BRAF inhibitors and their immunological effects in malignant melanoma

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ABSTRACT

Introduction: The treatment of cutaneous melanoma has been revolutionized by the development of small-molecule inhibitors targeting the MAPK pathway, including inhibitors of BRAF (BRAFi) and MEK (MEKi), and immune checkpoint blockade antibodies, occurring in tandem. Despite these advances, the 5-year survival rate for patients with advanced melanoma remains only around 50%. Although not designed to alter immune responses within the tumor microenvironment (TME), MAPK pathway inhibitors (MAPKi) exert a range of effects on the host immune compartment that may offer opportunities for therapeutic interventions.

Areas covered: We review the effects of MAPKi, especially BRAFi, on the TME, focusing on alterations in inflammatory cytokine secretion, recruitment of immune cells and their functions, both during response to BRAFi treatment and as resistance develops. We outline potential combinations of MAPKi with established and experimental treatments.

Expert opinion: MAPKi in combination or in sequence with established treatments such as checkpoint inhibitors, anti-angiogenic agents, or new therapies such as adoptive cell therapies, may augment their immunological effects, reverse tumor-associated immune suppression, and offer the prospect of longer-lived clinical responses. Refining therapeutic tools at our disposal and embracing ‘old friends’ in the melanoma treatment arsenal, alongside new target identification, may improve the chances of therapeutic success.

1. Introduction

The incidence of cutaneous melanoma is increasing, with an estimated 300,000 cases reported globally per annum [1]. While melanoma constitutes less than 5% of all skin cancers diagnosed, it remains the most lethal. Within the last decade, there has been significant expansion of the therapeutic arsenal for melanoma with the introduction of molecularly targeted therapies, in the form of mitogen-activated protein kinase (MAPK) pathway inhibitors, and immune modulatory therapies, in the form of checkpoint inhibitors, occurring in parallel [2]. The MAPK pathway has been identified as a critical signaling cascade underlying disease pathogenesis, with 50% of patients presenting with mutations in the signaling molecule BRAF [3]. Mutations in BRAF lead to constitutive activation of the MAPK pathway, promoting cell proliferation and inhibiting apoptosis, thus driving tumorigenesis [4]. Tumors are tested at diagnosis for BRAF mutations, and BRAF inhibitors (BRAFi) including Vemurafenib, introduced in 2011, and Dabrafenib, approved in 2013, initially demonstrated high efficacy as treatments, with a significant proportion of patients showing profound tumor regression. However, these responses are typically short-lived and development of acquired resistance follows [5,6]. This led to the development of MEK inhibitors (MEKi), which target the MAPK pathway downstream of BRAF. The combination of BRAFi and MEKi is now used as first-line treatment for unresectable or metastatic BRAFV600E/K mutant melanoma, leading to greater initial tumor response and delaying MAPK-driven acquired resistance compared with BRAFi treatment alone [7–9]. Although combinations of BRAFi and MEKi improve progression-free survival (PFS) when compared to monotherapy [10], therapeutic resistance remains a challenge.

Melanoma is well recognized as the archetypal immunogenic tumor, and the interactions between melanoma cells and immune cells within the TME contribute to tumor pathogenesis, invasion, and immune evasion [2]. The importance of these interactions has been demonstrated

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with the success of immune checkpoint inhibition. Immune checkpoint inhibitors (ICIs), developed at the same time as BRAFi, are monoclonal antibodies that target and block the cell-surface marker cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or the programmed death-1 pathway (PD-1/PD-L1). CTLA-4 is expressed on T cells, including regulatory T cells (Tregs), and inhibits T cell activation through binding of CD80 and CD86 expressed on antigen-presenting cells (APCs). Blocking this interaction with the antibody Ipilimumab results in T cell activation and enhanced effector function [11]. PD-L1 is highly expressed by tumor and stromal cells within melanoma lesions [12], while PD-1 is expressed on the T cell surface [11]. PD-L1/PD-1 interaction leads to negative regulation of T cells. Anti-PD-1 antibodies, such as Nivolumab or Pembrolizumab, and antibodies against PD-L1, such as Atezolizumab, inhibit PD-L1/PD-1 signaling, allowing T cell activation and reducing the number of exhausted T cells within the TME, augmenting anti-tumor immune responses [11,13]. The introduction of ICIs has resulted in significant improvements in PFS in advanced melanoma with up to 50% of patients experiencing unprecedented durable responses at 5 years following treatment with Ipilimumab and Nivolumab [14,15]. The success of ICIs, including in BRAF mutant melanoma [16], demonstrates that the manipulation of the immune system is a valuable therapeutic tool.

Although MAPK pathway inhibitors (MAPKi) were not designed to manipulate the immune system, they too appear to exert a range of immunological effects, which forms the focus of this review. Understanding the interactions between BRAF mutant melanoma cells and immune cells, both during response to MAPKi treatment and as resistance develops, may uncover new therapeutic avenues, ultimately lead to more sustained responses and improved clinical outcomes. This may include combining new treatments with MAPKi with the aim of identifying new synergistic effects or optimizing the combination and sequencing of already successful therapies, including ICIs.

