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REVIEWS

Combination of immunotherapy and other targeted therapies in advanced cutaneous melanoma

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ABSTRACT

Cutaneous Melanoma (CM) is an aggressive cancer whose incidence is increasing worldwide. However, the knowledge of its biology and genes driving cell growth and survival allowed to develop new drugs that have improved PFS and OS of advanced disease. Both BRAF targeting agents and immune checkpoint inhibitors (ICIs) have been adopted for the treatment of metastatic disease and the adjuvant setting. Several melanoma patients show innate or acquired drug-resistance and thus new strategies are required for overcoming this complication. New ICIs have been developed, and strategies of combination or sequencing are under investigation in ongoing clinical trials. In addition, pre-clinical data have demonstrated that many strategies induce the release of neoantigens within the tumor microenvironment, thus suggesting the combination of new agents with ICIs. Here, we review the ongoing strategies in advanced CM including a dedicated section on treatment of brain metastases.

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1. Introduction

Cutaneous melanoma (CM) is an aggressive skin cancer whose incidence is increasing worldwide. Melanoma treatment has been revolutionized over the past decade and either immune checkpoint inhibitors (ICIs) or targeted therapies are associated with durable survival. The introduction of numerous new cancer agents since 2011 has been associated with improved outcomes for patients with metastatic melanoma. In particular, robust data from phase-3 clinical trials and those from real-world treatments showed relevant impact on both progression-free survival (PFS) and overall survival (OS) in unresectable and metastatic CM. In contrast, only limited data are available for metastatic mucosal melanoma. Following a pilot study with anti-CTLA-4 (cytotoxic T lymphocyte associated protein-4) monoclonal antibody (mAb), Ipilimumab, and anti-BRAF agents (i.e. BRIM-3 trial[1]), an increasing number of combinations and strategies of sequencing have been explored, thus resulting in the development of many agents that are under investigation in phase-2 and −3 clinical trials. Current treatments have been associated with improved survival, but some patients still develop resistance and recurrent disease. Herein, we reviewed the most relevant treatments dedicated to the advanced CM, including brain metastases.

2. The landscape of melanoma treatment with immune checkpoint inhibitors

The blocking of immune checkpoints is a rapidly evolving anticancer option that provides meaningful efficacy in a high number of patients.[2] The original approach was based on the inhibition of negative signals of CTLA-4$^+$ T-cells. Ipilimumab, the first anti-CTLA-4 mAb, had changed the therapeutic landscape of metastatic CM although the objective response and long-term survival rates were only 20%.[3] However, the update of the molecular mechanisms driving checkpoint signals led to the development of agents blocking other critical receptors, such as the programmed death-1 (PD-1). In this context, nivolumab and pembrolizumab have significantly increased the survival of metastatic CM leading to long-term survival.[4] However, a significant number of patients recur or progress during ICIs and new combinations and/or sequences are under investigation in melanoma.[5] This effort leads to innovative drugs that include ICI agonist/antagonists, modulators of tumor microenvironment (TME) as well as targeted and epigenetic agents.

2.1. Combinations or sequencing

The rationale to combine anti-CTLA-4 and anti-PD-1/PD-L1 mAbs derives from their ability to target different sites and stages of T-cell activation. CTLA-4 is indeed mostly expressed by naïve T cells in the lymph nodes, whereas PD-1 is primarily expressed on antigen-experienced T cells in peripheral tissues.[6] Pre-clinical studies have demonstrated that the combination of ICIs is more effective than monotherapy in terms of melanoma control.[6–9] Hence, pre-clinical studies clearly proved that this strategy results in the infiltration within the TME of CD8 + T cells and the response of CD4+ effector T-cell via the expansion of an inducible T cell co-stimulator (ICOS)+ T helper (Th) 1-like CD4 subset.[6] Based on this evidence, the strategies of

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sequencing or combining nivolumab and ipilimumab have been investigated in metastatic CM.[10] In the phase-2 CheckMate 064 trial, unresectable stage III or IV patients were randomized to receive a sequential induction treatment with nivolumab followed by ipilimumab or ipilimumab followed by nivolumab.[11] After the induction phase, both cohorts received nivolumab until progression or unacceptable toxicity. Objective Response Rate (ORR) was higher for the sequence of nivolumab-ipilimumab than ipilimumab-nivolumab (41% vs. 20%), with a lower progression rate (38% vs. 60%). Notably, a higher 12-month OS rate was also obtained (76% vs. 54%). Treatment-related grade 3–4 adverse events (TRAEs) were almost similar between the two arms. However, sequential treatment does not offer a significant improvement over concurrent combination therapy.

In the CheckMate 067 study,[12] naïve CM and mucosal melanoma patients were randomly assigned to receive ipilimumab (3 mg/kg), nivolumab (1 mg/kg), or ipilimumab/nivolumab (3 mg/kg + 1 mg/kg). The OR rate was 58% in the nivolumab/ipilimumab group, 45% in the nivolumab group and 19% in the ipilimumab group. Median PFS was 11.5 months for nivolumab/ipilimumab, 6.9 months for nivolumab and 2.9 months for ipilimumab. The 5-years PFS rate was 36% for nivolumab/ipilimumab group, 29% for nivolumab and 8% for ipilimumab. The long-term follow-up has shown that median OS (mOS) was higher for the sequence of nivolumab-ipilimumab than ipilimumab-nivolumab, 36.9 months in the nivolumab group, and 19.9 months in the ipilimumab group. Overall survival at 5 years was 52% in the nivolumab plus ipilimumab group and 44% in the nivolumab group, as compared with 26% in the ipilimumab group. Therefore, this study has shown that patients receiving nivolumab as monotherapy or in combination with ipilimumab achieve an advantage in terms of OS, PFS and response rate (RR), regardless of the PD-L1 expression grade, as compared with those receiving ipilimumab. However, combinations are more toxic and the majority of AEs usually settle within 3–4 weeks. Notably, the 5-year OS was similar between patients who discontinued nivolumab/ipilimumab due to TRAEs. A separate consideration concerns mucosal melanoma since the 5-year outcome in CheckMate 067 suggested that those treated with nivolumab/ipilimumab have more favorable survival outcomes with respect to single agents.[13] However, the 5-year analysis also revealed that these patients had poorer long-term efficacy.[14] The CheckMate 511 study has further investigated the safety of the combination of nivolumab 3 mg/kg plus ipilimumab 1 mg/kg versus nivolumab 1 mg/kg plus ipilimumab 3 mg/kg.[15] The study met the primary end point, demonstrating a significantly lower incidence of grade 3–5 TRAEs for the nivolumab 3 mg/kg plus ipilimumab 1 mg/kg arm, thus suggesting with reasonable certainty that this combination of ipilimumab and nivolumab is a valid option in CM.

