless, there are several limitations regarding this treatment, such as being expensive and time consuming because of the selection of specific T cells acting against melanoma and the attrition rate noted during TIL generation as a result of tumor progression. The combination of checkpoint inhibition with TIL to

better control tumor progression during adoptive T cell transfer is under investigation.

Cytokines

L19IL2 (Darleukin)

Engineered cytokine products represent promising agents for the treatment of immunogenic tumors, such as malignant melanoma, in addition to immune checkpoint inhibitors. L19IL2 is a fully human fusion protein consisting of the L19 antibody specific to the alternatively spliced extra-domain B of fibronectin fused to human interleukin-2 [38]. A controlled, randomized phase II clinical trial assessed its potential in treating advanced metastatic melanoma. A group of patients enrolled received dacarbazine as a single agent, while two other groups received L19IL2 in two different schedules. Sixty-nine patients with stage IV melanoma were enrolled (24 in the dacarbazine arm, 23 and 22 in the other combination arms, respectively), and statistically significant benefit in terms of ORR was reported in patients receiving L19IL2 in combination with dacarbazine compared to dacarbazine as single agent [39].

Bempegaldesleukin (BEMPEG; NKTR-214)

Bempegaldesleukin is a first-in-class CD122-preferential IL-2 pathway agonist, designed to act through the IL-2 $\beta\gamma$ receptor and to selectively stimulate an antitumor response. A recent phase II randomized trial PIVOT-02 trial evaluating the combination treatment of bempegaldesleukin plus nivolumab as first-line therapy in metastatic melanoma showed positive results in term of efficacy and tolerability. A phase III, global randomized trial (PIVOT IO 001) comparing bempegaldesleukin plus nivolumab with nivolumab alone in patients with previously untreated, unresectable or metastatic melanoma is still going on, and the results have not been published yet [40].

Daromun Daromun is an intralesional immunocytokine obtained from the fusion of recombinant human proteins (cytokines IL-2 and TNF- α) to the monoclonal L19 antibody.

Although early clinical studies have already demonstrated its efficacy in local tumor destruction and in treating metastatic disease, the phase III Neo-DREAM study, is currently investigating the efficacy of neoadjuvant Daromun for resectable stage IIIB/c melanoma [41].

Bevacizumab

Systemic anti-vascular endothelial growth factor (VEGF) bevacizumab (Avastin, Genentech, San Francisco, CA, USA) has been studied in combination with other therapeutic agents for treating metastatic melanoma. An open-label randomized phase II trial characterized the efficacy and safety of bevacizumab in combination with carboplatin plus paclitaxel (CPB) in 114 patients with advanced melanoma. Results were significantly better in patients receiving bevacizumab in addition to CPB than in those who received CPB alone [42]. A phase III study should be performed to confirm these results (ClinicalTrials.gov identifier: NCT02023710).

Combination Treatment

Acquired resistance to both targeted therapy and immunotherapy has been frequently described, and, to avoid this problem, different combination therapies have been the objective of several studies. Numerous combinations including pairs and triplets of drugs with the highest clinical efficacy and minimum adverse events have been tested in unresponsive patients with advanced melanoma, especially for patients with BRAF V600e mutant disease, which constitutes 50% of advanced melanoma. Below, completed phase III trials of FDA-approved combination therapies in advanced melanoma will be reported.

Immunotherapy Combination

The immunotherapy combination of nivolumab (a programmed death [PD]-1 inhibitor) plus ipilimumab (a cytotoxic T-lymphocyte antigen-4 [CTLA-4] inhibitor) has been FDA-approved as first-line treatment for patients with BRAF-mutant melanoma. In the phase III CheckMate 067 study (NCT01844505),

involving patients with stage III/IV melanoma, a group was treated with ipilimumab plus nivolumab (4 doses at 3 mg/kg + 3 mg/kg every 2 weeks) and the other group with ipilimumab alone [43]. At 5-year follow-up the group of patients treated with the combination therapy reported an overall survival of 60% [8].