2. The MAPK pathway in melanoma and targeting with inhibitor drugs

The MAPK pathway is a family of intracellular signaling pathways activated through the binding of growth factors to receptor tyrosine kinases (RTK) [17]. Downstream events linked to these pathways include cellular differentiation, proliferation, apoptosis, and regulation of both innate and adaptive immune responses [18,19].

The pathway is arranged in three tiers containing sequentially acting serine threonine kinases: MAP kinase kinase (MAPKKK), MAP kinase kinase (MAPKK), and a downstream effector MAP kinase (MAPK) [20]. Sequential phosphorylation of these kinases (outlined in Figure 1) leads to the phosphorylation of downstream targets in the cytosol and nucleus, which mediate biological responses [18]. To prevent persistent activation of MAPK, upstream negative feedback occurs. In the absence of RTK-ligand engagement, members of the MAPK pathway remain in their non-phosphorylated, inactive states, preventing downstream signaling.

Aberrant activation of the MAPK pathway is a central step in melanoma pathogenesis [21], often driven by activating mutations of RAF (MAPKKK). The RAF family consists of ARAF, BRAF, and CRAF. While mutants of ARAF and CRAF are rare, activating mutations of BRAF are found in different malignancies including colorectal cancer, ovarian cancer, lung cancer, and melanoma [22]. The most common BRAF mutation, accounting for 90% of melanomas containing MAPK mutations, BRAFV600E, remains active in the absence of RAS, resulting in a constitutively active MAPK pathway that can drive aberrant cell growth and proliferation [4,18], and influence the TME [23]. This makes the MAPK pathway a valuable therapeutic target for melanoma.

Selective BRAF inhibitors with a high affinity for the BRAFV600E mutant include Vemurafenib, Dabrafenib, and Encorafenib [23]. Preclinical data for Vemurafenib (PLX4032) and Dabrafenib (GSK2118436) demonstrated significant antitumor activity against BRAFV600E mutant melanoma cell lines, including G1 cell cycle arrest, apoptosis, and blockage of ERK phosphorylation, reducing proliferation [23,24]. Both were also shown to inhibit other BRAFV600E mutant cell lines without inhibiting either wild-type BRAF or non-V600 BRAF mutants [24,25].

Encorafenib (LGX818), a second-generation BRAFi, produced prolonged target suppression and efficacy compared to first-generation BRAFi such as Vemurafenib and Dabrafenib [26]. Studies in xenograft mouse models of BRAFV600E expressing melanoma revealed that exposure to Vemurafenib, Dabrafenib, or Encorafenib resulted in a dose-dependent inhibition of tumor growth and in some cases tumor regression [24,25,27]. Clinical trials of BRAFi monotherapy demonstrated high rates of objective responses, improved PFS, and overall survival (OS) when compared to conventional cytotoxic chemotherapy [28]. Despite these results, a median PFS of only 6–7 months was
seen on BRAFi monotherapy due to both intrinsic and acquired resistance and subsequent relapse [5,6,29]. Resistance to BRAFi monotherapy is mediated by the reactivation of the MAPK pathway, which can occur through MAPK-dependent or MAPK-independent mechanisms. Common patterns of resistance include activating mutations of upstream components (NRAS mutants) and downstream components (MEK mutants) [30,31], activation of non-MAPK growth pathways (phosphatidylinositol-3-kinase/AKT pathway) [32], splice variants of BRAFV600E [33], and enhanced BRAF expression [34]. Vemurafenib, Dabrafenib, and, to a lesser extent, Encorafenib can all cause Ras-dependent paradoxical activation of the MAPK pathway, particularly in those tumors with preexisting Ras mutations [35,36]. Paradoxical activation is associated with hyperactive MAPK signaling, resulting in the emergence of hyperproliferative cutaneous events [37]. Resistance may also be precipitated by BRAFi treatment, creating an environment that favors the survival of non-BRAFV600E cells [35].

Preclinical studies demonstrate that acquired resistance to BRAFi therapy was associated with rapid recovery of MAPK signaling; this suggests that complete pathway inhibition may be required for therapeutic effect and thus MEK inhibitors (MEKi) are now used as first-line treatment in combination with BRAFi. This combination allows further inhibition of the MAPK pathway downstream of BRAF, and thus long-term inhibition of ERK (MAPK). Three phase III clinical trials demonstrated the clinical efficacy of BRAFi/MEKi combination therapy: the COLUMBUS trial compared the combination of Encorafenib + Binimetinib to Vemurafenib or Encorafenib monotherapy; and the COMBI-d and COMBI-v trials compared Trametinib and Dabrafenib combination therapy to Dabrafenib monotherapy. All demonstrated significant and clinically meaningful differences in OS when combinations were used, leading to these combinations to be approved as first-line therapy [38,39]. Furthermore, MEKi monotherapy was shown to block BRAFi-induced hyperproliferative cutaneous events in squamous cell carcinoma mouse models, indicating that addition of MEKi may have a role in preventing both BRAFi resistance and paradoxical MAPK activation [40]. As well as directly contributing to melanoma cell growth and survival, a constitutively active MAPK pathway contributes to the immunosuppressive TME seen in melanoma tumors. Inhibiting this pathway with BRAFi and MEKi can therefore have immunomodulatory effects, not just by targeting MAPK signaling in melanoma cells but by causing paradoxical activation in immune and stromal cells within the TME. The immunomodulatory activity of BRAFi may help to overcome the immunosuppressive microenvironment found in BRAF mutant melanomas [23], an effect reversed with inhibitor resistance.

