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Targeted agents and immunotherapies: optimizing outcomes in melanoma

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Abstract | Treatment options for patients with metastatic melanoma, and especially *BRAF*-mutant melanoma, have changed dramatically in the past 5 years, with the FDA approval of eight new therapeutic agents. During this period, the treatment paradigm for *BRAF*-mutant disease has evolved rapidly: the standard-of-care BRAF-targeted approach has shifted from single-agent BRAF inhibition to combination therapy with a BRAF and a MEK inhibitor. Concurrently, immunotherapy has transitioned from cytokine-based treatment to antibody-mediated blockade of the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and, now, the programmed cell-death protein 1 (PD-1) immune checkpoints. These changes in the treatment landscape have dramatically improved patient outcomes, with the median overall survival of patients with advanced-stage melanoma increasing from approximately 9 months before 2011 to at least 2 years — and probably longer for those with *BRAF*-V600-mutant disease. Herein, we review the clinical trial data that established the standard-of-care treatment approaches for advanced-stage melanoma. Mechanisms of resistance and biomarkers of response to BRAF-targeted treatments and immunotherapies are discussed, and the contrasting clinical benefits and limitations of these therapies are explored. We summarize the state of the field and outline a rational approach to frontline-treatment selection for each individual patient with *BRAF*-mutant melanoma.

The treatment landscape for advanced-stage, unresectable or metastatic melanoma has shifted dramatically over a short period of time. Before 2011, metastatic melanoma was considered a devastating disease and was almost uniformly fatal within 18 months of diagnosis. Standard-of-care treatments during this time included dacarbazine chemotherapy[1] and, in fit patients, immunotherapy with the cytokine IL-2 (REF. 2); the median overall survival of patients was ~9 months, and no treatment had been demonstrated to improve survival in a randomized phase III trial. Reflecting several decades of basic research into the genomics of cancer[3] and the fundamental underpinnings of the immune response against cancer[4], eight therapeutic agents have since been approved by the FDA, in rapid succession, for the treatment of melanoma (TABLE 1), with similar approvals made in other countries around the world. These agents include small-molecule inhibitors of BRAF or MEK, immunotherapeutic antibodies directed at cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell-death protein 1 (PD-1), and the modified oncolytic herpes virus talimogene laharparepvec (T-VEC). Indeed, after decades of trials with negative results in patients with metastatic melanoma, the primary end point of every phase III trial reported since 2011 has been achieved (FIG. 1). Importantly, in these phase III trials, seven of the eight approved agents have been associated with an improvement in overall survival, compared with that achieved with standard therapies — although T-VEC met the primary statistical end point of durable response rate, but was found to improve survival in only a subset of patients with injectable melanoma lesions in the skin and/or lymph nodes. Consequently, the goals of therapy in the metastatic setting have changed, shifting from a palliative delay in disease progression for a small minority, to durable clinical responses for a large minority and effective disease control and palliation for the majority.

Substantial heterogeneity exists in the natural history of metastatic melanoma, including differences in the pace of disease progression and the sites of metastatic lesions. Up to one-third of patients already have multifocal and rapidly progressing disease when metastatic melanoma is first detected. Such patients, who commonly have brain and visceral organ involvement, are largely incapable of achieving long-lasting remissions from either molecularly targeted treatments, or novel immunotherapies. Thus, the development of multimodality strategies, including radiation, surgery, and systemic therapy, as well as combined molecular targeted and immunological approaches, is a clinical imperative. By

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Key points

- Clinical therapeutics for advanced-stage melanoma have improved dramatically with the development of BRAF and MEK inhibitors, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell-death protein 1 (PD-1) blocking antibodies, and a modified oncolytic herpes virus that is delivered intratumourally
- The overall survival of patients with advanced-stage melanoma has improved from ~9 months before 2011 to an as yet undefined timeframe, with a subset of patients having ongoing long-term tumour control
- Melanoma, particularly cutaneous melanoma, is amendable to immunotherapy for various reasons, including extensive tumour infiltration by T cells, a high mutational load, and crosstalk between oncogenic signalling pathways and immunobiology
- Resistance mechanisms to BRAF-targeted treatments and immunotherapies are being elucidated; reactivation of the MAPK pathway is common after BRAF inhibition, whereas the effectiveness of both approaches might be limited by loss of tumour antigen presentation and T-cell trafficking
- To move the field of clinical therapeutics forward, a greater focus on specific patient populations (based on serum lactose dehydrogenase levels, ECOG performance status, and number of metastases), as well as on landmark progression-free and overall survival measures, will be required in clinical trials

contrast, most patients who develop metastatic disease following a diagnosis of high-risk primary or regionally advanced melanoma now have the possibility of surviving for years, owing to the availability of numerous effective treatment options. Moreover, the momentum of advances in effective systemic therapy for melanoma has raised expectations, and has motivated investigators to explore combination therapies to further increase clinical benefit; however, greater scrutiny of the results of clinical trials exploring combination therapies is required, in order to ensure that synergistic therapeutic interactions are achieved without synergistic toxicity.

Herein, we discuss the treatment of patients with unresectable or metastatic melanoma, paying particular attention to the frontline treatment of advanced-stage *BRAF*-mutant melanoma, comprising ~50% of cases[3,5,6] (FIG. 2). The phase III trial data supporting the use of BRAF–MEK inhibitor combinations, or anti-PD-1 antibodies — alone and in combination with an anti-CTLA-4 antibody — are reviewed. In addition, we explore the mechanistic considerations regarding resistance to therapy and biomarkers of efficacy, and the clinical considerations pertinent when choosing frontline therapy for patients with *BRAF*-mutant melanoma. Given the lack of effective targeted therapies available to patients with *BRAF*-wild-type tumours, the discussion of optimal patient selection for immunotherapy pertains equally to this large subgroup, with the possible exception of the uveal melanoma subpopulation[7,8].

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Genetic and immune landscape of melanoma

The genetic aetiology of the disease. Melanomas are associated with one of the greatest burdens of somatic genetic alterations of all human tumours[5,6]; however, the number of mutations per melanoma cell is highly variable between patients, highlighting that not all melanomas are associated with the DNA damage induced by chronic or even intermittent sun exposure. Nevertheless, melanomas arising on the head, neck, or upper extremities are typically associated with the highest rates of genetic alteration[9]; those that arise on the trunk or lower extremities fall in an intermediate range, while those that arise in acral, mucosal, or uveal surfaces generally have the lowest rates of mutation[9]. This spectrum of genetic complexity has two important ramifications with regard to treatment: the variable presence of mutations in pathways that confer resistance to therapies targeting the MAPK pathway (that is, BRAF and MEK inhibitors), and variable enrichment of neoepitopes that confer sensitivity to immunotherapy.

Highly focused genetic characterizations first identified *CDKN2A*[10] and *NRAS* mutations[11] as being common recurrent events in malignant melanomas. Subsequently, loss of *PTEN* was observed[12], and *BRAF* mutation was eventually demonstrated to be the most-common oncogenic event in melanomas[3]. Broader genetic profiling has revealed that aberrations affecting the tumour-suppressor genes *CDKN2A* (encoding both $p16^{INK4A}$ and $p14^{ARF}$, which restrain cell division via inhibition of cyclin-dependent kinases) or *PTEN* (which encodes a negative regulator of the PI3K pathway) can cooperate with oncogenic mutation of the MAPK-pathway genes *BRAF* or *NRAS* in driving melanomagenesis[5,6,13] (FIG. 2). This synergy was most powerfully corroborated in mouse transgenic models in which pairwise activation and inactivation of *NRAS* and *CDKN2A*, *BRAF* and *PTEN*, or *BRAF* and *CDKN2A*, respectively, gave rise to melanoma with high fidelity[14,15]. Characterization of tumours from large cohorts of patients with advanced-stage melanoma at the whole-genome level has provided greater granularity regarding the overall prevalence of these genetic alterations, as well as their overlap[5,9,16] (FIG. 2). Such studies have identified inactivating mutations in *NF1* — a negative regulator of RAS signalling — as defining another distinct melanoma subtype. Six RAS effector pathways have been described; thus, NF1 and NRAS mutations cannot be considered functionally or therapeutically in the same sense as those in BRAF, which is a central component of the MAPK pathway.

Activating mutations in *KIT* also define a mutually exclusive subset of advanced-stage melanomas, comprising ~1% of all cases[17]. These alterations are disproportionately found in acral and mucosal melanomas, being present in ~10% of these less-common forms of melanoma[18]. Among the well-described tumour-suppressor genes, *CDKN2A* mutation is detected in ~50% of advanced-stage melanomas, and can rarely overlap with *PTEN* deletion (FIG. 2). Both of these genetic events can

Table 1 | **Systemic therapies approved since 2011 for advanced-stage melanoma**

Agent	Mechanism	FDA-approved indications
Targeted therapies		
Vemurafenib	BRAF inhibitor	As monotherapy and in combination with cobimetinib for *BRAF*$^{V600E/K}$-mutant disease
Dabrafenib	BRAF inhibitor	As monotherapy and in combination with trametinib for *BRAF*$^{V600E/K}$-mutant disease
Trametinib	MEK inhibitor	As monotherapy and in combination with dabrafenib for *BRAF*$^{V600E/K}$-mutant disease
Cobimetinib	MEK inhibitor	In combination with vemurafenib for *BRAF*$^{V600E/K}$-mutant melanoma
Immunotherapies		
Ipilimumab	Anti-CTLA-4 antibody	As monotherapy and in combination with nivolumab
Pembrolizumab	Anti-PD-1 antibody	As a monotherapy
Nivolumab	Anti-PD-1 antibody	In combination with ipilimumab, or as monotherapy
Oncolytic viral therapy		
Talimogene laharparepvec	Modified oncolytic herpes virus	Local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with recurrent melanoma after surgery

CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; PD-1, programmed cell-death protein 1.

occur with or without *BRAF*, *NRAS*, or *NF1* alterations, but are known to cooperate in driving resistance to BRAF inhibitors in *BRAF*-mutant melanoma cell lines[19] and biopsy samples of resistant tumours[20,21].

Considerations for molecular diagnosis. In the context of current clinical melanoma practice, only molecular analysis of whether a *BRAF* V600 mutation is present is essential to guide treatment decision-making. V600E mutation is by far most common, comprising 74–86% of all *BRAF* mutations[5,6,22]. The prevalence of V600K mutations among the BRAF-V600-mutant population can range from 10% up to 30%[5,6,22]: V600K mutations are most frequently found in older patients (aged >65 years) and/or in those with evidence of chronic UV exposure[23], which contributes to demographic variations in prevalence. A sufficient body of evidence has been generated with the BRAF inhibitors vemurafenib and dabrafenib in patients harbouring V600K mutations for this subpopulation to be included, together with the V600E subgroup, in the regulatory approvals of both agents (TABLE 1). Other *BRAF* alterations are much rarer. A small proportion of patients with *BRAF*-mutant melanomas (3–5%) have other V600 amino acid substitutions, for example, V600M, V600D, or V600R[5,6,22]. Clinical evidence is currently insufficient to quote the likelihood that such patients will respond to the available BRAF inhibitors, although good response rates have been documented in case studies[24].

An additional 3–5% of melanomas harbour other forms of genetic alteration in BRAF that are also considered to be oncogenic[5]. These aberrations are divided between missense mutations in the region adjacent to V600 (within exon 15) or distant from this site (within exon 11), and *BRAF* translocations (also referred to as fusions)[25–28]. In melanomas and other cancers, dozens of sites in *BRAF* exons 11 and 15 (REFS 29–31) have been found to harbour missense mutations, but only a small number have been functionally characterized. Those that have been investigated seem to result in activation of the MAPK pathway, in cooperation with CRAF[31]. *BRAF* translocations generate proteins in which the kinase domain of BRAF is fused with regulatory domains of various other proteins[27]. Preclinical evidence indicates that the currently available BRAF inhibitors do not inhibit these non-V600-mutant forms of BRAF, nor BRAF fusion proteins[27,32]; however, MEK inhibitors are effective in suppressing signalling downstream of these BRAF mutants in experimental systems, and anecdotal reports of patients with such alterations responding to MEK inhibitors have been published[26–28].