2.2 The treatment of brain metastases

Brain metastases are the third most common origin of metastases. Brain metastases occur in 25% of patients at diagnosis of advanced melanoma and up-to 75% at the time of death.[16] Current treatments for limited brain metastasis setting include focal irradiation (gamma-knife and cyber-knife). The efficacy of the medical treatment of this clinical setting is limited in most of studies due to the modest ability of many drugs to cross the blood–brain barrier. The mechanisms leading to brain metastasis are partly known and, as recently described, alterations of pericytes provoke brain barrier remodeling, thus favoring the recruitment of immune suppressive cells. Moreover, astrocytes over-express interleukin-23 (IL-23) and melanoma cells release matrix metalloproteinase-2 (MMP-2) for the degradation of the extracellular matrix. In addition, the recruitment of myeloid-derived suppressor cells (MDSC), regulatory T cells (T-reg), and cancer-associated fibroblasts (CAF) restrain the co-stimulation activity exerted by CD80, CD86 and CD40, thus resulting in defective antigen presentation, limited presence of tumor-infiltrating lymphocytes (TIL) and T-cell exhaustion.[17] Indeed, high PD-1 expression by TIL has been demonstrated, whereas early clinical trials tested the combination of fotemustine with ipilimumab in asymptomatic patients.[18] Other studies have recently investigated the combination of nivolumab and ipilimumab in this melanoma population.[19,20] The phase-2 CheckMate 204 trial[21] enrolled patients with metastatic melanoma and at least one measurable, nonirradiated brain metastasis (tumor diameter, 0.5 to 3 cm) and no neurologic symptoms received nivolumab (1 mg per kilogram of body weight) plus ipilimumab (3 mg per kilogram) every 3 weeks for up to four doses, followed by nivolumab (3 mg per kilogram) every 2 weeks until progression or unacceptable toxic effects. The intracranial and extracranial ORR were 55% and 50%, respectively. An updated analysis of asymptomatic group at 20.6 months reported an intracranial and extracranial ORR of 54% and 49%, respectively, with a global ORR of 51%. The 18-month survival rate was 75%. In symptomatic patients, at a median follow-up of 5.2 months, the intracranial ORR was 16.7%, with a 6-month survival rate of 66%.[22] The Australian Brain Collaboration (ABC) phase-2 trial[23] enrolled asymptomatic patients with asymptomatic brain metastases with no previous local brain therapy to receive nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg) followed by nivolumab (3 mg/kg; Cohort A) or nivolumab (3 mg/kg; Cohort B), whereas those progressed after local therapy or with neurological symptoms or leptomeningeal spreading disease were included in cohort C (nivolumab 3 mg/kg) without randomization. At a median follow-up of 17 months, the intracranial ORR in cohorts A, B, and C was 46%, 20%, and 6%, respectively, with complete intracranial response in 17%, 12%, and 0%. The updated analysis after a follow-up of 34 months, revealed an intracranial ORR in cohorts A, B, and C of 51%, 20%, and 6%, respectively, with complete intracranial response in 26%,16%, and 0%, respectively. The 24-month intracranial PFS rate was 49% in cohort A, 15% in cohort B, and 6% in cohort C, with a 24-month survival rate of 63%, 51%, and 19%, respectively.[24] Another relevant study investigating the combination of nivolumab plus ipilimumab with or without local therapy in brain metastasis was completed by DeCOG[25] that included 31% of patients with symptomatic brain metastasis. The median follow-up was 18 months and the 2-years and 3-years OS rates were 41% and 3%, respectively. The best prognostic factors for OS were low lactate

dehydrogenase and S100B levels, limited number of metastases and a good ECOG performance status. There was no difference in OS between BRAF-mutated patients receiving in first-line a BRAF/MEK inhibitor or ICIs.

Local therapy with stereotactic radiosurgery or surgery improved OS regardless of the timepoint of the local treatment. Lastly, a recent metaanalysis revealed that combined immunotherapy increased long-term OS and PFS as compared to anti-PD1 mAb monotherapy or targeted therapy.[26] Thus, the combination of anti-CTLA-4 anti-PD-1 is to be considered the best option for the medical treatment of brain metastases and further clinical trials exploring novel combinations including radiotherapy are required.

2.3. New strategies of combination

Recent clinical trials are investigating the combination of anti-PD-1 mAb with agents that target other checkpoints (Table 1).

2.3.1. Anti-lymphocyte activation gene 3 (LAG-3)

LAG-3 is a surface molecule exerting a crucial role during T-mediated cytotoxicity while primarily expressed by exhausted T-cells and lymphocytes characterized by poor effector activity as well as Tregs. In a fashion almost similar to PD-L1 and CTLA-4, it binds MHC class II on dendritic cells (DCs) and negatively regulates T-cell proliferation and activation.[27,28] In addition, LAG-3 is also expressed by melanoma-infiltrating T-cells and often co-expressed with PD-1. LAG-3 level on T cells is increased, thereby restraining the T-cell activity and Interferon (IFN-γ production within the TME, under the influence of PD-1 co-stimulation).[29] Agents targeting LAG-3 are under investigation in many clinical trials and new combinations of anti-LAG-3 and anti-PD-1 mAbs have shown encouraging results for overcoming PD-1-driven resistance. A phase 1–2 clinical trial (NCT01968109) has explored the safety of the anti-LAG-3 monoclonal antibody relatlimab in combination with nivolumab in patients refractory to a previous anti-PD-1/PD-L1 therapy.[30] No relevant toxicities were reported. Moreover, early data showed an 11.5% ORR with a 49% disease control rate (DCR). Responses were more frequent in patients showing LAG-3 expression over 1%, while PD-L1 status was independent from the efficacy. A phase 2–3 study comparing Relatimab plus Nivolumab with respect to Nivolumab monotherapy in naïve patients with unresectable stage III or stage IV melanoma is currently ongoing (CA224-047 trial).

2.3.2. Anti-TIM-3

TIM-3 is a co-inhibitory receptor expressed by T-cells that exerts both inhibitory and activating functions. It induces apoptosis, anergy and T-cell exhaustion by interplaying with galectin-9 on immune cells and a phase 1–2 trial (NCT02817633, NCT02608268) is ongoing.[27]

2.3.3. Anti-CD276

It is a receptor of the CD28 and B7 family molecules expressed by DCs as well as melanoma microenvironment that favors tumor growth and confers resistance to apoptosis. Enoblituzumab has been tested in phase-1 trial in combination with pembrolizumab (NCT02475213) or ipilimumab (NCT02381314).[31]

2.3.4. V-domain Ig suppressor of T-cell activation (VISTA)

VISTA is a PD-L1 homolog and a co-inhibitory receptor of the B7 family that is mostly expressed by MDSCs, TAMs and DCs as well as naïve T-cells. VISTA activation inhibits the effector T-cell response through the binding of its ligand, VSIG-3. The simultaneous blockade of VISTA and PD-1 has been investigated in a phase-1 trial (NCT02812875), showing acceptable safety and promising results.[31]