3. Immunosuppressive environment created by BRAF mutant melanoma

BRAF mutant melanomas can create a TME that promotes both tumor growth and immune evasion by a range of mechanisms, including the secretion of immunosuppressive factors and the recruitment of immunoregulatory cells, outlined in Figure 2A.

BRAF mutant melanomas may recruit immunosuppressive immune cells via the secretion of chemokines, such as CCL2 [41]. The receptor for CCL2, CCR2, is expressed by myeloid-derived suppressor cells (MDSCs), regulatory T cells (T-regs), and monocytes, the latter of which are able to differentiate into macrophages upon entry into tumor tissue [42]. Together, these cells aid the establishment of a protumor TME. For example, tumor-associated macrophages (TAMs) are skewed towards a more immunoregulatory phenotype in BRAF mutant
melanoma, as demonstrated by the expression of marker genes such as Arg1, Chi3l3, M1r, and Mmp9 [43]. TAMs secrete factors such as IL-10, which can promote the expansion of T-regs and establish a positive feedback loop with further polarization of TAMs toward an immunosuppressive phenotype [44]; vascular endothelial growth factor (VEGF), which stimulates tumor growth and angiogenesis and promotes immunosuppressive TAMs [45]; and metalloproteases, which aids tumor growth and invasion [44]. As well as the recruitment of immunosuppressive cells, which secrete regulatory factors such as IL-6, IL-8, IL-10, and TGF-β [46,47], cells within the TME demonstrate altered cell–cell signaling, for instance, through impaired CD40:CD40L interactions. This prevents the maturation of APCs and CD8+ T cell activation [43]. The resultant effect of the combination of all the above mechanisms is increased immune evasion and therefore enhanced pathogenicity of melanoma lesions.

4. The immune effects of BRAF inhibition

Although not specifically designed to trigger anti-tumor immune responses, several preclinical and clinical studies (summarized in Table 1) suggest that MAPK pathway inhibition can alter the TME to improve anti-melanoma immune responses. It has been suggested that these effects may contribute to the anti-tumor efficacy of BRAFi. Indeed, immune-related adverse events (irAEs), such as vitiligo and panniculitis, have been postulated as a predictive marker of response to BRAFi treatment [48]. The effects of BRAF inhibition on cancer cell death may lead to the release of danger signals and of cancer-associated antigens. These could enhance anti-tumor immune responses by: (1) increasing inflammation and effector immune cell recruitment, e.g., cytotoxic T lymphocytes (CTLs); (2) altering the balance of cytokines to promote a more proinflammatory TME; and (3) enhancing the effector function of such immune cells, for example, by promoting antigen presentation. These effects are outlined in Figure 2B.

4.1. BRAF inhibition and immune cell recruitment

Multiple studies, in samples from both preclinical models of cancer and from patients undergoing BRAFi therapy, have shown that BRAFi promote tumor infiltration by lymphocytes [41,43,46,49,50]. Increased lymphocyte infiltration of melanoma, in particular CD8+ T cells, is correlated with more favorable outcomes [51]. BRAFi treatment has been associated with an increased ratio of cytotoxic CD8+ to CD4+ T cells, the latter of which may include immunosuppressive T-regs [41]. As well as promoting CTL infiltration, one small study
Table 1. BRAFi effect on immune function: preclinical data of the effect of BRAFi on the tumor microenvironment, specifically changes in immune mechanisms and pathways. TILS – tumor infiltrating lymphocytes; NK cells – natural killer cells; T-regs – regulatory T cells; MDSCs – myeloid-derived suppressor cells; APCs- antigen-presenting cells.

| BRAFi effect of immune function | Findings                                                                 | Model type                                                                                      | Papers/Refs |
|---------------------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------|
| Immune stimulatory factors      | Increased mRNA expression of IL-12a, IL-12b, IL-15, IL-18                | In vivo mouse model                                                                             | Bellmann et al. [50] |
|                                 | Increased mRNA expression of chemokines CCL2, CCL3, CCL4, CXCL9, CXCL10  | In vivo mouse model                                                                             | Bellmann et al. [50] |
|                                 | Increased production of IFNγ                                             | In vivo mouse model                                                                             | Ho et al. [43] |
| Immunosuppressive factors        | Decreased IL-6, IL-8                                                    | Tissue samples from patients                                                                    | Frederick et al. [46] |
|                                 | Decreased PD-1, PD-1, IL-18, VEGF                                       | In vitro human cell lines and in vivo mouse model                                               | Liu et al. [60] |
|                                 | Decreased IL-7, CX3CL1, GCSF, CXCL1, TGFa2, IL-8, VEGF, IFNa             | Human cell lines                                                                               | Whipple et al. [55] |
|                                 | Decreased VEGF                                                          | Tissue samples from patients                                                                    | Liu et al. [59] |
| Immune cell infiltration         | Increased TILs                                                          | Tissue samples from patients                                                                    | Frederick et al. [46] |
|                                 | Increased tumor infiltrating NK cells                                    | In vivo mouse model                                                                             | Knight et al. [41] |
|                                 | Increased CD8:CD4 ratio in TILs                                         | In vivo mouse model                                                                             | Wilmott et al. [49] |
|                                 | Decreased T-regs                                                        | In vivo mouse model                                                                             | Cooper et al. [74] |
|                                 | Decreased MDSCs                                                         | Blood samples from patients                                                                     | Knight et al. [41] |
| Immune cell function             | Increased melanoma antigen expression MART1, TYRP1, TYRP2, GP100        | In vivo mouse model                                                                             | Schilling et al. [53,54] |
|                                 | Increased MHC class I and II                                           | In vitro human cell lines and in vivo mouse model                                               | Ho et al. [43] |
|                                 | Increased markers of T cell cytotoxicity: increased perforin, granzyme B | Human cell lines                                                                               | Frederick et al. [46] |
|                                 | Increased maturation of APCs                                            | Human cell lines                                                                               | Bellmann et al. [50] |
|                                 | Increased expression of CD40L on CD4                                    | Tissue samples from patients                                                                    | Liu et al. [60] |
|                                 | Increased PD-1, PD-L1 and TIM3                                          | In vivo mouse model                                                                             | Whipple et al. [55] |
|                                 | Increased PD-L1 and PD-L2                                               | In vivo mouse model                                                                             | Bradley et al. [64] |