Beyond BRAF, data from phase II trials indicate a possibility of clinical responsiveness to KIT inhibitors in the *KIT*-mutant melanoma population[33–35]. Otherwise, testing for *NRAS* or *NF1* mutations is largely a matter of identifying patients who might be candidates for clinical trials. Emerging evidence supports the importance of *CDKN2A* and/or *PTEN* inactivation as mediators of resistance and a lower likelihood of achieving a durable response to BRAF-inhibitor-based therapy, but is not currently considered in clinical decision-making.

State of immune recognition in melanoma. Robust infiltration of lymphocytes into primary melanomas has long been associated with a reduced risk of metastasis. In the largest available population-based analysis of primary invasive melanomas[36], 15% of primary lesions had 'brisk' infiltration (lymphocytes throughout the tumour and/or along almost its entire base), 64% 'non-brisk', while 20% had no lymphocytic infiltration. Using this classification scheme, a dose–response relationship between an increasing density of lymphocytes and protection from melanoma fatality was observed[36]. Comparatively less evidence is available regarding the influence of lymphocytic infiltration into metastatic lesions on patient prognosis. In a cohort of patients with regional lymph-node metastases, however, broad gene-expression profiling identified

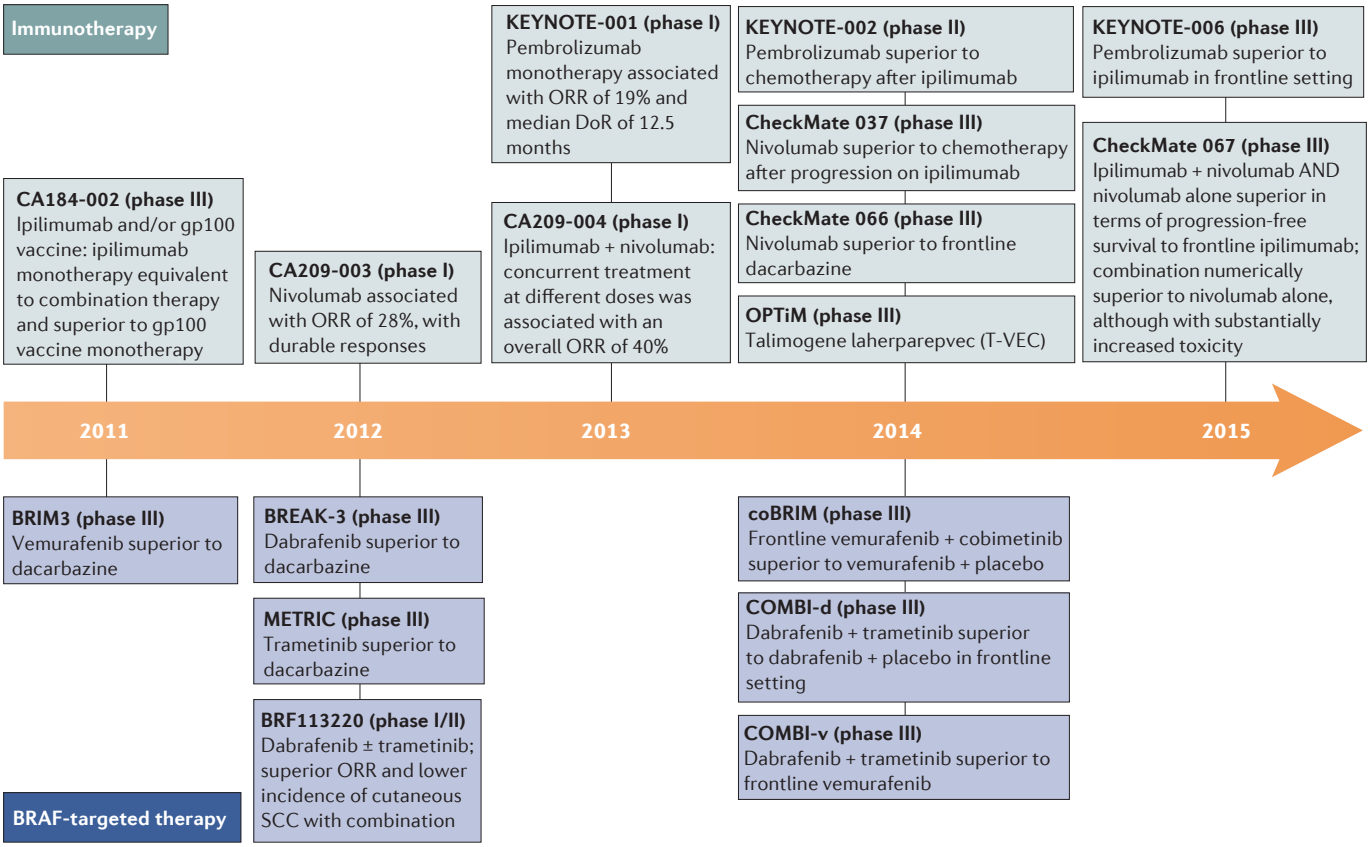


Figure 1 | **Timeline charting the seminal, practice-changing clinical trials in advanced-stage melanoma.** Over the past decade, rapid improvements in efficacy of melanoma therapeutics have been made. Since 2011, all reported large, randomized, phase III studies conducted in patients with advanced-stage disease have met their primary end points, ultimately providing a range of treatment options. Studies of immunotherapies have included ipilimumab, pembrolizumab, nivolumab, and the ipilimumab plus nivolumab combination. At the same time, monotherapy with BRAF (vemurafenib and dabrafenib) and MEK (trametinib) inhibitors, and subsequently combination therapy with BRAF and MEK inhibitors (dabrafenib plus trametinib, or vemurafenib plus cobimetinib) have been developed. In addition, Talimogene laherparepvec (T-VEC), a modified oncolytic herpes virus, has been clinically evaluated and approved. DoR, duration of response; GM-CSF, granulocyte macrophage colony-stimulating factor; ORR, objective response rate; SCC, squamous-cell carcinoma.

markers of immune infiltration as positive predictors of distant metastasis and unfavourable overall survival[37].

More-recent investigations have focused on specific antigens recognized by $CD8^+$ and $CD4^+$ T cells. Abundant data indicate that 'shared' melanocyte lineage antigens, such as tyrosinase[38], can be recognized by T cells in patients with melanoma, particularly following investigational vaccinations[39–41]. Larger-scale analyses are needed to understand the full repertoire of shared and tumour-specific epitopes that can be recognized in melanomas, even in untreated patients. Nevertheless, this type of deep analysis of tumour antigens has provided insight into potential relationships between the very high somatic mutation burden of melanomas and the elaboration of specific immune responses.

Given that immune recognition of primary and metastatic melanomas is common, although not ubiquitous, attention has turned to understanding the molecular features of tumour-mediated immunosuppression. Indeed, an extensive literature describes the expression of cell-surface factors that suppress T-cell effector functions[42], the production of immunomodulating cytokines, and the presence of immunosuppressive cell types[43], all of which can be exploited by tumour cells to evade and escape antitumour immunity. This understanding of therapeutic immune targets is now being used to guide novel drug development.

Clinical trial evidence

Both BRAF-targeted and immunotherapy approaches have been shown to substantially improve the overall survival of patients with advanced-stage melanoma (FIG. 3). To date, however, no prospective analysis of the optimal choice of frontline treatment has been completed. The approved anti-PD-1 antibodies nivolumab and pembrolizumab have demonstrated efficacy in patients with *BRAF*-mutant, BRAF-inhibitor-refractory disease[44,45], but similar data are lacking for ipilimumab[46], or for BRAF-inhibitor-based therapy in those refractory to anti-PD-1-antibody therapy. To inform decisions on frontline therapy, the outcomes of BRAF-targeted therapy followed by immunotherapy, or *vice versa,* are being compared directly in an

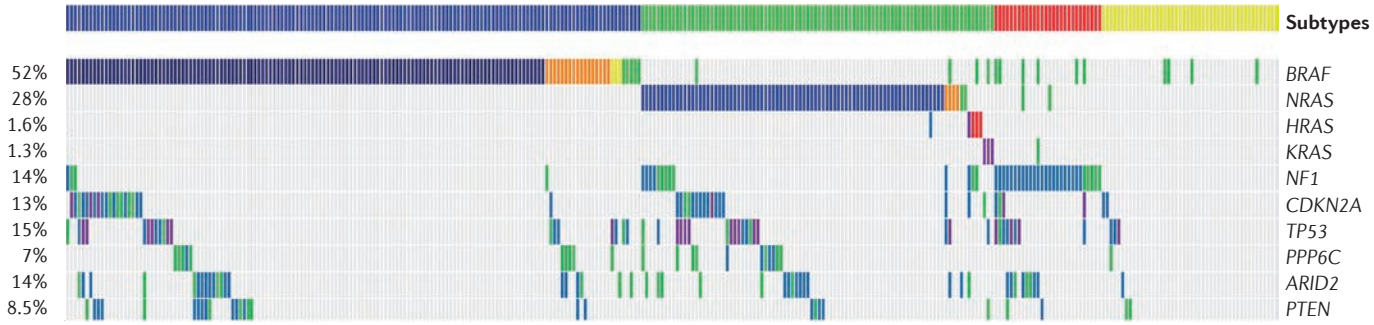


Figure 2 | **Frequency and overlap of alterations in driver and tumour-suppressor genes associated with melanoma.** Advanced-stage melanomas have been categorized according to mutational status into four main molecular subtypes, defined by mutations in either *BRAF*, *RAS*, *NF1*, or none of these genes. The effects of these driver mutations are additionally modified by mutations in other genes, such as *CDKN2A* and *PTEN*. Reproduced with permission from Elsevier © The Cancer Genome Atlas Network. *Cell* **161**, 1681–1696 (2015).

ongoing clinical trial (NCT02224781). In the absence of such data, clinicians aim to derive maximal benefit from all of the available therapies, with the hope that salvage therapy with either approach offers similar clinical benefit to that observed in the frontline setting in clinical trials.

BRAF-targeted therapies. The era of BRAF-targeted therapy dawned with the discovery that approximately half of all melanomas harbour *BRAF* mutations[3]. Initial attempts to block BRAF via non-RAF-isoform-selective inhibitors were unsuccessful[47]; however, subsequent structure-guided drug-discovery efforts facilitated the development of clinically active BRAF inhibitors[48]. The first-in-class agent was vemurafenib, a selective inhibitor of V600-mutant BRAF. This agent provided a clinical benefit not previously observed in metastatic melanoma: in the randomized phase III BRIM3 trial comparison against dacarbazine chemotherapy[49], the objective response rate (ORR) by RECIST criteria[50] was 48% versus 5%, and the median progression-free survival (PFS) was 5.3 months versus 1.6 months; a median overall survival of 13.3 months versus 10.0 months (hazard ratio (HR) 0.75; $P=0.0085$) was reported in an extended follow-up study[51]. The selective BRAF inhibitor dabrafenib was developed soon thereafter, showing very similar clinical benefit to vemurafenib when compared with dacarbazine — ORR 50% versus 6% and a median PFS of 5.1 months versus 2.7 months (HR 0.30; $P<0.0001$)[52]. The toxicities commonly associated with BRAF inhibitors include rash, photosensitivity (vemurafenib only), arthralgia, fatigue, and fever (specifically for dabrafenib)[49,51,52]. In addition, BRAF-inhibitor monotherapy has been associated with the development of secondary cutaneous lesions, including keratoacanthoma and squamous-cell carcinoma, in approximately 15–20% of patients[49,51,52].