2.3.5. Oncolytic viruses

Talimogene laherparepvec (T-VEC) is a genetically engineered herpes virus.[32] T-VEC lacks the ICP34.5 protein that allows the normal herpes virus to overcome stress response of the host cells, resulting in productive replication. Therefore, healthy cells infected by T-VEC can easily stop the viral replication by activating the stress response. On the other hand, melanoma cells often show a disrupted stress response, thus allowing T-VEC to replicate and finally lyse the cell releasing tumor-associated antigens (TAA). Moreover, T-VEC uses the cell translation machinery not only to replicate itself but also to synthesize GM-CSF recruiting DCs to the site, thus promoting an immune response toward cancer cells. Subsequently, the virus induces oncolysis that stimulates the release of TAAs, including neo-antigens.[33] The OPTiM phase-3 trial evaluated the efficacy of intra-lesional injection of T-VEC in patients with stage IIIB or IV melanoma.[34] The control arm received subcutaneous injections of recombinant GM-CSF. An ORR of 31.5% with 16.9% of patients experiencing a complete response was reported in the T-VEC arm as compared to an ORR of 6.4% in the control arm. Moreover, the mOS were 23.3 months and 18.9 months. However, 88% of patients achieving a complete response were estimated to survive in view of a 5-year analysis. These data suggest that the major role of T-VEC is not limited to the shrinkage of lesions due to its oncolytic capability but also induces a long-lasting disease control due to the development of a specific immune response. Moreover, T-VEC shows a considerable local immune activity, thus resulting in regression ≥50% that occurs in 64% of injected lesions. Moreover, a 50% reduction in tumor size was also seen in 34% of non-injected, non-visceral lesions and in 15% of visceral lesions. However, it has to be noted that only 8% of patients in this trial had widespread distant melanoma, the majority being patients with locoregional recurrence. It has been also shown that T-VEC may enhance the activity of ICI mAbs by stimulating the inflammation in cells surrounding the melanoma cells and increasing the recruitment of T cells.[35]

The first study evaluating this combination was a phase Ib trial by Puzanov et al., who administered T-VEC intratumorally in week 1, then in week 4 and every 2 weeks thereafter, while Ipilimumab (3 mg/kg) was administered intravenously every 3 weeks for four infusions, beginning in week 6. The objective response rate was 50%, and 44% of patients had a durable response lasting ≥6 months. Eighteen-month progression-free survival was 50%; 18-month overall survival was 67%.[36]

Table 1. Overview of clinical trials featuring therapies or combinations in melanoma patients. NIVO: Nivolumab; pts: patients; ORR: Overall Response Rate; OS: Overall Survival; DOR: Duration of Response; HR: Hazard ratio; PEMBRO: Pembrolizumab; PFS: Progression Free Survival; TRAEs: treatment related adverse events; ITT: Intention-to-treat; IPI: Ipilimumab.

Study	Patients	Design	Outcomes
NCT01968109	68 pretreated pts with unresectable or metastatic CM	Phase 1/2 trial of Relatimab (anti-LAG-3) plus NIVO	ORR = 11.5%, DCR = 49%. No major toxicities
CA224-047 (NCT03470922)	700 naïve pts with unresectable or metastatic CM	Phase 2/3 trial of of Relatimab (anti-LAG-3) plus NIVO vs NIVO	Ongoing
OPTiM (NCT00769704)	436 naïve pts with unresectable or metastatic CM	Phase 3 trial of intralesional T-VEC (oncolytic virus) vs subcutaneous GM-CSF	T-VEC improved ORR (31.5% vs 16.9%) and mOS (23.3 vs 18.9 mo, HR 0.79; 95% CI 0.62–1.00; $p = .0494$)
Illuminate-204 (NCT02644967)	49 pretreated pts with unresectable or metastatic CM	Phase 1/2 trial of Tilosotolimod (TLR-9 agonist) plus IPI in pts with advanced CM	ORR = 22.4%, DCR = 71%, mDOR = 11 mo, mPFS = 5.1 mo, mOS = 21 mo.
Illuminate-301 (NCT03445533)	Patients with unresectable or metastatic CM	Phase 3 trial of Tilosotolimod (TLR-9 agonist) plus IPI vs IPI	Ongoing
PIVOT-02 (NCT02983045)	38 naïve pts with metastatic CM	Phase 1/2 trial of Bempegaldesleukin (PEG-IL2) plus NIVO	ORR = 59.5% (18.9% complete responses)
PIVOT-IO-010 (NCT03635983)	760 naïve pts with metastatic CM	Phase 3 trial of Bempegaldesleukin (PEG-IL2) plus NIVO vs NIVO	Ongoing
PROPEL (NCT03138889)	Naïve pts with unresectable or metastatic CM	Phase 1/2 trial of Bempegaldesleukin (PEG-IL2) plus PEMBRO	Ongoing
IMspire170 (NCT03273153)	446 naïve pts with BRAFV600 mutated wild type unresectable or metastatic CM	Phase 3 trial Atezolizumab plus Cobimetinib versus PEMBRO	The combination not improved nor mPFS (5.5 vs 5.7 mo, HR 1.15; 95% CI 0.88–1.50; $p = .295$) or OS (NR in both arms, HR 1.06; 95% CI 0.69–1.61)
IMspire150 (NCT02908672)	514 naïve pts with BRAFV600 mutated unresectable or metastatic CM	Phase 3 trial Atezolizumab plus Vemurafenib and Cobimetinib vs Vemurafenib and Cobimetinib	The triplet improved mPFS (15.1 vs 10.6 mo, HR 0.78; 95% CI 0.63–0.97; $p = .025$) and mOS (21 vs 12 mo)
COMBI-i (NCT02908672)	532 naïve pts with BRAFV600 mutated unresectable or metastatic CM	Phase 3 trial of Spartalizumab, Dabrafenib, and Trametinib vs Dabrafenib and Trametinib	The triplet slightly improved mPFS in the ITT population (16.2 vs 12 mo, HR 0.82; 95% CI, 0.655–1.027; $p = .042$), but significantly improved mPFS in the TMBhigh subpopulation (23.9 vs 11.8 mo)
KEYNOTE-022 (NCT02130466)	120 naïve pts with BRAFV600 mutant unresectable or metastatic CM	Phase 2 trial of PEMBRO, Dabrafenib, and Trametinib vs Dabrafenib and Trametinib	The triplet increases mPFS (16.9 vs 10.7 mo, HR 0.53; 95% CI 0.34 to 0.83) and mOS (NR vs 26.3 mo, HR 0.64; 95% CI 0.38 to 1.06), but increased grade 3–5 TRAEs (58% vs 25%)
LEAP-004 (NCT03776136)	104 pretreated pts with unresectable or metastatic CM	Phase 2 trial of PEMBRO and Lenvatinib	ORR = 21.4%, DCR = 65%, mPFS = 4.2 mo

T-VEC efficacy was explored also in combination with pembrolizumab in the phase-1b part of the MASTERKEY-265 clinical trial. This sequential treatment was associated with an ORR of 57% and a CR rate of 24% while in a follow-up efficacy analysis after a median follow-up of 38.6 months, ORR was 67% with a CR rate increased to 43%.[37]