Looking at sequential melanoma biopsies demonstrated a direct effect of BRAFi on the expansion of T cell populations within the TME [52]. Together, these mechanisms may create a T cell population with a richer and more diverse T cell receptor repertoire [52]. Furthermore, immunosuppressive MDSC populations decrease with BRAFi [43,53,54]. BRAFi cause a reduction in CCL2 secreted by tumor cells [41]. Although CCR2 is expressed on T-regs and MDSCs, it is not expressed on natural killer (NK) cells and CD8+ T cells [41]. The reduction in CCL2 caused by BRAFi, therefore, may support the preferential recruitment of effector cells over immunoregulatory cells, aiding the anti-tumor response. Aside from changes in the CCL2/CCR2 chemokine pathway, BRAFi can also increase the expression of the chemokines CXCL9 and CXCL10, which promote the recruitment of NK cells and T cells [50,55]. Together these effects compound a change in the TME toward one which promotes anti-tumor responses.

Interestingly, the immune cell compartment can also be interrogated to predict response to MAPKi. One study of pre-treatment biopsies of melanomas from patients who subsequently were treated with BRAFi, with or without additions MEKi, demonstrated a correlation between high intratumoural CD8+ T cells with concomitantly low CD163+ myeloid cells, likely representing TAMs, and higher probability of response as well as longer PFS and OS, when compared to those with fewer infiltrating CD8+ T cells and high frequency of TAMs [56].

4.2. Changing the balance to create a more proinflammatory and less immunoregulatory TME

As well as altering the immune cell compartment, BRAFi can influence the inflammatory milieu of the TME, upregulating proinflammatory cytokines, such as IL-1 and IL-2, and IL-15 and IL-18, which promote the activation of T cells and NK cells [50,55], while decreasing the secretion of cytokines such as IL-6, which contributes to cancer cell invasion, and IL-8, an important chemokine in melanoma growth, cell motility, invasion, and angiogenesis [46,57,58]. BRAFi can reduce VEGF [55,59,60], a strong angiogenic mediator normally secreted by melanoma and tumor-associated stromal cells. Tumor-associated neovascularization is largely abnormal and tortuous. It can restrict the penetration of drugs, impair the infiltration of immune effector cells [61], and increase hypoxia within the TME which promotes immunosuppressive TAMs, T-regs, and MDSCs [55,62]. Aside from this, VEGF has a direct effect on melanoma cell growth [63]. The reduced expression of VEGF seen with BRAF inhibition, along with the decreased secretion of chemokines that attract ‘protumor’ immune cells, can
therefore create a more hospitable environment for effector cell trafficking and effector function against cancer cells [45].

4.3. BRAFi and immune cell function

Not only can BRAFi recruit effector immune cells and create a more favorable environment for their activation and proliferation, but they can also improve the cancer killing mechanisms of such cells. The increased infiltration of CD8+ T cells seen with BRAFi is associated with tumor shrinkage and necrosis and improved survival in preclinical models, and BRAFi have been shown to actively improve CTL function, as measured by an increase of granzyme B and perforin, co-localized with CD8 + T cells [46,49]. A key mechanism driving enhanced CTL function is improved antigen presentation: BRAFi promote the maturation of APCs, such as TAMs and dendritic cells (DCs), through enhanced CD40:CD40L signaling [43]: BRAFi decreases major histocompatibility complex (MHC-I) internalization from the melanoma cell surface, thereby increasing the expression of MHC-I on tumor cells [64]; and BRAF inhibition has been reported to lead to increased expression of melanoma specific antigens: glycoprotein 100 (gp100), melanoma antigen recognized by T cells (MART-1), dopachrome tautomerase (DCT), and tyrosinase-related protein 1 (TYRP1), enhancing immune recognition of melanoma cells [46]. Aside from improving CTL function, it has been demonstrated that CD4 + T effector function (through the secretion of IFNγ) and CD4 + cell helper function (via signaling through CD40L) are increased with BRAFi [43].