Owing to the successes with BRAF inhibitors and the appreciation that BRAF signalling is dependent on downstream activation of MEK1/2, the development of MEK inhibitors became a priority. Trametinib was the first MEK inhibitor to gain regulatory approval for use as a single agent. In the phase III METRIC trial[53], this agent was associated with an ORR of 22% and a median PFS of 4.8 months (TABLE 2), with common toxicities that included skin manifestations, diarrhoea, and fatigue, and less-common cardiac and ocular toxicities (such as cardiomyopathy and retinopathies, respectively). In parallel with the clinical development of BRAF and MEK inhibitors, translational investigations were elucidating the mechanistic underpinnings of molecular signalling through the MAPK pathway, and mechanisms of resistance to BRAF-inhibitor monotherapy[54]. This work was essential in identifying MEK as being of particular importance in BRAF-inhibitor resistance and, thus, the potential synergy between BRAF and MEK inhibitors[55]. The mechanistic specificity of this work additionally led to the description of a phenomenon dubbed 'paradoxical activation' of the MAPK pathway via BRAF inhibition in *BRAF*-wild-type cells, for example, keratinocytes[56–59]. This discovery suggested that some of the hyperproliferative cutaneous manifestations associated with BRAF inhibition in the clinic might be mitigated by combination therapy incorporating a MEK inhibitor. As hypothesized, a subsequent randomized phase I/II study of dabrafenib in combination with trametinib demonstrated an ORR of 76%, a median PFS of 9.4 months[60], and a median overall survival of 27.4 months[61] among patients with metastatic melanoma treated with the recommended dosing regimen (versus 54%, 5.8 months, and 20.2 months, respectively, among those treated with dabrafenib alone). Additionally, the cutaneous adverse effects of BRAF monotherapy were attenuated (7% with dual therapy versus 19% with BRAF-inhibitor monotherapy), although the incidence of fever related to dabrafenib was increased (71% versus 26%)[60].

The combination of dabrafenib and trametinib was further investigated in two international phase III clinical trials: COMBI-d[62] and COMBI-v[63]. In COMBI-d[62], 423 patients received either dabrafenib and trametinib, or dabrafenib and placebo; the ORR was 76% versus 54%, the median PFS was 11.0 months versus 8.8 months, and the median overall survival was 25.1 months versus 18.7 months (HR 0.71; $P=0.0107$). Toxicity profiles were similar to those seen in previous trials of these agents, with febrile syndrome more common, and hyperkeratotic cutaneous manifestations less common in the combination group[62]. In COMBI-v[63], the same BRAF–MEK inhibitor combination used in COMBI-d was compared

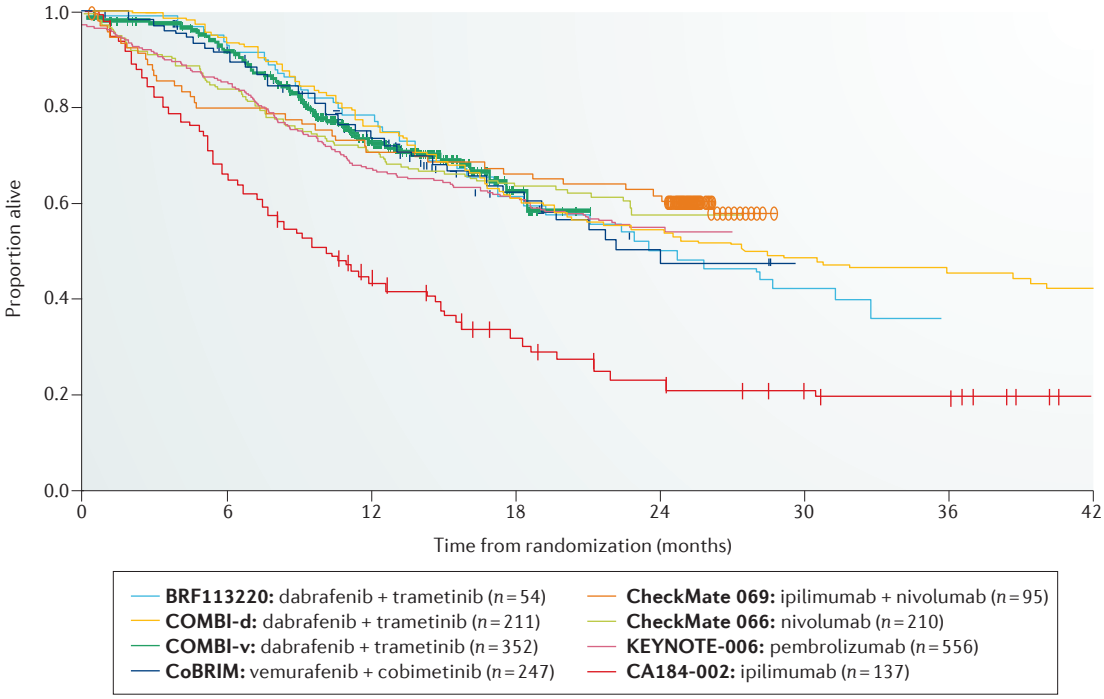


Figure 3 | **Summary of overall survival by Kaplan–Meier analysis across seminal clinical trials in patients with advanced-stage melanoma.** The clinical trials that have defined modern clinical care of patients with advanced-stage melanoma are shown with colour-coding to identify each study. The BRF113220, COMBI-d, COMBI-v and CoBRIM trials were performed only in patients with *BRAF*$^{V600E/K}$-mutant melanoma. Median and landmark overall survival outcomes have been improved with both BRAF-directed treatment and immunotherapy approaches. *n*, number of patients treated.

to BRAF monotherapy with vemurafenib (rather than with dabrafenib). Among the 704 patients randomized, the ORR to dabrafenib plus trametinib was 64% versus 51% with vemurafenib, the median PFS was 11.4 months versus 7.3 months, and the median overall survival was 25.1 months versus 18.7 months (HR 0.69; $P = 0.005$)[64].

Similar to the results seen with dabrafenib plus trametinib, the combination of vemurafenib and the MEK1/2 inhibitor cobimetinib had substantial efficacy in a phase I study[65]: the ORR was 87% and the median PFS was 13.7 months in patients who were naive to prior treatment with a BRAF inhibitor. As anticipated, the incidence of cutaneous hyperproliferative manifestations was substantially lower with this combination than that expected with BRAF-inhibitor monotherapy[65]. The most common toxicities included diarrhoea, rash, liver-enzyme abnormalities, fatigue, and nausea[65]. These data led to initiation of the phase III coBRIM trial[66–68], in which 495 patients received vemurafenib plus cobimetinib, or vemurafenib plus placebo, with ORRs of 70% versus 50%, a median PFS of 12.3 months versus 7.2 months, and a median overall survival of 22.3 months versus 17.4 months (HR 0.70; $P = 0.005$). The toxicities observed were similar to those seen in the phase I study[65–68].

With the data from these phase III trials, BRAF-inhibitor and MEK-inhibitor combination therapy has become the standard-of-care BRAF-targeted treatment for *BRAF*-mutant melanoma. The choice between the two BRAF–MEK-inhibitor regimens depends on patient-related factors, such as the ability to tolerate fever associated with dabrafenib and trametinib versus the cutaneous and gastrointestinal adverse events associated with vemurafenib and cobimetinib. These differences have been reviewed elsewhere[69,70]; these papers are a recommended resource when further considering these combinations for an individual patient.

MEK-based therapy for melanoma without BRAF V600 mutation. As noted, MEK inhibitors have demonstrated single-agent efficacy in the *BRAF*-V600-mutant population, with ORRs of >20% with trametinib[53] as well as other MEK inhibitors evaluated in phase II clinical trials[71]. In addition, preclinical evidence indicates that MAPK-pathway dependency and the resultant therapeutic vulnerability to MEK inhibitors exist in a substantial portion of melanomas that lack *BRAF* V600 mutations. Clinically, this vulnerability has been evaluated most thoroughly in patients with *NRAS*-Q61-mutant melanoma with the MEK inhibitor binimetinib: results of a signal-finding phase II study showed an ORR of 20%, with a median PFS of 4 months[71]. Subsequently, the first report of a phase III trial of binimetinib versus chemotherapy confirmed a statistically superior PFS with binimetinib (median of 2.8 months versus 1.5 months; $P < 0.001$), although overall survival was not significantly improved in an interim analysis (HR 0.81)[72].

On the basis of these phase III results, MEK-inhibitor monotherapy is moving towards regulatory approval in the non-*BRAF*-mutant melanoma setting; however, the clinical benefit is modest. Beyond monotherapy,

a growing body of literature supports the existence of positive mechanistic interactions of MEK inhibitor with cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors[73], MDM2 antagonists[74], or PI3K/AKT-pathway inhibitors[75], leading to phase I and II trials investigating these combinations. Responses to MEK inhibitors have also been observed in patients with tumours harbouring non-V600 *BRAF* mutations, or a *BRAF*/*NRAS*-wild-type status[26–28]; however, the numbers of patients included were small, limiting comparisons between the outcomes of these groups and those observed with MEK-inhibitor monotherapy in *BRAF*-V600-mutant and *NRAS*-mutant populations. Nevertheless, the current evidence in *NRAS*-mutant melanoma indicates that investigation of MEK-inhibitor-based combination regimens seems justified.

Immunotherapy with immune-checkpoint inhibitors. Historically, immunotherapy with interferon and interleukin cytokines has been used in the treatment of advanced-stage melanoma, but with substantial toxicity and modest clinical benefit[76]. With the elucidation of specific immune-regulatory molecules, modern immunotherapeutic approaches have focused on augmentation of cell-mediated immunity using monoclonal antibodies. This approach, termed 'immune-checkpoint blockade', was pioneered with the development of the anti-CTLA-4 antibodies ipilimumab and tremelimumab. These antibodies were developed in parallel and share many biochemical features; however, only ipilimumab gained regulatory approval for melanoma therapy.

The efficacy of ipilimumab has been evaluated in the phase III CA184-002 and CA184-024 trials, as a single agent[77] and in combination with dacarbazine chemotherapy[78], respectively (TABLE 2). These studies demonstrated ORRs of 11–15%, a median PFS of ~3.0 months, and a median overall survival of 10.1–11.2 months (ipilimumab monotherapy versus a gp100 vaccine: HR 0.68, $P<0.001$; ipilimumab plus dacarbazine versus dacarbazine, HR 0.72, $P<0.001$)[77,78]. In these studies, however, ipilimumab was used at a different dose: 3 mg/kg in the monotherapy trial versus 10 mg/kg in the combination therapy trial. Ipilimumab monotherapy at 10 mg/kg and 3 mg/kg has been compared directly in the CA184-169 trial[79], which involved 727 patients; the median overall survival was improved with the higher dose (15.7 months versus 11.5 months; HR 0.84; $P<0.04$), but at the expense of increased toxicity (rate of grade 3–4 events: 34% versus 19%).The toxicities associated with ipilimumab include immune-related phenomena, such as dermatitis, diarrhoea, colitis and, less commonly, hepatitis, uveitis, and hypophysitis[80].