2.4. Combinations of ICIs with co-stimulatory molecules and cytokines

A milestone in immune response concerns the efficacy of T-cell stimulation that is mostly regulated through signals driven by the TCR and co-signaling receptors as OX40, CD137, and ICOS. These are possible targets for combination strategies with anti-CTLA-4 and anti-PD-1 mAbs.[38,39] The ENGAGE-1 trial (NCT02528357) is exploring the combination of OX40 agonist mAb and pembrolizumab whereas the JAVELIN (NCT02554812) is investigating the combination with Avelumab, or the INDUCE-1 with an anti-ICOS receptor agonist (NCT02723955). Based on the earliest studies with either interferon or IL-2, the new compound Bempegaldesleukin (NKTR-214), a PEGylated interleukin-2, has been designed to activate CD8 + T cells and NK cells.[40] The PIVOT-02 phase-1-2 trial tested the combination of Bempegaldesleukin and Nivolumab in naïve stage IV melanoma patients and an ORR of 59.5% was reported.[41] A phase-3 trial comparing the combination of Bempegaldesleukin and Nivolumab with Nivolumab monotherapy in 760 naïve patients is currently ongoing (PIVOT IO-010, CT03635983). Moreover, Bempegaldesleukin has been combined with Pembrolizumab in the phase 1–2 PROPEL trial (NCT03138889).

2.5 Combinations of ICIs with modulators of the TME

Many pieces of evidence have highlighted the role of TME in favoring melanoma cell proliferation and, therefore, the efficacy of immunotherapy. Among these modulators, indoleamine 2,3-dioxygenase (IDO) and members of the Toll-Like Receptors (TLRs) family have been investigated in clinical trials. An additional pathway implicated in the regulation of immune system is that mediated by the enzyme arginase whose major role includes the impairment of T-cell functions, and its inhibition represents an alternative strategy for improving the effectiveness of immunotherapy. IDO is an immunosuppressive enzyme involved in the tryptophan catabolism and degradation into kynurenine. Notwithstanding the promising results of the phase 1–2 trial, the phase-3 study (ECHO-301) combining the IDO inhibitor epacadostat with pembrolizumab failed in terms of PFS and OS.[42–44] Among the TLR family, TLR-9 recognizes unmethylated cytosine–phosphate–guanine (CpG) dinucleotide motifs in bacterial and viral (DNA) and is highly expressed by DCs and B cells. Signaling mediated by TLR-9 stimulates the IFN-α production, the proliferation of B-cell and co-stimulation, thus suggesting TLR-9 agonists for the treatment of cancer. Thus, Tilsotolimod (IMO-2125), a synthetic phosphorothioate oligonucleotide, was engineered to exert direct agonistic activity toward TLR 9 for the stimulation of either innate or adaptive immunity and the modulation of antigen presentation. In addition, IMO-2125 induces high production of IFN-α by DCs along with a number of cytokines and chemokines, B-cell proliferation, and activation of TLR 9 by either B cells or DCs in the TME as well as a systemic immune response when administered by intratumoral injection.[44–46] The Illuminate-204 phase 1–2 trial has recently exploited the efficacy and tolerability of IMO-2125 in patients with unresectable stage III or stage IV melanoma who failed previous therapy with anti-PD1 mAb.[47] Tilsotolimod was administered intralesionally in combination with systemic Ipilimumab. The results of the Illuminate-204 trial showed an ORR of 22.4% and a DCR of 71%. The median duration of response was 11 months. As a secondary endpoint, PFS and OS were evaluated resulting of 5.1 months and 21 months, respectively. Given the efficacy and the excellent tolerability profile demonstrated, a phase-3 study comparing the combination of Tilsotolimod and Ipilimumab versus Ipilimumab monotherapy after the failure of anti-PD1 mAb is currently ongoing (Illuminate-301, NCT03445533). Instead, another TLR-9 agonist, SD-101, is under investigation in a phase-1 study evaluating its safety in combination with Pembrolizumab in naïve patients with unresectable or metastatic melanoma.[48]

3. Immune checkpoint inhibitors and targeted therapy in BRAF-mutated patients

Oncogene-targeted therapy with B-Raf proto-oncogene (BRAF) and mitogen-activated protein kinase kinase (MEK) inhibitors has modified the natural history of '*oncogene addicted*' metastatic melanoma. It is noteworthy that combination of targeted agents induces a high early response in patients with *BRAFV600* mutated melanoma, with a median duration of response of approximately one-year.[49–51] Although BRAF and MEK inhibitors are associated with a higher ORR as compared with immunotherapy, acquired resistance results in relapse with a median PFS of 11.5 months.[52] Preclinical and translational data, however, have shown that BRAF inhibition has an immune-modulating effect and, moreover, potentiate the anti-cancer immune activity. It occurs within the TME by increasing the antigen presentation, the antigen-specific T cell recognition, the homing of immune effector cells nearby melanoma tumor and improving the activity of T cell effectors.[52–56] Also MEK inhibitors exert immunomodulating effect but the real impact on immune cell activity is still debated. Pre-clinical trials with MEK inhibitors have demonstrated potential immune stimulation through the up-regulation of tumor-derived antigens, whereas other *in vitro* studies suggested a negative effect on T-cell proliferation and activity.[53,57–60] These findings suggested to combine in CM targeted agents with ICIs in order to obtain a synergistic effect. Preclinical and phase 1–2 trials evaluated the combination of BRAF/MEK inhibitors with anti-PD1 or anti-PDL1 mAbs in CM, thus showing acceptable toxicity profile.[61,62] These preliminary results led to the development of phase-3 clinical trials (Table 1), including the Keynote-022-part 3, COMBI-I, and IMspire150.

The Keynote 022-part 3 of the Keynote-022 was a multicenter phase 1–2 trial designed to optimize the dose and efficacy. In Keynote 022-part 3 BRAFV600 mutated

metastatic CM patients were randomized to receive dabrafenib plus trametinib plus pembrolizumab or dabrafenib plus trametinib.[63] In a first analysis completed after 9.5 months of follow-up, the study did not meet the primary endpoint of PFS (16.0 months in the triplet arm versus 10.3 months in the doublet arm and a hazard ratio [HR] of 0.66). In a post hoc analysis completed after 36.6 months of follow-up, a clinical advantage was observed in the triplet arm in terms of PFS (16.9 months vs 10.7 months), median duration of response (25.1 months vs 12.1 months; HR: 0.32) and mOS (not reached versus 26.3 months in the doublet arm [HR: 0.64]). The ORR was 63% in the triplet arm and 72% in the doublet arm. Exploratory subgroup analysis of PFS showed that the HR for the subgroups favored the triplet over the doublet arm in patients aged ≤65 years, men, ECOG 0 and elevated lactate dehydrogenase levels. Grade 3–5 AEs occurred in 70% of patients in the triplet arm and 58% were treatment related. COMBI-I was a randomized phase 3 study in which BRAFV600 melanoma patients were randomized to receive triplet experimental combination dabrafenib plus trametinib plus spartalizumab, or standard target combination dabrafenib plus trametinib.[64] The primary end point was PFS that has not been met, with a median follow-up from the time of randomization of 16.2 months in the triplet arm that was not statistically different from the standard treatment arm. In PFS subgroup analysis, triplet treatment was superior in patients with a high tumor burden or high number of metastatic sites. The ORR was 68.5% in triplet arm and 64.2% in dabrafenib plus trametinib arm. Median duration of response was not reached in triplet arm and was 20.7 months in control arm. Median OS was not reached in each arm (HR 0.785). In dabarafenib plus trametinib plus spartalizumab arm, grade 3 or higher adverse event were 70.4%, and 54% of them were treatment related. In the control arm, grade 3 or higher adverse event were 57.2%, and 33.3% were treatment related.