5. Development of BRAFi resistance – the contribution of immune cells

Although a favorable TME following BRAFi treatment might suggest the creation of an effective immune response to the tumor, as mentioned, BRAFi resistance is common and typically observed clinically after around 6 months of treatment [10]. An important resistance mechanism is the paradoxical activation of MAPK pathways in BRAF wild-type cells in the TME, including immune and stromal cells, as outlined in Figure 2C. For example, there is evidence to suggest that tumor-associated fibroblasts can be paradoxically activated by BRAFi and MEKi, leading to matrix remodeling [65] and the secretion of growth factors, such as hepatocyte growth factor (HGF), enabling the survival and growth of melanoma cells [66]. Importantly, due to their abundance in the TME and their protumor functions, TAMs can also be paradoxically activated and are thought to be important contributors to BRAFi resistance, secreting growth factors such as VEGF, which can in turn cause reactivation of MAPK pathway and enhance tumor growth [67].

As tumors develop resistance to MAPKi, the initial upregulation of melanoma antigens seen on initiation of BRAFi treatment is reversed, and melanoma antigen expression is reduced [68]. This results in reduced CD8+ cell activation and CTL function. Aside from this, the reactivation of MAPK leads to increased expression of PD-L1 on melanoma cells [69], which can impair T cell activation and aid immune evasion. Despite an initial increase in CD8+ T cell infiltration, at the point of progression on BRAFi, patient samples demonstrate a low immune infiltrate [70], and CD8+ T cells with an exhausted phenotype, with increased TIM-3 (T cell immunoglobulin and mucin domain-containing protein 3) and PD-1 expression [46].

6. Overcoming BRAF resistance

Combinations of BRAFi and MEKi have been used to overcome BRAFi resistance, yet, since both inhibitor types act on the same pathway, resistance still develops by compensatory pathway activation, eventually leading to disease progression. The immunomodulatory effects of MAPKi create the potential for combining BRAFi with treatments that target cancer immunity, to promote a more sustained response to MAPK pathway inhibition, thus prolonging the time to resistance. We outline below potential combinations of both new and established treatments, which could offer the chance of longer-lived responses in patients (summarized in Figure 2D) and have summarized ongoing clinical trials in Table 2.

6.1. Combination with ICIs

The rationale for combining BRAFi/MEKi with ICI is attractive given the frequent but short-lived responses to BRAFi/MEKi, and the less predictable but more durable responses seen with ICIs. Preclinical studies have demonstrated that markers of T cell exhaustion, such as PD-1, are upregulated by BRAFi and MEKi. Combined with the fact that these targeted drugs do not impact negatively on the viability, proliferation, or signaling of human lymphocytes suggests that BRAFi/MEKi could be utilized to prime the tumor and TME for therapy with ICIs [46,71]. Furthermore, there is evidence to suggest that BRAFi may in fact increase the effector function of human lymphocytes, potentially making the TME more amenable to immune activation with ICIs [72]. As discussed, BRAFi can be shown to increase CD8+ T cell tumor infiltration; however, this infiltration diminishes with time. Timing of administration of ICIs may therefore be optimized to coincide with this proinflammatory response to maximize potential anti-tumor efficacy [70]. Studies published to date have focused on concurrent administration of ICI and BRAFi/MEKi, the efficacy of which has been suggested in preclinical models but in practice has been limited by unacceptable toxicity [73,74].

Overlapping side effect profiles have caused several initial trials examining the combination of ICI and BRAFi/MEKi to be discontinued early. A phase I study combining Vemurafenib and Ipilimumab closed to recruitment after six of the first ten patients developed grade 3 hepatic adverse events [75]. A further trial examining the role of doublet therapy of Dabrafenib and Ipilimumab and triplet therapy of Dabrafenib, Trametinib, and Ipilimumab demonstrated that two of the seven patients receiving triplet therapy developed colitis with intestinal perforation [76]. The lower toxicity of BRAFi when given in combination with MEKi, along with the development of anti-PD-L1/anti-1 agents
Table 2. Clinical trials of combination therapies in metastatic melanoma with BRAFi/MEKi with another therapeutic intervention.