Disease progression before obtaining disease control or tumour shrinkage occurs in approximately 10–15% of patients treated with ipilimumab; these atypical patterns of treatment response were formalized into a set of radiological imaging criteria termed the immune-related response criteria (irRC)[81]. Assessment of response using the irRC has become common practice for immune-checkpoint inhibitors, with a particular emphasis on the need to confirm progression at least 4 weeks from the initial imaging scan that indicated progressive disease. A consistent observation throughout the clinical trials of anti-CTLA-4 antibodies was that a proportion of patients had long-term survival independent of the extent of response. In a pooled-analysis of 1,861 patients who had participated in the phase II and III clinical trials of ipilimumab[82], 22% were alive at 3 years and a plateau on the survival curve suggested the likelihood of longer-term survival thereafter. A clinical point of interest surrounding ipilimumab and other checkpoint-inhibitor immunotherapies relates to the use of concomitant steroid administration. This question has not be thoroughly investigated, although findings from a phase II study of ipilimumab in patients with brain metastases suggested a detrimental effect on the response rate in a cohort receiving concurrent steroids[83]. More broadly, however, data from multiple patient series have demonstrated that administration of steroids at the time of immune-related toxicity does not affect the potential long-term benefit from immune-checkpoint blockade[84]. Immune-related toxicities observed in clinical practice should therefore be managed intensely at the time of initial observation.

After CTLA-4 blockade provided proof of concept for immune-checkpoint inhibition, anti-PD-1 antibodies underwent rapid clinical development. Large phase I clinical trials of pembrolizumab and nivolumab showed response rates ranging from 20–40% in patients with melanoma, depending on the line of therapy[85,86] (FIG. 1). The spectrum of toxicities with these agents was similar to that observed for ipilimumab, although the frequency and severity of adverse events was lower (approximately 10–15% for grade 3–4 adverse events)[80,85,86]. Subsequent clinical trials demonstrated the efficacy of both anti-PD-1 agents in terms of ORR and PFS in the second-line setting after ipilimumab treatment[87,88], and in terms of overall survival with frontline nivolumab[89], compared with chemotherapy in each case.

Phase III trials have also demonstrated clinical benefits from pembrolizumab and from nivolumab in the first-line setting. In the KEYNOTE-006 study[45], 2-weekly and 3-weekly schedules of pembrolizumab both provided an ORR, PFS, and overall survival benefit compared with standard ipilimumab therapy, but were not statistically different from each other (TABLE 2). Adverse events were similar to those seen in other trials of immune-checkpoint inhibitors, with grade 3–4 adverse events observed in 10.1–13.3% of patients treated with pembrolizumab and 19.9% of those treated with ipilimumab. Regarding nivolumab monotherapy, the first interim analysis of the phase III CheckMate 067 trial[90] revealed considerable ORR and PFS improvements with this agent, compared with those of ipilimumab monotherapy (the overall survival data remain immature; TABLE 2). Adverse events were as expected for agents of this class: 16.3% of patients had grade 3–4 adverse events with nivolumab versus 27.3% with ipilimumab[90].

As the clinical development of anti-PD-1 antibodies proceeded, a growing body of research suggested an added benefit in tumour response when administered in combination with an anti-CTLA-4 antibody[91]. From a mechanistic perspective, this finding was consistent with the contrasting role of PD-1 in regulating the effector

Table 2 | **Phase III trials with results that have shaped the landscape of frontline therapy for advanced-stage melanoma**

Trial	Primary end point	Treatment arms (*n*)	Baseline characteristics (proportion of patients)					RECIST ORR	PFS		OS		
			BRAF$^{V600E/K}$ mutated	Previously untreated	LDH >ULN	ECOG PS>0	M1c		Median	1-year	Median	1-year	2-year
CA184-002 (REF. 77)	OS	gp100 vaccine (136)	NR	0%	38.2%	48.5%	72%	1.5%	2.8 mo	NR	6.4 mo	25%	13.7%
		gp100 vaccine + ipilumumab (403)	NR	0%	37%	42.4%	70%	5.7%	2.8 mo	NR	10.0 mo	44%	21.6%
		Ipilimumab (137)	NR	0%	38.7%	47.4%	73%	11%	2.9 mo	NR	10.1 mo	46%	23.5%
CA184-024 (REF. 78)	OS	Dacarbazine (252)	NR	100%	43.7%	29%	55%	10.3%	3 mo	NR	9.1 mo	36%	17.9%
		Dacarbazine + ipilimumab (250)	NR	100%	37.2%	29.2%	57%	15.2%	3 mo	NR	11.2 mo	47%	28.5%
CA184-169 (REF. 79)	OS	Ipilimumab (10 mg/kg)	22%	44%	36%	28%	63%	15%	2.8 mo	15%	15.7 mo	54%	38%
		Ipilimumab (3 mg/kg)	22%	43%	38%	30%	61%	12%	2.8 mo	15%	11.5 mo	48%	31%
BRIM3 (REFS 49, 51)	PFS + OS	Dacarbazine (338)	100%	100%	58%	32%	65%	5%	1.6 mo	NR	9.7 mo	NR	NR
		Vemurafenib (337)	100%	100%	58%	32%	66%	48%	5.3 mo	NR	13.6 mo	NR	NR
BREAK-3 (REFS 52, 159)	PFS	Dacarbazine (63)	100%	2%	30%	25%	63%	7%	2.7 mo	NR	NR	NR	NR
		Dabrafenib (187)	100%	3%	36%	33%	66%	50%	5.1 mo	NR	20 mo	NR	45.0%
CheckMate 066 (REF. 89)	OS	Dacarbazine (208)	0%	100%	35.6%	41.8%	61%	13.9%	2.2 mo	8%	10.8 mo	41%	NR
		Nivolumab (210)	0%	100%	37.6%	29.1%	61%	40%	5.1 mo	44%	Not reached	73%	NR
METRIC[53]	PFS	Chemotherapy (108)	100%	65%	39%	36%	58%	8%	1.5 mo	NR	NR	NR	NR
		Trametinib (214)	100%	67%	36%	36%	67%	22%	4.8 mo	NR	NR	NR	NR
COMBI-d‡ (REF. 62)	PFS	Dabrafenib (212)	100%	100%	33%	29%	65%	51%	8.8 mo	34%*	18.7 mo	68%	42%
		Dabrafenib + trametinib (211)	100%	100%	36%	27%	67%	67%	11 mo	44%*	25.1 mo	74%	51%
COMBI-v (REF. 63, 64)	OS	Vemurafenib (351)	100%	100%	32%	30%	59%	51%	7.3 mo	29%*	18 mo	64%	38%
		Dabrafenib + trametinib (351)	100%	100%	34%	29%	63%	64%	11.4 mo	50%*	25.6 mo	73%	51%
coBRIM[68]	PFS	Vemurafenib (248)	100%	100%	43%	33%	62%	50%	7.2 mo	30%*	17.4 mo	64%	38%
		Vemurafenib + cobimetinib (247)	100%	100%	46%	24%	59%	69.6%	12.3 mo	50%*	22.3 mo	75%	48%
KEYNOTE-006 (REFS 45, 103)	PFS + OS	Ipilimumab (278)	38.5%	65.1%	32.7%	32.4%	64%	11.9%	2.8 mo	19%	16 mo	58%	43.0%
		Pembrolizumab 2-weekly (279)	35.1%	65.6%	29%	29.7%	64%	33.7%	5.5 mo	39%	Not reached	74%	55.0%
		Pembrolizumab 3-weekly (277)	35.0%	66.8%	35.4%	31.8%	68%	32.9%	4.1 mo	38%	Not reached	68%	55.0%

Table 2 (cont.) | **Phase III trials with results that have shaped the landscape of frontline therapy for advanced-stage melanoma**

Trial	Primary end point	Treatment arms (*n*)	Baseline characteristics (proportion of patients)					RECIST ORR	PFS		OS		
			BRAF$^{V600E/K}$ mutated	Previously untreated	LDH >ULN	ECOG PS>0	M1c		Median	1-year	Median	1-year	2-year
CheckMate 067 (REF. 90)	PFS+OS	Ipilimumab (315)	30.8%	100%	36.5%	28.9%	58%	19%	2.9 mo	18%	NR	NR	NR
		Nivolumab (316)	31.6%	100%	35.4%	24.7%	58%	43.7%	6.9 mo	42%	NR	NR	NR
		Ipilimumab + nivolumab (314)	32.2%	100%	36.3%	26.7%	58%	57.6%	11.5 mo	49%	NR	NR	NR
OPTiM[98]	Durable response lasting ≥6 months	GM-CSF (141)	NR[§]	46%	4%	23%	21%	NR[‖]	NR[‖]	NR[‖]	18.9 mo	NR	40%
		T-VEC (295)	NR[§]	47%	5%	28%	23%	NR[‖]	NR[‖]	NR[‖]	23.3 mo	NR	50%

ECOG PS, Eastern Cooperative Oncology Group performance status; GM-CSF granulocyte-macrophage colony-stimulating factor; LDH > ULN, serum lactate dehydrogenase level greater than upper limited of normal; mo, months; *n*, number of patients; NR, not reported; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RECIST, Response Criteria In Solid Tumours; T-VEC, Talimogene Laherparepvec; *Approximately, based on reading of Kaplan-Meier plots. [‡]3-year PFS and OS were 22% and 44% respectively in the combination arm of this trial[156]. [§]Data collected was incomplete. [‖]Neither RECIST nor WHO criteria were used to assess response.

phase of the immune response, as opposed to the role of CTLA-4 in mediating the priming phase. The results from a phase I clinical trial evaluating the safety of combination therapy with ipilimumab and nivolumab supported a phase II dosing regimen of 3-weekly ipilimumab (3 mg/kg) and nivolumab (1 mg/kg) for four doses, followed by 2-weekly nivolumab maintenance at 3 mg/kg thereafter[92]. In comparison with historical controls for either agent as monotherapy, an apparent increase in the toxicity profile of the combination therapy was observed, with approximately 50% of patients experiencing grade 3–4 adverse events[92]. Importantly, clinical efficacy seemed to be improved, with an ORR of 53% at the recommended phase II dose[92]. In the subsequent phase II CheckMate 069 study[93], the efficacy of the combination regimen was compared with that of standard ipilimumab monotherapy in 142 patients (randomized 2:1). Clinical end points were statistically improved in those who received combination therapy: ORR of 61% versus 11%; median PFS not-reached versus 4.4 months (HR 0.40, $P<0.001$). The adverse-events profile was similar to that of the phase I study of the combination[92], with 54% of patients experiencing grade 3–4 adverse events compared with 24% treated with ipilimumab only[93]. An even more robust analysis of this combination approach was performed in the aforementioned CheckMate 067 trial[90], which included a nivolumab plus ipilimumab treatment arm in addition to the previously described monotherapy arms. The results of this study demonstrated substantially improved ORR and PFS with combination therapy compared with either nivolumab or ipilimumab alone (TABLE 2). The spectrum and incidence of adverse events was consistent with those noted in prior trials, with grade 3–4 toxicity in 55% of patients treated with the combination versus 27.3% of those treated with ipilimumab only[90]. Although the overall survival data remain immature, landmark survival data from the phase II CheckMate 069 study indicate survival rates of 73% with the combination versus 65% with ipilimumab monotherapy at 1 year, and 64% versus 54% at 2 years[94].

Before the emergence of ipilimumab plus nivolumab combination therapy, the default treatment paradigm in metastatic melanoma had been sequential therapy using the available agents. The question has arisen as to whether concurrent use of ipilimumab plus nivolumab offers benefits that are not gained with sequential single-agent anti-PD-1 and anti-CTLA-4 therapy. This question has been addressed indirectly, to some extent, by data from the randomized phase II CheckMate 064 study[95], wherein the investigators assessed the safety of sequential administration of these agents as a primary end point, but also obtained preliminary data surrounding the efficacy of this approach. In this study, patients were treated with nivolumab induction then a forced switch to ipilimumab followed by nivolumab maintenance therapy, or with ipilimumab induction then a forced switch to nivolumab with subsequent nivolumab maintenance[95]. Although toxicity was statistically similar between the two arms throughout the entire treatment period, and broadly consistent with that observed in CheckMate 067 and CheckMate 069, efficacy outcomes were superior in the nivolumab-first arm relative to the ipilimumab-first arm[95]. In the nivolumab-first arm, the ORR after both nivolumab and ipilimumab treatment was 56%, the median overall survival was not reached, and 12-month survival was 76% (compared with 31%, 16.9 months, and 54%, respectively in the ipilimumab-first arm)[95]. These data must be interpreted cautiously relative to those obtained with upfront combination therapy with nivolumab and ipilimumab, given the sample size limitations and the lack of primary statistical analyses to directly address this question. Nevertheless, sequential administration of nivolumab then ipilimumab seems to preliminarily compare favourably to the concurrent combination of these agents. More overall survival data for the combination approach are needed, although one can confidently state that offering ipilimumab plus nivolumab therapy to patients who are not candidates for sequential therapy (for example, those with a high disease burden or rapid progression) is reasonable.