The IMspire170 phase-3 trial compared the combination of Atezolizumab and Cobimetinib versus Pembrolizumab in patients with unresectable or metastatic BRAFV600 wild-type CM. The study showed no significant difference, with a PFS of 5.5 months in the combination versus 5.7 months in the monotherapy arm (HR = 1.15, p = .295). Similar results are derived from the first ad-interim analysis of OS (HR = 1.06). Moreover, AEs were more frequent in the experimental arm.[65] The IMspire150 was a randomized, double-blind, placebo-controlled phase 3 study. Patients with unresectable stage IIIc-IV, BRAFV600 mutated CM patients were randomized to receive vemurafenib, and cobimetinib (BRAF and MEK inhibitors) plus atezolizumab (anti-PDL1 mAb) or vemurafenib and cobimetinib plus placebo.[66] Patients were randomly assigned to the atezolizumab group (n = 256) or control group (n = 258). At a median follow-up of 18.9 months, PFS was significantly prolonged with atezolizumab as compared to control arm (15.1 vs. 10.6 months; HR: 0.78). Common treatment-related AEs in the atezolizumab and control groups were blood creatinine phosphokinase increase, diarrhea, rash, arthralgia, pyrexia, alanine aminotransferase increase and lipase increase. Atezolizumab, vemurafenib and Cobimetinib combination received the Food and Drug Administration approval in 2020. Finally, the LEAP004 is a phase-2 trial evaluating the combination of Pembrolizumab and Lenvatinib (a VEGFR and FGFR inhibitor) in patients with unresectable or metastatic CM who failed at least a previous line with anti-PD1 mAb. The rationale for the use of Lenvatinib is the shift to an immunostimulatory microenvironment due to the inhibition of VEGFR and FGFR. An ORR of 21.4% and a DCR of 65% were reported, with a median PFS of 4.2 months.[67] This study suggests that Lenvatinib in combination with immunotherapy could be a valid option for those patients who progressed beyond first-line immunotherapy or a first-line target-therapy, offering a possible therapy in an orphan clinical setting.

Therefore, triplet combinations obtained an ORR that was comparable to standard combo-targeted but with a longer durable response in responding patients when compared with targeted therapy alone. Probably, this difference in median duration of response constitutes the principal difference in efficacy between triplet and targeted therapy whereas a major difference in efficacy and PFS might be observed after a longer follow-up. Safety profile is a critical issue for this combination in daily clinical practice and also the patient selection is a critical issue. Therefore, some subgroups of patients may benefit from triplet as those with poor baseline prognostic factors including high tumor burden or high baseline LDH level. The comparison between triplet BRAF/MEK inhibitors plus PDL1/PD1 blockers and other immunotherapy combos (anti-CTLA4 + anti-PD1) is still an open question. Future evaluations are required to evaluate the best algorithm and optimize the first-line strategy.

4. Conclusions

The adoption of ICIs has profoundly changed the landscape of advanced CM treatment and the results obtained are a milestone for developing new options in both neoadjuvant and adjuvant setting. The discovery of new receptors driving the activation of T-cells that may be potentially druggable has opened to new sequencing and combination strategies, involving different ICIs. Also, the manipulation of key regulators of the immune response such as TLRs, along with the development of complex immune stimulators such as T-VEC, is providing promising agents for those patients who are refractory to anti-PD1 mAbs. Lastly, results from the Checkmate-204 and ABC trials focused on the role of combo immune therapy for the treatment of brain metastases that, along with radiosurgery, represents a viable option for this severe complication. Therefore, expected results from ongoing clinical trials will represent a critical breakthrough to apply innovative therapies to the current strategies in unresectable and metastatic CM.