| Study (NCT) | Phase | Clinical Setting | Line of Treatment | Arms | Primary and Secondary End Points | Outcome |
|-------------|-------|-----------------|-------------------|------|----------------------------------|---------|
| Ribas et al. (73) | 1 | BRAF<sup>V600E</sup> mutant positive metastatic melanoma | First line | Arm 1 (n = 6): Vemurafenib (high dose) run in + Ipilimumab Arm 2 (n = 6): Vemurafenib (low dose) + Ipilimumab | Evaluate safety and dose administration | Discontinued due to hepatotoxicity |
| Minor et al. (74) NCT01767454 | 1/2 | BRAF<sup>V600E</sup> mutant positive unresectable or metastatic melanoma | Unspecified | Arm 1: Dabrafenib + Ipilimumab Arm 2: Dabrafenib + Trametinib + Ipilimumab | Evaluate safety of combinations | Discontinued due to intestinal perforation (n = 2) in the triplet arm |
| KEYNOTE-022 NCT02130466 | 1/2 | Metastatic melanoma | Unspecified | BRAF mutant: Dabrafenib + Trametinib + Pembrolizumab | Number of participants with dose-limiting toxicities Objective response rate in those without the BRAF<sup>V600E,K</sup> mutations PFS in those with the mutation | Phase 1: ORR 67% in BRAF-mutant Phase 2: Dabrafenib + Trametinib + Pembrolizumab versus Dabrafenib + Trametinib + placebo) in BRAF mutant; ORR 63% versus 72% 73% of patients with grade 3–4 treatment related toxicities |
| TRIDEnt NCT02910700 | 2 | BRAF<sup>V600E</sup> mutant positive unresectable or metastatic melanoma (stage III/ stage IV) | Unspecified | Binimetinib: MEK 1/2 inhibitor Arm 1: Dabrafenib + Trametinib + Nivolumab Arm 2: Trametinib + Nivolumab Arm 3: Binimetinib + Encorafenib + Nivolumab | Objective Response Rate Secondary: Incidence of adverse events Complete response Partial response | ORR 91% 21% discontinued study due to toxicity (hepatitis + nephritis) |
| COMBI-I, NCT02967692 | 3, part 1 | Previously untreated patients with unresectable locally advanced disease or metastatic melanoma BRAF<sup>V600E</sup> mutant positive | First line | Spartializumab: anti-PD-1 antibody Arm 1: Spartializumab + Dabrafenib + Trametinib Arm 2: Pembrolizumab + Dabrafenib + Trametinib | Safety run in Part 1 – evidence of dose limiting toxicities Biomarker cohort Randomized PFS | ORR 100% 22% discontinued Spartializumab due to toxicity (hepatitis + transaminis) |
| TRILOGY, NCT02908672 (IMspire150) | 3 (double blind, randomized) | Previously untreated patients with unresectable locally advanced disease or metastatic melanoma BRAF<sup>V600E</sup> mutant positive | First line | Placebo Arm: Vemurafenib + Cobimetinib (n = 256) Experimental Arm: Vemurafenib + Cobimetinib + Atezolizumab (n = 258) | PFS Secondary: Percentage of participants with objective response rate Duration of response Overall Survival | Active HR = 0.72 (p = 0.025) |
| BRAF/MEKi and ICI – Lead In NCT01656642 | 1b (open label) | BRAF<sup>V600E</sup> mutant positive melanoma | First line | Arm 1: Vemurafenib + Cobimetinib 1 month run in followed by Atezolizumab Arm 2: Vemurafenib + Cobimetinib + Atezolizumab | Percentage of Participants with Dose Limiting Toxicities Percentage of participants with adverse events Secondary: Pharmacokinetics Measurements Percentage of Participants with Objective Response | Enrollment complete ORR 83.3% 44.1% of patients with grade 3–4 treatment related adverse events (Continued) |
| Study (NCT)          | Phase | Clinical Setting                     | Line of Treatment                                           | Arms                                                                 | Primary and Secondary End Points                                                                 | Outcome                                                                 |
|----------------------|-------|--------------------------------------|-------------------------------------------------------------|----------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|
| NCT02027961          | 1     | BRAF<sup>V600E</sup> mutant positive | Unspecified                                                 | Durvalumab: anti-PD-L1 antibody                                      | Number of participants with dose-liming toxicities and treatment-related adverse events           | ORR 76% in BRAF<sup>V600E</sup> mutant                                  |
|                      |       |                                      |                                                             | BRAF<sup>V600E</sup> concurrent; Trametinib + Durvalumab         | Secondary: Percentage of Participants with Objective Response Duration of Response                  | ORR 21% in BRAF<sup>V600E</sup> concurrent                                  |
|                      |       |                                      |                                                             | BRAF<sup>V600E</sup> sequential: Trametinib → Durvalumab          | Safety of different schemes of continuous/intermittent Dabrafenib + Trametinib during treatment with Pembrolizumab | ORR 50% in BRAF<sup>V600E</sup> sequential                                     |
|                      |       |                                      |                                                             | Arm 1: Pembrolizum + Dabrafenib + Trametinib (short)              | Feasibility of different schemes of continuous/intermittent Dabrafenib-Trametinib                | After a median follow-up of 17.4 months, the median PFS of patients treated with PEM monotherapy was 10.6 months compared to 27.0 months for patients treated with PEM and short-term/intermittent Dabrafenib + trametinib (p = 0.13) |
|                      |       |                                      |                                                             | Arm 2: Pembrolizum + Dabrafenib + Trametinib (intermediate)       | Secondary: Determine PFS                                                                          |                                                                                       |
|                      |       |                                      |                                                             | Arm 3: Pembrolizum + Dabrafenib + Trametinib (long)               | Determine rates of response                                                                     |                                                                                       |
|                      |       |                                      |                                                             | Arm 4: Pembrolizum + Dabrafenib + Trametinib (long)               | Determine long term toxicities                                                                   |                                                                                       |
|                      |       |                                      |                                                             | First Line (n = 32)                                               |                                                                                                 |                                                                                       |
|                      |       |                                      |                                                             | Arm A: Nivolumab + Ipilimumab (induction), Nivolumab (maintenance) | Overall survival rate                                                                           | Active, recruiting                                                              |
|                      |       |                                      |                                                             | Arm B: Dabrafenib + Trametinib, followed by Nivolumab + Ipilimumab | Secondary: PFS                                                                                   |                                                                                       |
|                      |       |                                      |                                                             | Arm C: Dabrafenib + Trametinib                                   | Objective Response Rate                                                                         |                                                                                       |
|                      |       |                                      |                                                             | Arm D: Nivolumab + Ipilimumab (induction), Nivolumab (maintenance) | Toxicity Rate for irAEs                                                                         |                                                                                       |
|                      |       |                                      |                                                             |                                                                                                 |                                                                                       |
| IMPemBra (NCT02625337) | 2     | Stage IV BRAF<sup>V600E</sup> or BRAF<sup>V600E</sup> positive metastatic melanoma | Unspecified                                                 | Arm A: Nivolumab + Ipilimumab (induction), Nivolumab (maintenance) | Overall survival rate                                                                           | Active, not recruiting                                                   |
|                      |       |                                      |                                                             | Arm B: Dabrafenib + Trametinib, followed by Nivolumab + Ipilimumab | Secondary: Total PFS, best overall response rate and duration of response                        |                                                                                       |
|                      |       |                                      |                                                             | Arm C: Dabrafenib + Trametinib                                   |                                                                                                 |                                                                                       |
|                      |       |                                      |                                                             |                                                                                                 |                                                                                       |
| DREAMseq (NCT02224781) | 3     | Locally advanced unresectable melanoma or stage IV melanoma | Unspecified                                                 | Arm A: Nivolumab + Ipilimumab (induction), Nivolumab (maintenance) | Overall Survival                                                                               | Active, recruiting                                                              |
|                      |       |                                      |                                                             | Arm B: Dabrafenib + Trametinib, followed by Nivolumab + Ipilimumab | Secondary: Total PFS, best overall response rate and duration of response                        |                                                                                       |
|                      |       |                                      |                                                             | Arm C: Dabrafenib + Trametinib                                   |                                                                                                 |                                                                                       |
|                      |       |                                      |                                                             |                                                                                                 |                                                                                       |
| SECOMBIT (NCT 02631447) | 2     | Metastatic melanoma with the BRAF<sup>V600E</sup> mutation | Unspecified                                                 | Arm A: LGX818 MEX: MEK162 PD = progressive disease                | Overall Survival                                                                               | Active, not recruiting                                                   |
|                      |       |                                      |                                                             | Arm A: LGX818 + MEK162 until PD, followed by Nivolumab + Ipilimumab | Secondary: Total PFS, best overall response rate and duration of response                        |                                                                                       |
|                      |       |                                      |                                                             | Arm B: Nivolumab + Ipilimumab until PD, followed by LGX818 + MEK162 |                                                                                                 |                                                                                       |
|                      |       |                                      |                                                             | Arm C: LGX818 + MEK162 for 8 weeks, Nivolumab + Ipilimumab until PD, then LGX818 + MEK162 |                                                                                                 |                                                                                       |
|                      |       |                                      |                                                             | Three Arms: Arm 1: Dabrafenib + Trametinib, then Pembrolizumab     | Pathological Response Rate                                                                      | Active, not recruiting                                                   |
|                      |       |                                      |                                                             | Arm 2: Dabrafenib + Trametinib + Pembrolizum (6 w), then Pembrolizumab | Secondary: Objective Clinical Response Rate                                                         |                                                                                       |
|                      |       |                                      |                                                             | Arm 3: Pembrolizum                                                | Relapse Free Survival                                                                          |                                                                                       |
|                      |       |                                      |                                                             |                                                                                                 | Overall Survival                                                                         |                                                                                       |
|                      |       |                                      |                                                             |                                                                                                 |                                                                                       |