Talimogene laherparepvec (T-VEC). The most recently approved agent for the treatment of patients with advanced-stage, unresectable melanoma is T-VEC, an injectable modified herpes virus genetically engineered to selectively replicate in tumour cells, and to produce granulocyte-macrophage colony-stimulating factor (GM-CSF)[96]. The virus itself is known to elicit immune responses and, when incorporated into tumour cells, induces tumour-specific production and secretion of GM-CSF, which independently enhances antigen presentation by tissue-resident macrophages[97]. Thus, the combination of the virus and cytokine production is intended to induce T-cell recognition of virally-infected tumour cells and to promulgate a broader immune response to tumour antigens released during the first wave of the immune response.

T-VEC must be administered directly into tumours and, therefore, was initially tested in patients with cutaneous and subcutaneous melanoma lesions (notably a population with in-transit disease). For such visible lesions, regression of the injected tumours was reproducibly demonstrated. Unlike with other injectable agents, responses to T-VEC were observed in adjacent uninjected lesions, and occasionally at distant metastases[96,98].

The rate of durable response (lasting ≥6 months, although a patient could initially have disease progression by RECIST criteria) in injected and non-injected lesions was the primary end point of the phase III OPTiM trial[98], in which T-VEC was compared to subcutaneously administered GM-CSF. By this end point, 16.3% of patients treated with T-VEC had a protocol-defined durable response compared with 2.1% with GM-CSF[98]. OPTiM had eligibility parameters chosen to select for patients with a relatively limited extent of distant metastatic disease; many patients had regionally advanced disease only. Differences in the durable response rates with the use of T-VEC versus GM-CSF were more pronounced in patients with stage IIIB–C (33% versus 0%) or IVM1a disease (16% versus 2%) than in those with stage IVM1b (3% versus 4%) or IVM1c disease (7% versus 3%)[98]. Accordingly, the systemic utility of this approach must be considered in the context of the other available systemic agents associated with higher rates of disease control. Comparison of the entire treatment cohorts did not reveal any statistically significant difference in overall survival between the two arms (TABLE 2), but a HR of 0.57 for overall survival was reported for the large subgroup of patients with stage IIIB–C and IVM1a disease[98]. Of note, most of the patients with a limited extent of disease would have had access to additional lines of therapy following disease progression; therefore, this difference in overall survival might be at least partially explained by an enhancement of responsiveness to subsequent therapies, as opposed to an intrinsic benefit from T-VEC alone. Toxicities associated with T-VEC are modest compared with those of any of the systemically administered therapies. Most patients experience some degree of inflammation at injection sites, sometimes painful. Fever and chills are infrequent and typically short-lived. No autoimmune toxicities similar to those seen with immune-checkpoint inhibitors have been reported[98].

On the basis of its mechanism of action, hypothetically, T-VEC could enhance the immunogenicity of melanomas for which baseline immune recognition is lacking or not robust. Given the emerging evidence suggesting that the efficacy of anti-PD-1 antibodies is largely confined to patients with tumours that have robust baseline $CD8^+$ T-cell infiltrates[99,100], T-VEC could potentially be deployed before or concomitantly with immune-checkpoint inhibitors in those with few or no infiltrating T cells at baseline. Of note, preliminary reports have described high response rates in patients with advanced-stage melanoma treated using the combination of T-VEC and ipilimumab (~50%)[101], or pembrolizumab (~46%)[102]; a phase III trial of pembrolizumab with and without T-VEC is underway (NCT02263508).

Combination targeted and immunotherapy — first principles. Substantial evidence has established the benefit of BRAF-inhibitor-based therapy, at least in the short term, for most patients with *BRAF*-V600-mutant melanoma, whereas ~20% and ~35% of patients with an objective or stable-disease response to ipilimumab[82] and nivolumab[86] or pembrolizumab[103], respectively, maintain disease control for many years (up to 5 years based on the most up-to-date follow-up data). Thus, from a clinical perspective alone, the potential role of combination regimens containing molecularly targeted and immunotherapies is of considerable interest. Furthermore, evidence supports a positive effect of MAPK-pathway-targeted therapies on immune recognition.

Before the introduction of BRAF and MEK inhibitors into the clinic, oncogenic activation of the MAPK pathway was known to suppress the expression of microphthalmia-associated transcription factor (MITF)[104]. In turn, MITF downregulation can result in suppression of melanocyte-lineage antigen expression, some of which are known to be recognized by T cells in patients with advanced-stage melanoma[105]. In preclinical models and in patients, expression of MITF is upregulated following BRAF inhibition, with associated large-magnitude, although variable, increases in the expression of lineage antigens, such as gp100, melan-A, and tyrosinase-related proteins 1 and 2 (REFS 106,107). Whether increased expression of these antigens alone offers the opportunity for pre-existing tumour-infiltrating $CD8^+$ T cells to recognize and contribute to the eliminaton of melanoma cells remains unknown; however, the observation that tumour-infiltrating lymphocyte counts are increased early in the course of BRAF-inhibitor therapy, when compared with those of tumour biopsy samples taken from the same patients immediately before therapy, supports this concept[107,108]. The characteristics of these lymphocytes have been described only preliminarily[109], therefore, their contributions to therapeutic responses are unclear — markers of T-cell activation have been documented, but so have markers of T-cell exhaustion[104]. Current research is aimed at identifying specific T-cell clones within these infiltrates that can be evaluated *ex vivo* to demonstrate tumour specificity and monitored over time in correlation with exceptional responses.

Ex vivo, BRAF and MEK inhibitors have the expected effect on MAPK signalling in T cells from mice, namely stimulation and inhibition of this pathway, respectively[110]. In co-cultures of autologous melanoma cells and T cells from the same mice, BRAF inhibition stimulates T-cell proliferation and IFNγ production as a sign of net T-cell activation; additional MEK inhibition impairs these effects[110]. These observations raised concerns that MEK inhibitors might not be a useful targeted-therapy backbone for combination immunotherapy, and that BRAF–MEK combination therapy might not be as capable of potentiating an immune response as BRAF-inhibition alone. However, increasing evidence from preclinical *in vivo* experience with MEK inhibitors[110], and analyses of serial tumour-biopsy specimens from patients receiving combined BRAF–MEK-inhibitor therapy suggest a positive effect on the expression of MITF and melanocyte lineage antigens, and that T-cell infiltration persists under such treatment[107]. Moreover, a compelling additional observation indicates that MEK inhibitors can disrupt a deleterious signalling circuit between tumour cells and the so-called 'M2-like' macrophage population, which impair effector T-cell entry into tumours and drive melanoma-cell growth[111]. These findings suggest that combined BRAF–MEK inhibition could enhance immune recognition of melanoma cells, and that these effects on antitumour immunity could be enhanced through immune-checkpoint inhibition. Nevertheless, despite an increase in the number of clinical trials of combination therapy with immune-checkpoint inhibitors and BRAF and/or MEK inhibitors in the advanced-stage melanoma population, many mechanistic questions persist.

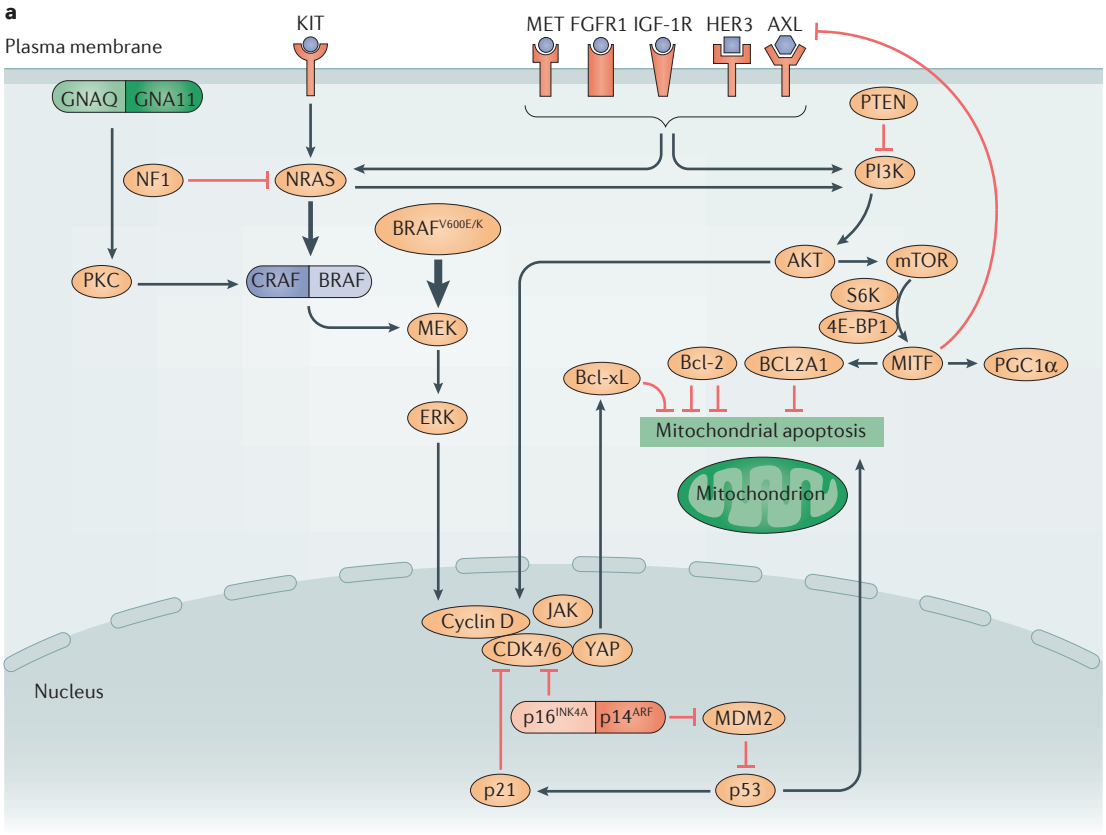
Another major gap in our knowledge pertains to the optimal scheduling and sequencing of targeted agents and immunotherapies. As discussed, evidence suggests that MAPK-pathway-targeted therapy positively affects immune responses, although the findings have generally been generated at very early time points in the course of therapy; when tumours are analysed months later, at the time of disease progression, the effects on melanoma antigen expression and T-cell infiltration have shown to have dissipated[108,109]. Limited data is available, however, on antigen expression and the state of the tumour microenvironment in patients who remain in response. Such information would provide critical guidance on the prioritization of treatment schedules to explore clinically. At present, ongoing clinical trials are exploring the safety of concomitant and continuous administration of BRAF, MEK, and PD-1 or PD-L1 inhibitors (NCT02130466, NCT02967692, NCT02908672). In addition, a neoadjuvant study is underway to explore such continuous triple therapy, as well as a short induction period of BRAF–MEK inhibition, followed by anti-PD-1 therapy (NCT02858921). Initial reports on these triplet combinations have suggested that they are well tolerated, with response rates similar to those observed with BRAF–MEK inhibition[112–114]. Of note, the very slow clearance of therapeutic antibodies might make it difficult to manipulate the immunotherapy administration schedule, but the small-molecule targeted agents might be intermittently dosed with sufficiently rapid clearance to provide a treatment-free interval. Such a strategy has several merits, most notably, the potential to minimize the risk of toxicities associated with combination therapy, and the ability to explore whether the effects of the targeted therapies on antigen expression and T-cell infiltration could be engaged repeatedly throughout the course of immune-checkpoint blockade.