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References

1. Chapman PB, Robert C, Larkin J, Haanen JB, Ribas A, Hogg D, Hamid O, Ascierto PA, Testori A, Lorigan PC, et al. Vemurafenib in patients with BRAFV600 mutation-positive metastatic melanoma: final overall survival results of the randomized BRIM-3 study. Ann Oncol. 2017;28(10):2581–87. doi:10.1093/annonc/mdx339.
2. Topalian SL. Targeting immune checkpoints in cancer therapy. JAMA. 2017;318(17):1647–48. doi:10.1001/jama.2017.14155.
3. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):1290. doi:10.1056/NEJMoa1003466.
4. Larkin J, Minor D, D'Angelo S, Neyns B, Smylie M, Miller WH Jr, Gutzmer R, Linette G, Chmielowski B, Lao CD, et al. Overall survival in patients with advanced melanoma who received nivolumab versus investigator's choice chemotherapy in CheckMate 037: a randomized, controlled, open-label phase III trial. J Clin Oncol. 2018;36(4):383–90. doi:10.1200/JCO.2016.71.8023.
5. Gide TN, Wilmott JS, Scolyer RA, Long GV. Primary and acquired resistance to immune checkpoint inhibitors in metastatic melanoma. Clin Cancer Res. 2018;24(6):1260–70. doi:10.1158/1078-0432.CCR-17-2267.
6. Wei SC, Levine JH, Cogdill AP, Zhao Y, Anang NAS, Andrews MC, Sharma P, Wang J, Wargo JA, Pe'er D, et al. Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade. Cell. 2017;170(6):1120–33. doi:10.1016/j.cell.2017.07.024.
7. Rotte A, Jin JY. Lemaire V mechanistic overview of immune checkpoints to support the rational design of their combinations in cancer immunotherapy. Ann Oncol. 2018;29(1):71–83. doi:10.1093/annonc/mdx686.
8. Tarhini A. Immune-mediated adverse events associated with ipilimumab ctla-4 blockade therapy: the underlying mechanisms and clinical management. Scientifica (Cairo). 2013;2013:857519.
9. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, Berdelou A, Varga A, Bahleda R, Hollebecque A. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer. 2016;54:139–48. doi:10.1016/j.ejca.2015.11.016.
10. Rotte A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. J Exp Clin Cancer Res. 2019;38(1):255. doi:10.1186/s13046-019-1259-z.
11. Weber JS, Gibney G, Sullivan RJ, Sosman JA, Slingluff CL Jr, Lawrence DP, Logan TF, Schuchter LM, Nair S, Fecher L, et al. Sequential administration of nivolumab and ipilimumab with a planned switch in patients with advanced melanoma (CheckMate 064): an open-label, randomised, phase 2 trial [published correction appears in Lancet Oncol. 2016 Jul;17 (7): e270]. Lancet Oncol. 2016;17(7):943–55. doi:10.1016/S1470-2045(16)30126-7.
12. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob -J-J-J-J, Rutkowski P, Lao CD, Cowey CL, Schadendorf D, Wagstaff J, Dummer R, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2019;381(16):1535–46. doi:10.1056/NEJMoa1910836.
13. D'Angelo SP, Larkin J, Sosman JA, Lebbé C, Brady B, Neyns B, Schmidt H, Hassel JC, Hodi FS, Lorigan P, et al. Efficacy and safety of nivolumab alone or in combination with ipilimumab in patients with mucosal melanoma: a pooled analysis. J Clin Oncol. 2017;35(2):226–35. doi:10.1200/JCO.2016.67.9258.
14. Shoushtari AN, Wagstaff J, Ascierto PA, Butler MO, Lao CD, Marquez-Rodas I, Chiarion-Sileni V, Dummer R, Ferrucci PF, Lorigan P, et al. CheckMate 067: long-term outcomes in patients with mucosal melanoma. J Clin Oncol. 2020;38(15_suppl):10019. doi:10.1200/JCO.2020.38.15_suppl.10019.
15. Lebbé C, Meyer N, Mortier L, Marquez-Rodas I, Robert C, Rutkowski P, Menzies AM, Eigentler T, Ascierto PA, Smylie M, et al. Evaluation of two dosing regimens for nivolumabin nivolumab in combination with ipilimumab in patients with advanced melanoma: results from the phase IIIb/IV CheckMate 511 trial. J Clin Oncol. 2019;37(11):867–75. doi:10.1200/JCO.18.01998.
16. Cagney DN, Martin AM, Catalano PJ, Redig AJ, Lin NU, Lee EQ, Wen PY, Dunn IF, Bi WL, Weiss SE, et al. Incidence and prognosis of patients with brain metastases at diagnosis of systemic malignancy: a population-based study. Neuro-Oncology. 2017;19(11):1511–21. doi:10.1093/neuonc/nox077.
17. Di Giacomo AM, Valente M, Cerase A, Lofiego MF, Piazzini F, Calabrò L, Gambale E, Covre A, Maio M. Immunotherapy of brain metastases: breaking a "dogma." J Exp Clin Cancer Res. 2019;38(1):419. doi:10.1186/s13046-019-1426-2.
18. Di Giacomo AM, Ascierto PA, Pilla L, Santinami M, Ferrucci PF, Giannarelli D, Marasco A, Rivoltini L, Simeone E, Nicoletti SV, et al. Ipilimumab and fotemustine in patients with advanced melanoma (NIBITM1): NIBIT-M1): an open-label, single-arm phase 2 trial. Lancet Oncol. 2012;13(9):879–86. doi:10.1016/S1470-2045(12)70324-8.
19. Di Giacomo AM, Ascierto PA, Queirolo P, Pilla L, Ridolfi R, Santinami M, Testori A, Simeone E, Guidoboni M, Maurichi A, et al. Three-year follow-up of advanced melanoma patients who received ipilimumab plus fotemustine in the Italian Network for Tumor Biotherapy (NIBIT)-M1 phase II study. Ann Oncol. 2015;26(4):798–803. doi:10.1093/annonc/mdu577.
20. Di Giacomo AM, Chiarion Sileni V, Del Vecchio M, Ferrucci PF, Guida M, Quaglino P, Guidoboni M, Marchetti P, Cutaia O, Amato G, et al. Efficacy of ipilimumab plus nivolumab or ipilimumab plus fotemustine vs fotemustine in patients with melanoma metastatic to the brain: primary analysis of the phase III NIBIT-M2 trial. Ann Oncol. 2020;31:S672–S710. doi:10.1016/j.annonc.2020.08.1205.
21. Tawbi HA, Forsyth PA, Algazi A, Hamid O, Hodi FS, Moschos SJ, Khushalani NI, Lewis K, Lao CD, Postow MA, et al. Combined nivolumab and ipilimumab in melanoma metastatic to the brain. N Engl J Med. 2018;379(8):722–30. doi:10.1056/NEJMoa1805453.
22. Tawbi HA, Forsyth PAJ, Hodi FS, Lao D, Moschos SJ, Hamid O, Atkins MB, Lewis KD, Thomas RP, Glaspy JA, et al. Efficacy and safety of the combination of nivolumab (NIVO) plus ipilimumab (IPI) in patients with symptomatic melanoma brain metastases (CheckMate 204). J Clin Oncol. 2019;37(15_suppl):9501–950. doi:10.1200/JCO.2019.37.15_suppl.9501.
23. Long GV, Atkinson V, Lo S, Sandhu S, Guminski AD, Brown MP, Wilmott JS, Edwards J, Gonzalez M, Scolyer RA, et al. Combination nivolumab and ipilimumab or nivolumab alone in melanoma brain metastases: a multicentre randomised phase 2 study. Lancet Oncol. 2018;19(5):672–81. doi:10.1016/S1470-2045(18)30139-6.
24. Long GV, Atkinson VG, Lo S, Sandhu SK, Brown M, Gonzalez M, Guminski A, Scolyer RA, Emmett L, Menzies AM, et al. Long-term outcomes from the randomized phase II study of nivolumab (nivo) or nivo+ ipilimumab nivo+ipilimumab (ipi) in patients (pts) with melanoma brain metastases (mets): anti-PD1 brain collaboration (ABC). Ann Oncol. 2019;30:v533–v563. doi:10.1093/annonc/mdz255.001.
25. Amaral T, Kiecker F, Schaefer S, Stege H, Kaehler K, Terheyden P, Gesierich A, Gutzmer R, Haferkamp S, Uttikal J, et al. Combined immunotherapy with nivolumab and ipilimumab with and without local therapy in patients with melanoma brain metastasis: a DeCOG* study in 380 patients. J Immunother Cancer. 2020;8(1):e000333. doi:10.1136/jitc-2019-000333.
26. Rulli E, Legramandi L, Salvati L, Mandala M. The impact of targeted therapies and immunotherapy in melanoma brain metastases: a systematic review and meta-analysis. Cancer. 2019;125(21):3776–89. doi:10.1002/cncr.32375.