(Continued)
| Study (NCT) | Phase | Clinical Setting | Line of Treatment | Arms | Primary and Secondary End Points | Outcome |
|-------------|-------|------------------|-------------------|------|----------------------------------|---------|
| BRAFi/MEKi and antiangiogenic agent | NCT01495988 | 2 | Stage IV BRAF<sup>V600E</sup> positive melanoma | First line | Arm 1: Vemurafenib + Cobimetinib + Placebo | Maximum tolerated dose |
| | | | | | Arm 2: Vemurafenib + Cobimetinib + Bevacizumab | Median PFS |
| | NCT03175432 | 2 | Untreated melanoma with brain metastasis | First line | Arm 1: Bevacizumab + Atezolizumab + Placebo | Secondary: Overall Survival + Response Rates |
| | | | | | Arm 2: Bevacizumab + Atezolizumab + Cobimetinib | Objective intracranial response rate |
| | | | | | | Safety and tolerability of the arms in trial |
| | | | | | | Secondary: Incidence of adverse events |
| | | | | | | Overall response rates |
| | | | | | | Duration of response |
| BRAFi/MEKi and cell-based therapy | NCT03455764 | 1 + 2 | Advanced melanoma BRAF<sup>V600E/K</sup> positive | Second line (following BRAFi/MEKi treatment) | Lacnotuzumab (MCS110): Colony-stimulating factor-1 (CSF-1) inhibitor antibody Lacnotuzumab + Dabrafenib + Trametinib | Dose-Limiting Toxicity |
| | NCT03101254 | 1 + 2 | Advanced melanoma BRAF<sup>V600E/K</sup> positive | First line | LY3022855: Colony-stimulating factor-1 receptor (CSF-1 R) inhibitor LY3022855 + Vemurafenib and Cobimetinib | PFS (Complete Response Rate) |
| | | | | | | Secondary: Side Effects from Therapy |
| | | | | | | Overall Response Rate |

NCT = clinicaltrials.gov number.
(themselves associated with lower rates of rAEs than CTLA-4 inhibition), has renewed interest in combination therapy, with a number of clinical trials in progress or recently completed.