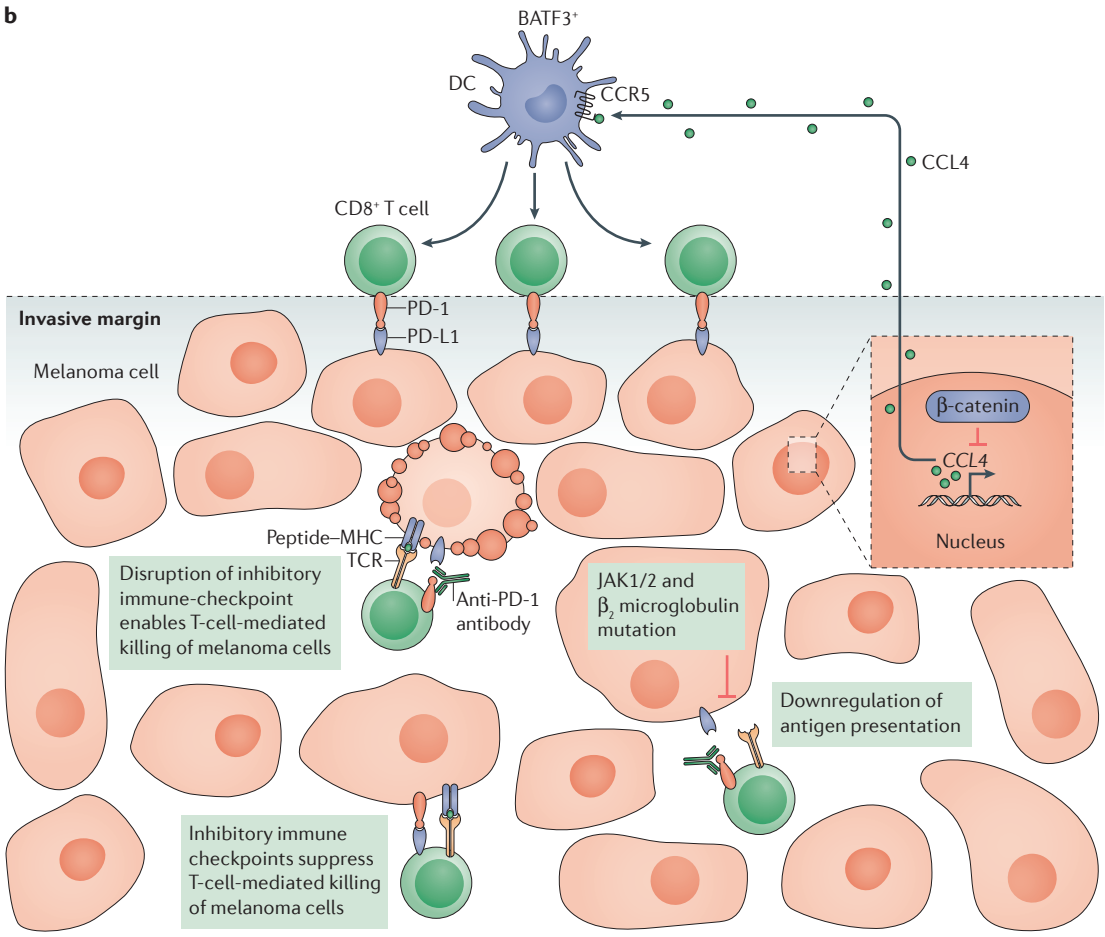
At present, combinations of molecularly targeted agents and immunotherapies must be considered investigational. Each approach alone has clear effectiveness, and translational research findings support potential positive interactions; however, combination therapy might substantially increase toxicity, or even undermine efficacy, in the long term. Combination regimens are particularly attractive in the setting of a very high disease burden (specifically in patients with a *BRAF* V600 mutation), but in the absence of more-extensive data regarding safety and early efficacy, the use of such regimens in routine practice cannot be endorsed.

Resistance and biomarkers of activity

Despite progress in the clinical management of advanced-stage melanoma, many patients eventually development resistance to treatment. Major efforts have been made to better identify factors that could be used to guide patient selection for a specific treatment, as well as the molecular mechanisms underpinning the lack of benefit observed either initially (primary resistance), or secondarily (acquired resistance).

BRAF–MEK-inhibitor resistance and biomarkers. In the arena of molecular approaches to the treatment of melanoma, and especially those directed at BRAF, a patient-selection biomarker is immediately obvious: the presence or absence of the targetable mutation, in particular, a *BRAF* V600 mutation. *BRAF* mutation is not a standalone predictor of effective treatment, however, given the heterogeneity of response observed in the clinic, and the eventual failure of the therapy in most patients. The elucidation of secondary mechanisms of resistance to BRAF-directed therapies has generated a substantial literature[115], although larger datasets and meta-analyses have revealed that only a handful of changes underlie the preponderance of genomic resistance mechanisms. In the largest studies to date[20,116–118], involving 132 samples obtained at the time of clinical resistance, one or more genomic causes of resistance were identified in 58% of patients: *NRAS/KRAS* mutation in 20%, *BRAF* splice variants in 16%, *BRAF* amplification in 13%, *MEK1/2* mutation in 7%, and non-MAPK-pathway alterations in 11%. Similar resistance mechanisms have been described with combined BRAF–MEK inhibition[119,120]. These genomic data have been confirmed in other studies; however, a large fraction of BRAF–MEK inhibitor resistance might also be driven through non-genomic or immune mechanisms of tumour escape[121]. In particular, aberrant methylation of CpG sites within the melanoma genome and other transcriptomic changes that affect the expression of several molecules (such as MET, LEF1, and YAP1), and decreases in antigen presentation and effector-T-cell function (among other immunological changes) might contribute to therapy resistance. With this possibility in





mind, a summary hypothesis postulates that, based on our current understanding, ~38% of tumour resistance to *BRAF*-targeted therapy can be attributed to non-genomic mechanisms, and 56% to both genomic and non-genomic aberrations, with the mechanisms remaining unknown in the final 6% of cases[121]. These findings will be of substantial importance when triplet inhibitor combinations are considered and the intersection between BRAF-directed therapy and immunotherapy is investigated.

As data on genomic and non-genomic resistance mechanisms accumulates, further combination regimens will be considered. The clinical feasibility of clinical trials is, however, daunting: the degree of heterogeneity of resistance observed between patients, as well as within different tumours in the same patient, is a major barrier to therapeutic progress. This hurdle could potentially be overcome through real-time monitoring for changes associated with resistance to BRAF–MEK-inhibitor therapy (for example, analysis of the predominant melanoma-cell clone at progression), and reactive use of targeted therapeutics based on the biological changes observed[122]. Such an approach would necessitate intermittent assessment of the ongoing status of the tumour; therefore, the substantial progress that has been made in the development of non-invasive monitoring technologies, such as 'liquid biopsy' of circulating tumour cells (CTC)[123] and circulating cell-free tumour DNA (ctDNA)[124], is noteworthy. To date, however, no specific approach has obtained regulatory approval for use in patients with melanoma.

During BRAF–MEK-inhibitor treatment, multiple escape pathways associated with metabolism and oxidative phosphorylation have been described, and might be amenable to targeted interventions[125,126]. In some tumours, BRAF and MEK inhibition has been observed to increase signalling through the YAP pathway[127], leading to escape from cell death via increased expression of the anti-apoptotic protein Bcl-xL[128] (FIG. 4a). If this change could be observed non-invasively via CTC analysis, a Bcl-xL inhibitor might be rationally added to the treatment regimen.

Figure 4 | **Molecular signalling and immunological interactions relevant to the clinical treatment of melanoma. a** | Schematic summary of the key molecular signalling pathways related to melanoma tumorigenesis. Advanced-stage melanoma can be categorized according to mutational profiles. These include mutations in either the *BRAF*, *RAS*, or *NF1* genes in approximately 50%, 20%, and 15% of patients, respectively, and rarer mutations in *KIT*, *GNAQ*, or *GNA11*. Generally, these mutations result in activation of ERK signalling, and most melanomas are, therefore, broadly amendable to therapeutic inhibition at the levels of BRAF, MEK, or KIT. **b** | Summary of immune interactions in the tumour microenvironment of melanoma. Following priming by activated dendritic cells (DCs), antigen-specific T cells can migrate to melanoma tumours. In response to cytokines, particularly of IFNγ, T-cell-inflamed tumours upregulate immune-inhibitory molecules, such as programmed cell-death 1 ligand 1 (PD-L1). Binding of PD-L1 to programmed cell-death protein 1 (PD-1) on T cells at the invasive margins of melanomas can suppresses their antitumour activity. Upon the application of anti-PD1 antibody, however, these T cells are released from inhibition leading to tumour infiltration and antitumour activity. Mechanisms of resistance to anti-PD1 immunotherapy have been described to involve loss of IFNγ signalling via JAK mutations, loss of antigen presentation owing to mutations in β_2 microglobulin (a component of the major histocompatibility complex class I (MHC I)), and activation of β-catenin signalling (leading to loss of DC recruitment and T-cell priming). BATF3, basic leucine zipper transcriptional factor ATF-like 3; CCL4, C–C chemokine 4; CCR5, C–C-chemokine receptor 5; TCR, T-cell receptor.

Similarly, although ERK phosphorylation is consistently suppressed after BRAF–MEK inhibition, levels of phosphorylated S6K are often unaffected[129], probably owing to crosstalk between the MAPK and PI3K/AKT/mTOR pathways (FIG. 4a). Thus, addition of an mTOR inhibitor to treatment might be an appropriate response to this observation. Restoration of MITF expression in melanoma cells after inhibition of mutant BRAF has been associated with increased expression of the downstream antiapoptotic protein BCL2A1 (REF. 130), as well as peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α), a master regulator of mitochondrial biogenesis and, thus, oxidative phosphorylation[131] (FIG. 4a). In this context, mTOR inhibition has also been shown to suppress the outgrowth of clones with resistance to MAPK inhibition[126]. Finally, in those melanomas in which MITF expression is not restored[132], many receptor tyrosine kinases can be upregulated and contribute to the resistant phenotype[133,134] (FIG. 4a). This 'MITF^{low}' phenotype is also potentially amenable to targeted intervention, as many MITF outputs are under the control of JAK signalling and could, therefore, be amendable to JAK-inhibitor therapy[135].