27. Huard B, Prigent P, Tournier M, Bruniquel D, Triebel F. CD4/major histocompatibility complex class II interaction analyzed with CD4- and lymphocyte activation gene-3 (LAG-3)-Ig fusion proteins. Eur J Immunol. 1995;25(9):2718–21. doi:10.1002/eji.1830250949.
28. Taube JM, Young GD, McMiller TL, Chen S, Salas JT, Pritchard TS, Xu H, Meeker AK, Fan J, Cheadle C, et al. Differential expression of immune-regulatory genes associated with PD-L1 display in melanoma: implications for PD-1 pathway blockade. Clin Can Res. 2015;21(17):3969–76. doi:10.1158/1078-0432.CCR-15-0244.
29. He Y, Rivard CJ, Rozeboom L, Yu H, Ellison K, Kowalewski A, Zhou C, Hirsch FR. Lymphocyte-activation gene-3, an important immune checkpoint in cancer. Cancer Sci. 2016;107(9):1193–97. doi:10.1111/cas.12986.
30. Ascierto PA, Bono P, Bhatia S, Melero I, Nyakas MS, Svane I, Larkin J, Gomez-Roca C, Schadendorf D, Dummer R, et al. Efficacy of BMS-986016, a monoclonal antibody that targets lymphocyte activation gene-3 (LAG-3), in combination with nivolumab in pts with melanoma. Ann Oncol. 2017;28:v605–v649. doi:10.1093/annonc/mdx440.011.
31. Burugu S, Dancsok AR, Nielsen TO. Emerging targets in cancer immunotherapy. Semin Cancer Biol. 2018;52:39–52.
32. Fukuhara H, Ino Y, Todo T. Oncolytic virus therapy: a new era of cancer treatment at dawn. Cancer Sci. 2016;107(10):1373–79. doi:10.1111/cas.13027.
33. Jiang H, Rivera-Molina Y, Gomez-Manzano C, Clise-Dwyer K, Bover L, Vence LM, Yuan Y, Lang FF, Toniatti C, Hossain MB, et al. Oncolytic adenovirus and tumor-targeting immune modulatory therapy improve autologous cancer vaccination. Cancer Res. 2017;77(14):3894–907. doi:10.1158/0008-5472.CAN-17-0468.
34. Andtbacka RHI, Collichio F, Harrington KJ, Middleton MR, Downey G, Öhrling K, Kaufman HL. Final analyses of OPTiM: a randomized phase III trial of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in unresectable stage III-IV III–IV melanoma. J Immunother Cancer. 2019;7(1):145. doi:10.1186/s40425-019-0623-z.
35. Liu Z, Ravindranathan R, Kalinski P, Guo ZS, Bartlett DL. Rational combination of oncolytic vaccinia virus and PD-L1 blockade works synergistically to enhance therapeutic efficacy. Nat Commun. 2017;8(1):14754. doi:10.1038/ncomms14754.
36. Puzanov I, Milhem MM, Minor D, Hamid O, Li A, Chen L, Chastain M, Gorski KS, Anderson A, Chou J, et al. Talimogene laherparepvec in combination with ipilimumab in previously untreated, unresectable stage IIIB-IV melanoma. J Clin Oncol. 2016;34(22):2619–26. doi:10.1200/JCO.2016.67.1529.
37. Long GV, Dummer R, Andtbacka RH Follow-up analysis of MASTERKEY-265 phase 1b (ph1b) study of talimogene laherparepvec (T-VEC) in combination (combo) with Pembrolizumab (pembro) in patients (pts) with unresectable stage IIIB–IV M1c melanoma (MEL). Presented at: the 2018 SMR International congress; 2018 Oct 24-27; Manchester, EN; 2018.
38. Murciano-Goroff YR, Warner AB, Wolchok JD. The future of cancer immunotherapy: microenvironment-targeting combinations. Cell Res. 2020;30(6):507–19. doi:10.1038/s41422-020-0337-2.
39. Segal NH, Logan TF, Hodi FS, McDermott D, Melero I, Hamid O, Schmidt H, Robert C, Chiarion-Sileni V, Ascierto PA, et al. Results from an integrated safety analysis of urelumab, an agonist anti-CD137 monoclonal antibody. Clin Cancer Res. 2017;23(8):1929–36. doi:10.1158/1078-0432.CCR-16-1272.
40. Charych D, Khalili S, Dixit V, Kirk P, Chang T, Langowski J, Rubas W, Doberstein SK, Eldon M, Hoch U, et al. Modeling the receptor pharmacology, pharmacokinetics, and pharmacodynamics of NKTR-214, a kinetically-controlled interleukin-2 (IL2) receptor agonist for cancer immunotherapy. PLoS One. 2017;12(7):e0179431. doi:10.1371/journal.pone.0179431.
41. Diab A, Tannir NM, Bentebibel S-ES-E, Hwu P, Papadimitrakopoulou V, Haymaker C, Kluger HM, Gettinger SN, Sznol M, Tykodi SS, et al. Bempegaldesleukin (NKTR-214) plus nivolumab in patients with advanced solid tumors: phase I dose-escalation study of safety, efficacy, and immune activation (PIVOT-02). Cancer Discov. 2020;10(8):1158–73. doi:10.1158/2159-8290.CD-19-1510.
42. Grzywa TM, Sosnowska A, Matryba P, Rydzynska Z, Jasinski M, Nowis D, Golab J. Myeloid cell-derived arginase in cancer immune response. Front Immunol. 2020;11:938.
43. Labadie BW, Bao R, Luke JJ. Reimagining IDO pathway inhibition in cancer immunotherapy via downstream focus on the tryptophan-kynurenine-aryl tryptophan–kynurenine–aryl hydrocarbon axis. Clin Cancer Res. 2019;25(5):1462–71. doi:10.1158/1078-0432.CCR-18-2882.
44. Long GV, Dummer R, Hamid O, Gajewski TF, Caglevic C, Dalle S, Arance A, Carlino MS, Grob -J-J-J-J, Kim TM, et al. Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study. Lancet Oncol. 2019;20(8):1083–97. doi:10.1016/S1470-2045(19)30274-8.
45. Wang D, Jiang W, Zhu F, Mao X, Agrawal S. Modulation of the tumor microenvironment by intratumoral administration of IMO-2125, a novel TLR 9 agonist, for cancer immunotherapy. Int J Oncol. 2018;53:1193–203.
46. Middleton MR, Hoeller C, Michielin O, Robert C, Caramella C, Öhrling K, Hauschild A. Intratumoural immunotherapies for unresectable and metastatic melanoma: current status and future perspectives. Br J Cancer. 2020;123(6):885–97. doi:10.1038/s41416-020-0994-4.
47. Haymaker C, Andtbacka RHI, Johnson DB, Shaheen MF, Rahimian S, Chunduru S, Gabrail N, Doolittle G, Puzanov I, Markowitz J, et al. 1083MO Final results from ILLUMINATE-204, a phase I/II trial of intratumoral tilsotolimod in combination with ipilimumab in PD-1 inhibitor refractory advanced melanoma. Ann Oncol. 2020;31:S672–S710. doi:10.1016/j.annonc.2020.08.1207.
48. Milhem MM, Long GV, Hoimes CJ, Amin A, Lao CD, Conry RM, Hunt J, Daniels GA, Almubarak M, Shaheen MF, et al. Phase 1b/2, open label, multicenter, study of the combination of SD-101 and pembrolizumab in patients with advanced melanoma who are naïve to anti-PD-1 therapy. J Clin Oncol. 2019;37(15_suppl):9534. doi:10.1200/JCO.2019.37.15_suppl.9534.
49. Larkin J, Ascierto PA, Dréno B, Atkinson V, Liszkay G, Maio M, Mandalà M, Demidov L, Stroyakovskiy D, Thomas L, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med. 2014;371(20):1867–76. doi:10.1056/NEJMoa1408868.
50. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, De Braud F, Larkin J, Garbe C, Jouary T, Hauschild A, Grob JJ, et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N Engl J Med. 2014;371(20):1877–88. doi:10.1056/NEJMoa1406037.
51. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, Lichinitser M, Dummer R, Grange F, Mortier L, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med. 2015;372(1):30–39. doi:10.1056/NEJMoa1412690.
52. Ascierto PA, Dummer R. Immunological effects of BRAF+MEK inhibition. Onco Targets Ther. 2018;7:e1468955.
53. Boni A, Cogdill AP, Dang P, Udayakumar D, Njauw CN, Sloss CM, Ferrone CR, Flaherty KT, Lawrence DP, Fisher DE, et al. Selective BRAFV600E BRAF V600E inhibition enhances T cell T-cell recognition of melanoma without affecting lymphocyte function. Cancer Res. 2010;70(13):5213. doi:10.1158/0008-5472.CAN-10-0118.
54. Sapkota B, Hill CE, Pollack BP. Vemurafenib enhances MHC induction in BRAF V600E homozygous melanoma cells. Oncoimmunology. 2013;2(1):e22890. doi:10.4161/onci.22890.
55. Frederick DT, Piris A, Cogdill AP, Cooper ZA, Lezcano C, Ferrone CR, Mitra D, Boni A, Newton LP, Liu C, et al. BRAF inhibition is associated with enhanced melanoma antigen