Targeted Therapy Combination

The introduction of BRAF inhibitors for patients with BRAF V600-mutated metastatic melanoma has enlarged the armamentarium of treatment for clinicians; however, resistance to BRAF inhibitors has been described. Combining BRAF and MEK inhibition has represented a new strategy for better inhibition of the MAPK pathway compared to BRAF inhibition alone. The association of cobimetinib (a MEK inhibitor) and vemurafenib (a BRAF inhibitor) has been FDA approved as standard treatment for patients with BRAF^{V600}-mutated melanoma. Clinical data pooled from the BRIM-2, BRIM-3, BRIM-7, and coBRIM studies, involving patients treated with vemurafenib or cobimetinib plus vemurafenib, have demonstrated the combination therapy to be more effective in terms of tumor reduction and progression-free survival, supporting the combination BRAF and MEK inhibition as a standard of care in this disease setting [44]. Moreover, the association of the BRAF inhibitor dabrafenib (at a dose of 150 mg twice daily) plus the MEK inhibitor trametinib (2 mg once daily) studied in the COMBI-d and COMBI-v trials also showed excellent results leading to long-term benefit in approximately one third of the patients who had unresectable or metastatic melanoma with BRAF V600E or V600K mutation. In particular, a total of 563 patients were randomly assigned to receive dabrafenib plus trametinib (211 in the COMBI-d trial and 352 in the COMBI-v trial), reporting PFS rates of 21% (95% confidence interval [CI], 17–24) at 4 years and 19% (95% CI, 15–22) at 5 years. The OS rates were 37% (95% CI, 33–42) at 4 years and 34% (95% CI, 30–38) at 5 years. A complete response occurred in 109 patients (19%) and was associated with an improved long-term outcome, with an OS rate of 71% (95% CI, 62–79) at 5 years [45]. Another FDA-approved BRAF/MEK inhibitor

combination is encorafenib and binimetinib (450 mg once daily + 45 mg twice daily). Phase III data of 577 patients randomized to encorafenib plus binimetinib, encorafenib alone, or vemurafenib alone showed an ORR of 63%, 51%, and 40% and PFS of 14.9, 9.6, and 7.3 months, respectively. Interestingly, grade 3 to 4 AEs occurred less frequently in the combination group (58%) compared to encorafenib alone (66%) and vemurafenib alone (63%) [46].

CONCLUSIONS

In the last decade clinical research on melanoma has completely changed the scenario of therapeutic approaches for patients with unresectable advanced melanoma. Recent publications have reported outstanding results in the adjuvant treatment of stage IIIA to fully resected stage IV melanoma. Multiple targets for drug development have been identified and are currently undergoing development. Targeting immunological pathways (CTLA4, PD1, TNF, and TNFR superfamilies, immunoglobulin superfamily, B7, CD28, TLR-9, TLR-3, NK cells, and other mediators like IDO, TGF β , CXCR4, adenosine pathway, VEGF), driver mutations (BRAF, NRAS, ckit and corresponding signaling pathways), and genetically engineered viruses and vaccines have been shown to be valid treatment options used alone or in combination to avoid resistance [5]. Nivolumab remains an efficacious adjuvant treatment for patients with resected high-risk melanoma, with a safety profile that is more tolerable than that of ipilimumab [9]. Moreover, whether all patients at stage III or IV should receive adjuvant therapy and how to treat patients at stage IIB, IIC, or IIIA in adjuvant therapy remain controversial issues. In the last years, targeted nanoformulations have been explored for their potential in melanoma treatment, and promising results have been obtained [47–49]. Finally, regarding the decision-making on the appropriate adjuvant therapy for patients with resected stage III or IV melanoma, not only the efficacy but also the toxicity profile and medical history of the patient should be taken into account. Further reports of ongoing trials and research are

needed to improve the efficacy of neoadjuvant and adjuvant therapy and to determine the standard adjuvant therapy for patients with high-risk advanced melanoma.

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Data Availability. Data are available in the manuscript; tables are included in the article.

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