Immunotherapy resistance and biomarkers. In comparison with the mechanisms of resistance to molecular therapies directed at BRAF, those related to immunotherapy are less well understood; owing to the relatively low ORRs with immunotherapy, greater effort has been placed on the development of predictive markers of response. In the context of anti-PD-1 therapy, the most obvious predictive biomarker has been PD-L1 expression, assessed by immunohistochemical staining. Indeed, assays for PD-L1 have been approved as complementary diagnostic tests for use in the context of melanoma and other tumour types; however, at present, agreement on the definition of 'positivity' for PD-L1 expression is lacking. This confusion results from the use of different antibodies for the purpose of testing, as well as varying diagnostic specifications for determining the results. This variation is emphasized within the clinical trial data described previously. In the KEYNOTE-006 study of pembrolizumab versus ipilimumab[45], a positive result for PD-L1 testing was defined using the Merck 22C3 antibody as membranous cell staining of PD-L1 in ≥1% of cancer cells (Allred proportional score of 2–5). With this cut-off point, 80.5% of patients in the trial had PD-L1-positive tumours[45]. By contrast, in the CheckMate 067 study of nivolumab and ipilimumab[90], PD-L1 antibody testing was performed using the Bristol-Myers Squibb (Dako) 28–8 antibody, with positivity defined as ≥5% of tumour cells showing PD-L1 staining of any intensity on the cell surface in a section containing ≥100 evaluable tumour cells. According to these criteria, only 23.6% of the patients had a PD-L1-positive status[90]. The effect of PD-L1 positivity therefore varies substantially depending on which assay is used, and which treatment is being considered. In the frontline trial of nivolumab versus chemotherapy[89], the PD-L1-negative cohort of patients treated with nivolumab had superior outcomes relative to those in the chemotherapy group, suggesting that PD-L1 status (at least by this definition) is not a good stratification

Subgroup	Deaths/number of patients	Median OS (95% CI), months
Normal LDH, <3 organ sites with metastasis	60/237	45.5 (45.5–NE)
Normal LDH, ≥3 organ sites with metastasis	77/161	25.6 (21.3–NE)
LDH: ≥1 to <2×ULN, ECOG PS=0	51/93	19.1 (16.2–NE)
LDH: ≥1 to <2×ULN, ECOG PS ≥1	45/56	10.8 (8.0–14.1)
LDH: ≥2×ULN	54/70	8.8 (7.1–12.8)

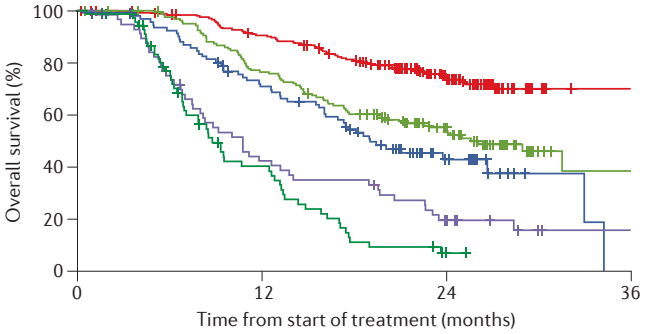


Figure 5 | **Factors associated with overall survival in patients with melanoma treated with dabrafenib and trametinib.** Clinical factors associated with outcomes to BRAF inhibitor treatment in patients with advanced-stage melanoma have been identified. In a regression-tree analysis, serum lactate dehydrogenase (LDH) levels (normal, ≥1 to <2 x ULN, or ≥2 x ULN), ECOG PS (0 or ≥1), and the number of organ sites with metastases (<3 or ≥3) were found to significantly correlate with outcome. CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; NE, not evaluable; OS, overall survival; ULN, upper limit of normal. Modified with permission from Elsevier © Long, G. V. *et al. Lancet Oncol.* **17**, 1743–1754 (2016).

marker in this context. Interestingly, however, in an under powered subgroup analysis of the CheckMate 067 study data, the median PFS of patients with PD-L1-positive melanomas treated with nivolumab was identical (14 months) to that of PD-L1-positive patients treated with nivolumab in combination with ipilimumab[90]. This finding suggests that PD-L1 status deserves further evaluation as a biomarker for patient stratification (and might define a subgroup who can forego combination immunotherapy in favour of single-agent anti-PD-1 immunotherapy), or perhaps indicates an underlying biology worthy of further research.

Beyond PD-L1 staining as a biomarker of response or resistance to immune-checkpoint inhibitors, more-comprehensive models to explain the tumour microenvironment are under investigation. In particular, the results of elegant histopathological work have indicated that the presence of adaptive immune resistance, manifest by $CD8^+$ T cells that cause upregulation of PD-L1 expression on melanoma cells at the invasive tumour margin (FIG. 4b), might more clearly explain responsiveness or primary resistance to anti-PD-1 immunotherapy[136]. In this study[136], tumour biopsy samples obtained from patients before and after anti-PD-1 therapy suggested that responsiveness was correlated with high numbers of cells expressing CD8, PD-1, and/or PD-L1 located at the invasive tumour margin, together with a more-clonal T-cell receptor (TCR) repertoire. Using these data, the investigators were able to develop a high-fidelity multivariate predictive model to predict the likelihood of response using an individual patient sample[136].

From a more holistic viewpoint, the gene-expression profile of the tumour microenvironment has been proposed as a biomarker of response[137]. On the basis of the observation that intratumoural antitumour inflammation is triggered after secretion of IFNγ by infiltrating $CD8^+$ T cells[99], a profile referred to as the 'T-cell-inflamed tumour microenvironment' has been associated with response to diverse immunotherapies, including vaccines, IL-2, as well as anti-CTLA-4 and anti-PD-1 antibodies[138–141]. This concept has been applied broadly in melanoma and beyond (in gastric, head and neck, and urothelial cancers), with multiple reports from the 2015 ASCO meeting indicating not only that increasing IFNγ-associated gene-expression scores are predictive of a response to pembrolizumab, but also, perhaps more importantly, that the lack of IFNγ-associated gene expression is very highly correlated with lack of clinical benefit[100,142–144].

Interest in the intersection between intratumoural genomic changes and the immune response increased with the discovery that tumour-intrinsic signalling pathways are associated with immune exclusion. Analyses of tumours with a very low IFNγ-associated gene-expression score, or a 'non-T-cell-inflamed' tumour microenvironment have revealed roles for activation of the WNT/β-catenin-signalling pathway[145], and an enrichment for mutations in *PTEN*[146] in immune evasion. Deleterious mutations in the genes encoding JAK1 and JAK2 (which are involved in IFNγ signalling), or β_2 microglobulin (an MHC class I subunit), as well as loss of expression of interferon regulatory factor 1 (IRF1) have also been described in anti-PD-1-antibody-resistant patient samples and cell lines[147,148]. These intratumoural changes are associated with deficits in autophagy, antigen presentation, and the type I interferon response, suggesting that rational drug combinations should be explored to either suppress these pathways using β-catenin or PI3Kβ inhibitors, and/or amplify antigen presentation, perhaps via an approach such as FLT3-ligand agonism.

Interpreting the available evidence

Owing to the absence of randomized clinical trial data comparing BRAF-directed therapy versus immunotherapy, and on the sequencing of these treatments, clinicians must use the best available clinical trial data, clinical judgement, and patient and disease characteristics in treatment decision-making. Naturally, clinicians make informal intertrial comparisons, with an inherent risk of overinterpretation of the data. Thus, important considerations are: the similarity and differences in baseline patient characteristics that can be consistently and objectively measured, for example, using serum lactate dehydrogenase (LDH) levels; the relative size of the studies and power to measure the end points reported (for instance, a three-arm study that is not powered to compare the outcomes of arms two and three, yet the end points are necessarily reported for all three arms, such as in the CheckMate 067 trial[90]); and the method used to report a specific end point (such as median PFS versus landmark PFS[149]), or the use of unusual end points (such as the durable response rate as defined in the OPTiM trial[98]).

The importance of baseline characteristics. The end points of phase III randomized trials conducted in patients with advanced-stage melanoma should be compared carefully, taking into account the relative proportions of patients with an elevated LDH level, M1c-stage disease, and an ECOG performance status >0, and whether the trial included only treatment-naive patients (TABLE 2). Indeed, for both BRAF-directed and anti-PD-1 therapies, the long-established prognostic factors[150] remain the most important baseline features associated with long-term patient benefit. In a multivariate hierarchical analysis of data from 617 patients with advanced-stage melanoma who had received dabrafenib combined with trametinib in one of three randomized trials, LDH level was the baseline factor most strongly associated with PFS and overall survival[151] (FIG. 5). Among those with an LDH level in the normal range, the number of organ sites involved at baseline was most strongly associated with PFS and overall survival: in patients with a normal LDH level and <3 disease sites, the 2-year PFS and overall survival were 46% and 75%, respectively, whereas for those with an LDH level ≥2 times the upper limit of normal (≥2 x ULN), the values were 2% and 7%, respectively[151]. Similarly, a multivariate analysis of data from 411 patients with advanced-stage melanoma who received pembrolizumab in the phase I KEYNOTE-001 study demonstrated that the sum of the diameters of target metastases (as per RECIST criteria), LDH level, and ECOG performance status were baseline factors independently associated with survival, although a hierarchical analysis was not undertaken[152]. The importance of LDH levels as a predictive factor has also been underscored by analyses within the reported phase III trials. A subgroup analysis of the CheckMate 067 trial[153] revealed that no patient with an LDH level ≥2 × ULN responded to ipilimumab in the frontline setting, although some did respond to nivolumab (ORR 21.6%), or nivolumab combined with ipilimumab (ORR 37.8%). Together, these data suggest that intervention earlier in the course of metastatic disease is the most-effective approach, but also highlight that effective drug therapies for patients with elevated LDH levels (particularly ≥2 × ULN) are a major unmet clinical need.

Patients with LDH levels ≥2 × ULN commonly have rapidly dividing melanoma cells and, thus, rapid disease progression; therefore, the window of opportunity for a response to any drug therapy might be short, and the kinetics of response becomes important. BRAF-directed therapy is associated with a rapid onset of response, even in patients with an elevated LDH level; however, tumour shrinkage is short-lived in such patients, as demonstrated by the shorter median PFS and overall survival after BRAF-directed therapy in this group (5.5 months and 10.8 months, respectively) compared with those of patients with normal LDH levels (17.8 months and not reached, respectively) in the COMBI-v trial[64]. Notably, in contrast to all other trials, ≤5% of patients enrolled the OPTiM trial had elevated LDH levels at baseline and, thus, the median overall survival of 23.3 months in the T-VEC group should not be over interpreted[98] (TABLE 2).

Box 1 | **End points for future melanoma trials**

The following end points should be assessed and reported in future melanoma clinical trials, for the total populations of evaluable patients, as well as separately for patients with baseline serum lactose dehydrogenase (LDH) levels within the upper limit of normal (ULN), for those with LDH > ULN, and for those with LDH ≥ 2 × ULN.

Response criteria

- Objective response rate (by RECIST criteria)
- Complete response rate
- Median duration of response among the 25% of patients with the longest duration of response (in total population of evaluable patients only)

Progression-free survival (PFS)

- Median PFS
- 1-Year PFS
- 2-Year PFS
- 3-Year PFS
- 5-Year PFS

Overall survival (OS)

- Median OS
- 1-Year OS
- 2-Year OS
- 3-Year OS
- 5-Year OS

Trial end point considerations. The interpretation of the duration of response (DoR) as an end point has many pitfalls. DoRs are longer with anti-PD-1 therapy (median not reached for the ~33% of patients who responded to pembrolizumab in KEYNOTE-006)[45] than with BRAF-directed therapy (median of 12.9 months in the 69% of patients who responded to dabrafenib plus trametinib in COMBI-d)[62]. Emerging data indicate, however, that ~43% of patients who have a RECIST objective response to pembrolizumab, experienced progressive disease by 36 months — although, the rates of progression in patients with a complete response were very low (<2%)[154]. Importantly, the DoR end point ignores the fact that only 20–40% of patients with advanced-stage melanoma respond to anti-PD-1 therapy, whereas nearly 70% of patients with *BRAF*$^{V600E/K}$-mutant melanoma respond to BRAF-directed therapy. Furthermore, the available data do not suggest that only immunotherapy has an effect on long-term survival: among previously untreated patients, 3-year landmark overall survival was 45% in the large phase I KEYNOTE-001 study of pembrolizumab[154], and was 45% and 44% after combined dabrafenib and trametinib treatment in COMBI-v and COMBI-d[155,156], respectively.

The interpretation of trials reporting the median PFS and/or overall survival versus others reporting only landmark data is another potential pitfall in making evidence-based decisions: both median and landmark outcomes should be reported. For example, the median PFS and overall survival were numerically similar between the experimental and standard-therapy arms

of trials with ipilimumab (approximately 3 months and 9–11 months, respectively)[78]; the differences in landmark survival analyses better reflected the statistically significant hazard ratios in favour of ipilimumab (TABLE 2). In the absence of a mature landmark PFS data, the most appropriate and least biased measure of therapeutic benefit[149], the median DoR in the 25% of patients with longest response durations, might be the best comparison to perform in order to negate the differences in response rates.