expression and a more favorable tumor microenvironment in patients with metastatic melanoma. Clin Cancer Res. 2013;19 (5):1225–12231. doi:10.1158/1078-0432.CCR-12-1630.
56. Donia M, Fagone P, Nicoletti F, Andersen RS, Høgdall E, Straten PT, Andersen MH, Svane IM. BRAF inhibition improves tumor recognition by the immune system: potential implications for combinatorial therapies against melanoma involving adoptive T-cell transfer. Oncoimmunology. 2012;1(9):1476. doi:10.4161/onci.21940.
57. Long GV, Wilmott JS, Haydu LE, Tembe V, Sharma R, Rizos H, Thompson JF, Howle J, Scolyer RA, Kefford RF. Effects of BRAF inhibitors on human melanoma tissue before treatment, early during treatment, and on progression. Pigment Cell Melanoma Res. 2013;26(4):499–508. doi:10.1111/pcmr.12098.
58. Comin-Anduix B, Chodon T, Sazegar H, Matsunaga D, Mock S, Jalil J, Escuin-Ordinas H, Chmielowski B, Koya RC, Ribas A. The oncogenic BRAF kinase inhibitor PLX4032/RG7204 does not affect the viability or function of human lymphocytes across a wide range of concentrations. Clin Cancer Res. 2010;16(24):6040–48. doi:10.1158/1078-0432.CCR-10-1911.
59. Kono M, Dunn IS, Durda PJ, Butera D, Rose LB, Haggerty TJ, Benson EM, Kurnick JT. Role of the mitogen-activated protein kinase signaling pathway in the regulation of human melanocytic antigen expression. Mol Cancer Res. 2006;4(10):779–92. doi:10.1158/1541-7786.MCR-06-0077.
60. Vella LJ, Pasam A, Dimopoulos N, Andrews M, Knights A, Puaux A-LA-L, Louahed J, Chen W, Woods K, Cebon JS. MEK inhibition, alone or in combination with BRAF inhibition, affects multiple functions of isolated normal human lymphocytes and dendritic cells. Cancer Immunol Res. 2014;2(4):351–60. doi:10.1158/2326-6066.CIR-13-0181.
61. Deken MA, Gadiot J, Jordanova ES, Lacroix R, Van Gool M, Kroon P, Pineda C, Geukes Foppen MH, Scolyer R, Song J-YJ-Y, et al. Targeting the MAPK and PI3K pathways in combination with PD1 blockade in melanoma. Oncoimmunology. 2016;5(12): e1238557. doi:10.1080/2162402X.2016.1238557.
62. Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Goedert L, Pinheiro EM, Koya RC, Graeber TG, Comin-Anduix B, et al. Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) BRAF V600E melanoma. Sci Transl Med. 2015;7(279):279ra41. doi:10.1126/scitranslmed.aaa4691.
63. Ferrucci PF, Di Giacomo AM, Del Vecchio M, Atkinson V, Schmidt H, Schachter J, Queirolo P, Long GV, Stephens R, Svane IM, et al. KEYNOTE-022 international team. KEYNOTE-022 part 3: a randomized, double-blind, phase 2 study of pembrolizumab, dabrafenib, and trametinib in BRAFmutant BRAF-mutant melanoma. J Immunother Cancer. 2020;8(2):e001806. doi:10.1136/jitc-2020-001806.
64. Nathan P, Dummer R, Long GV, Ascierto PA, Tawbi HA, Robert C, Rutkowski P, Leonov O, Dutriaux C, Mandala M, et al. Spartalizumab plus dabrafenib and trametinib in patients with previously untreated BRAF V600–mutant unresectable or metastatic melanoma: results from the randomized part 3 of the Phase III COMBI-I trial. Ann Oncol. 2020;31:S1142–S1215. doi:10.1016/j.annonc.2020.08.2273.
65. Arance AM, Gogas H, Dreno B, Flaherty KT, Demidov L, Stroyakovskiy D, Eroglu Z, Ferrucci PF, Pigozzo J, Rutkowski P, et al. Combination treatment with cobimetinib (C) and atezolizumab (A) vs pembrolizumab (P) in previously untreated patients (pts) with BRAFV600 wild type (wt) advanced melanoma: primary analysis from the phase 3 III IMspire170 trial. Ann Oncol. 2019;30: v851–v934. doi:10.1093/annonc/mdz394.066.
66. Gutzmer R, Stroyakovskiy D, Gogas H, Robert C, Lewis K, Protsenko S, Pereira RP, Eigentler T, Rutkowski P, Demidov L, et al. Atezolizumab, vemurafenib, and cobimetinib as first-line treatment for unresectable advanced BRAFV600 mutation-positive melanoma (IMspire150): primary analysis of the randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2020;395(10240):1835–44. doi:10.1016/S0140-6736(20)30934-X.
67. Arance AMF, O'Day SJ, De La Cruz ML, Petrella T, Jamal R, Ny L, Carneiro A, Berrocal A, Márquez-Rodas I, Spreafico A, et al. LBA44 - Lenvatinib (len) plus pembrolizumab (pembro) for advanced melanoma (MEL) that progressed on a PD-1 or PD-L1 inhibitor: initial results of LEAP-004. Ann Oncol. 2020;31:S1142–S1215.