Mature landmark PFS data are now emerging from phase III trials in patients with advanced-stage melanoma, facilitating comparisons of drug efficacy without bias relating to differences in post-trial therapy, which can affect overall survival. Bearing in mind all the caveats regarding differences in baseline patient characteristics between trials, the 2-year PFS was 30% with dabrafenib and trametinib[62], 31% with pembrolizumab[103], 39% with nivolumab in patients with *BRAF*-wild-type melanoma only[89], and 14% with ipilimumab[103].

As discussed, with all available drug therapies, patients with good prognostic features at baseline comprise most of the long-term responders and survivors. Additionally, data from multiple retrospective analyses suggest that initiation of treatment earlier after the development of metastatic disease, defined by smaller sum diameters of metastases in studies of PD-1 blockade[152,157] and by lower overall numbers of disease sites in trials of BRAF and MEK inhibition[151,158] (FIG. 5), is correlated with improved clinical outcomes. Thus, trial end points should be reported accordingly, in order that investigational drugs can be more efficiently integrated into the emerging treatment paradigm for advance-stage melanoma; we have proposed end points that should be reported for every large phase II or phase III trial in the future (BOX 1). The recommended landmark end points emphasize the importance of ongoing analyses within a trial after the primary end point is met.

Box 2 | **Research needs for melanoma therapeutics**

BRAF/MEK inhibition

Benefits of frontline approach:

- Rapid onset of treatment response
- Immune priming and tumour debulking effects
- Efficacy against brain metastases

Future needs:

- Understanding of long-term clinical outcomes
- On-treatment biomarkers

Immunotherapy

Benefits of frontline approach:

- Immunological memory and potential to discontinue treatment
- Avoids potential adaptive cross-resistance from prior therapy
- Combination immunotherapy has highest benefit of any treatment in patients with high serum lactate dehydrogenase (LDH) levels

Future needs:

- Comparison of long-term outcomes of anti-PD-1 monotherapy versus combined CTLA-4–PD-1 blockade
- Refinement and comparison between baseline biomarkers to improve patient selection

CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; PD-1, programmed cell-death protein 1.

The choice of first-line therapy

Two goals of therapy have emerged in the management of patients with metastatic melanoma: short-term palliation and induction of durable remission. These goals are not mutually exclusive, but how available therapies can be used to optimize both remains unclear. BRAF-targeted therapy and immunotherapy each have substantial clinical benefits, and the evidence base to inform the choice of frontline therapy for individual patients lacks clarity, necessitating further research (BOX 2).

Arguments for frontline BRAF-targeted therapy. Reports of rapid symptomatic relief within 1–2 weeks of initiating vemurafenib or dabrafenib therapy have become commonplace. The exact kinetics of response have not been documented, although analyses of serial 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F-FDG)-PET scans performed before and after 2 weeks of therapy suggest a rapid and widespread antitumour effect within this timescale. Thus, a pressing clinical question is whether BRAF-targeted therapy can be offered to patients with a high burden of metastatic disease at baseline as a prelude to immunotherapy. This strategy has several potential merits, including the potential upregulation of melanocytic antigens and $CD8^+$ T-cell infiltration with BRAF inhibition, but rigorous clinical trials have not been conducted to define the appropriate duration of therapy.

Mature clinical trial data indicate that the 3-year survival of patients with previously untreated, advanced-stage, *BRAF*-V600-mutant melanoma who receive vemurafenib[51] or dabrafenib[159] monotherapy is 21–31%; for dabrafenib and trametinib combination therapy, only mature 2-year data are available (53% of patients alive[155,156]; TABLE 2), but data from a small phase II cohort of patients suggest that the 3-year survival rate might be 38%[160]. These data indicate that BRAF-inhibitor-based therapy can result in long-term benefit, but the issue remains as to whether this likelihood is greater than with immunotherapy for the overall population.

Brain metastases have long been recognized as a predictor of an unfavourable prognosis and responsiveness to previous standard therapies in patients with melanoma. Hence, patients with brain metastases — ~33% of the overall advanced-stage melanoma patient population — have predominately been excluded from clinical trials. Emerging evidence indicates, however, that patients with brain metastases are nearly as likely to initially respond to BRAF-inhibitor therapy as those without brain metastases. In uncontrolled phase I–II trials of monotherapy with dabrafenib or vemurafenib in patients with melanoma brain metastasis[161–163] (TABLE 3), ORRs and PFS were only marginally inferior to those observed in trials that included only patients without active brain metastases. These benefits are presumed, although not yet proven, to

Table 3 | **Clinical studies of BRAF-targeted drugs and immunotherapies in patients with melanoma brain metastases**

Agent	Number of patients treated	Primary end point	Baseline characteristics (%age of patients)				RECIST ORR for brain lesions	Median PFS	Median OS	1-Year OS
			BRAF$^{V600E/K}$ mutated	No previous systemic treatment	LDH >ULN	ECOG PS >0				
Vemurafenib[163]	24	Safety	100%	0%	NR	NR	37%	3.9 mo	5.3 mo	0%
Dabrafenib[161]	89 with no prior brain-directed treatment)	IRR	100%	34%	55%	46%	39% (V600E); 7% (V600K)	4.0 mo (V600E); 2.0 mo (V600K)	8.3 mo (V600E); 4.1 mo (V600K)	94% (V600E); 68% (V600K)*
	83 with prior brain-directed treatment	IRR	100%	63%	53%	39%	31% (V600E); 22% (V600K)	4.1 mo (V600E); 4.0 mo (V600K)	7.9 mo (V600E); 5.5 mo (V600K)	94% (V600E); 89% (V600K)*
Dabrafenib[162]	10	Safety	100%	40%	60%	70%	80%	4.2 mo	NR	10%
Ipilimumab[83]	36	Disease control at 12 weeks	NR	29%	NR	33%	24% ‡	1.5 mo	7.0 mo	31%
Pembrolizumab[171]	18	ORR	33%	22%	39%	67%	22%	NR	Not reached	NR

ECOG PS, Eastern Cooperative Oncology Group performance status; IRR, intracranial response rate; LDH >ULN, serum lactate dehydrogenase level greater than upper limited of normal; mo, months; NR, not reported; ORR, objective response rate; OS, overall survival; PFS progression-free survival; RECIST, Response Criteria In Solid Tumors. *Estimated based on Kaplan-Meier overall survival curves. ‡Modified WHO criteria were used for response assessment.

translate into prolonged overall survival, and have led to BRAF-inhibitor therapy becoming a mainstay treatment for patients with untreated metastatic melanoma involving the brain. Whether surgical resection or stereotactic radiosurgery for *BRAF*-mutant brain metastases at baseline is of benefit to patients remains unclear; however, BRAF-inhibitor therapy is largely supplanting the use of whole-brain radiation as an initial treatment approach in patients with multiple brain metastases. The findings of ongoing studies of BRAF-directed therapy (with dabrafenib plus trametinib; NCT02039947), as well as trials of immunotherapies (nivolumab plus ipilimumab; NCT02320058 and NCT02374242), which also seem to have some activity against brain metastases (TABLE 3), will provide greater insight into the roles of these treatments in the management of patients with disease involving the brain.

If biomarkers of long-term benefit from BRAF-targeted therapy prove to be elusive, an additional strategy for optimizing treatment is emerging in the form of therapeutic response monitoring. Analysis of overall survival in relation to response to dabrafenib and trametinib indicates that patients who achieve a complete response do exceptionally well compared with partial esponders or non-responders (88%, 55% and <40% survival at 2 years, respectively)[151]. Considering this information, the advent of blood-based monitoring of response or resistance has provided a new opportunity for timely therapeutic switching. Indeed, intriguing evidence indicates that liquid biopsy measurement of CTCs, ctDNA, or tumour-derived exosomes can inform on disease burden and the likelihood of a response to therapy[164]. Similarly, persistence of phosphorylated S6K following 2 weeks of BRAF-inhibition predicts short-lived disease control and could be developed as an on-therapy response biomarker, to help navigate patients to immunotherapy before disease progression[129].

Arguments for frontline immunotherapy. Turning the immune system against cancer enables engagement of several unique properties of this system that cannot be achieved with any chemical drug. In particular, if an effective antitumour immune response is induced in a patient with metastatic melanoma, immunological 'memory' offers the potential for long-lasting, possibly life-long, therapeutic responses. In this context, a major advantage of immunotherapy is the possibility to discontinue treatment and maintain antitumour responses.

Importantly, the effectiveness of immunotherapy, particularly PD-1 blockade, is independent of the presence or absence of *BRAF* mutations[136], but could in theory be modified by the genomic instability and non-genomic evolutionary selective pressures that tumours are exposed to during cancer treatment. A paucity of robust data on the outcomes of patients treated with immunotherapy following BRAF-targeted therapy limits our understanding of this issue; however, small series and genomic studies of immunotherapy resistance suggest that resistance to BRAF inhibition might attenuate, rather than augment, the benefit of immunotherapy[165–167].

Previously, the hypothesis that patients with fast-growing, *BRAF*-mutated melanomas and high LDH levels were best treated with BRAF-targeted therapy prevailed. Now, combined BRAF and MEK inhibition therapy is known to have the lowest durable benefit in patients with a high baseline LDH levels[151,158], whereas the ipilimumab plus nivolumab regimen has impressive efficacy in this population[153]. Therefore, in this setting, BRAF-directed therapy might only evoke short-lived tumour responses, and immunotherapy is more likely to provide long-term benefit.

Ideally, the selection of patients to receive anti-PD-1 antibody monotherapy would be based on the presence of pre-existing intratumoural melanoma-antigen-specific T cells that are suppressed by PD-1–PD-L1 interactions,

as opposed to the current trial-and-error approach[168]. Long-term clinical outcomes of patients treated in clinical trials comparing combined CTLA-4–PD-1 blockade versus anti-PD-1 monotherapy are eagerly awaited, and the argument for frontline immunotherapy will broaden as other immunotherapy combinations are developed. As new combinations become available, we envision that the choice of frontline therapy will ultimately be tailored for individual patients based on a rational understanding of tumour immune microenvironment and how best to redirect the immune response for optimal benefit.

Conclusions

Over a relatively short period of time, the number of effective treatment options for patients with advanced-stage melanoma has increased considerably; now multiple treatment options are available, with both BRAF-targeted and immunotherapeutic modalities associated with improved overall survival. Clinical trials to determine the optimal treatment choice for the 'average' patient are ongoing (NCT02224781). At present, consideration of patient-specific features, such as comorbidities, biochemical or other clinical parameters of the kinetics of melanoma (such as baseline LDH and performance status), and patient tolerance of toxicity should be weighed as the highest priorities when considering frontline therapy.

Despite the wealth of options now available for the treatment of patients with advanced-stage melanoma, the future of drug development in this disease is bright. Combination clinical trials of BRAF, MEK, and PD-1/PD-L1 antagonists suggests an overlapping benefit between BRAF-targeted approaches and immunotherapy[112]. Dosing strategies of available agents, such as anti-CTLA-4 and anti-PD-1 antibodies, are being further evaluated to optimize clinical benefit and minimize toxicity[169]. Moreover, preliminary evidence of benefit and minimal toxicity has been obtained for several novel immunotherapeutics, such as indolamine 2,3-dioxygenase inhibitors and oncolytic viruses, when combined with an anti-PD-1 antibody[102,170]. Finally, surgical resection and radiotherapy remain relevant, and should be incorporated into patient care in the context of a multidisciplinary approach tailored to each patient. Importantly, in this rapidly evolving therapeutic landscape, patients should continue to be encouraged to consider participating in a clinical trial at all decision points during therapy.

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