The KEYNOTE-022 (NCT02130466) trial compared the combination of Dabrafenib, Trametinib, and Pembrolizumab to Dabrafenib, Trametinib, and placebo [77]. While statistical significance was not reached for the primary end point of PFS, there was a numerical trend toward improved PFS. The median duration of responses was also numerically longer in triplet therapy than in the placebo arm. However, this was associated with a 58% rate of grade 3–5 treatment-related adverse events (tAEs), which would not typically be accepted in clinical practice [78]. Further to this, the findings of the TRIDeNT (NCT02910700) study, adding Nivolumab to Dabrafenib and Trametinib, showed encouraging efficacy signals at the cost of a 78% rate of grade 3–4 tAEs [79]. The COMBI-I trial (NCT02967692) did not demonstrate any benefit from the addition of the anti-PD-1 monoclonal antibody Spartializumab to Dabrafenib and Trametinib, with the authors commenting that adverse event management was challenging and frequent dosing adjustments were required [80].

For BRAFi/MEKi/ICI combinations to be safely used in clinical practice, there is a need to identify predictive biomarkers for toxicity as well as response. Preplanned subgroup analyses within the TRILOGY/IMSpire150 trial (NCT0290867) evaluating combinations of Vemurafenib plus Cobimetinib with or without Atezolizumab (Table 2) suggested benefit in those patients with high serum LDH, more than three sites of metastasis and M1c disease, the latter referring to metastatic spread to locations other than the central nervous system [81]. It may be that the benefits of anti-tumor efficacy outweigh those of tAEs in patients with a higher disease burden [77,80]. In addition to improved patient selection, modifications to treatment dose and schedule with ‘lead in’ or priming therapy, whereby BRAFi/MEKi are given prior to ICIs, or ‘on–off’ dosing regimens where BRAFi/MEKi are given for short periods while patients are maintained on ICIs or vice versa, may lead to better tolerated regimens and more durable responses.

As discussed, preclinical studies support the principle of lead in/priming therapy with BRAFi/MEKi prior to ICI therapy. In a phase 1b study (NCT01656642) including a BRAFi/MEKi lead in/priming phase with Vemurafenib and Cobimetinib, followed by the addition of the antibody Atezolizumab (anti-PD-L1), CD4+ T cell proliferation was increased without an increase in T-reg, priming the TME for immunotherapy as demonstrated by increased helper T cells and CTLs in the TME following the administration of Atezolizumab [82]. This has been recapitulated in the IMSpire150 trial, which demonstrated an improved median PFS, when Atezolizumab was added after a BRAFi/MEKi run in, with little difference in adverse events reported. The US Food and Drug Administration (FDA) have now approved the combination, but it is yet to be approved by the European Medicines Agency (EMA) or the Medicines and Healthcare products Regulatory Agency (MHRA) in the UK [81] (Table 2).

The IMPemBra trial (NCT02625337) examined the impact of short-term/intermittent dual MAPK pathway inhibition (Dabrafenib and Trametinib) with Pembrolizumab in the hope of reducing the high frequency of grade 3–4 tAEs in triplet therapy and high rates of treatment discontinuation. This trial demonstrated improved median PFS of patients from 10.6 months in the Pembrolizumab monotherapy arm to 27.0 months in patients with Pembrolizumab combined with short-term intermittent Dabrafenib and Trametinib therapy with improved tolerability compared to traditional triplet therapy [83]. There are ongoing studies exploring the appropriate sequencing of these agents within the DREAMseq trial (NCT02224781), NEMOCRIT (NCT02631447), and NEMOTRIO (NCT02858921) studies, and preliminary results have been recently published.

In contrast to preclinical studies, early data from the DREAMseq trial seem to demonstrate that patients, in fact, benefit from ICI treatment prior to BRAFi/MEKi. Their unique trial design enrolled patients to either receive Nivolumab/Ipilimumab or Dabrafenib/Trametinib until disease progression, whereby they switched to the other treatment. Superior OS was seen in the patients receiving ICI first, which became evident at 10 months, with more ongoing responses than patients who started with MAPKi [84]. In addition, preliminary results from the phase 2 SECOMBIT trial, which compared a similar treatment sequencing, appears to support the use of ICI prior to MAPK inhibition [85]. In addition to the treatment arms used in DREAMseq, SECOMBIT has a third arm, in which patients receive priming with BRAFi/MEKi for eight weeks, before switching to ICI therapy until disease progression, and switching back to treatment with BRAFi/MEKi thereafter. This third arm also demonstrated better outcomes compared with patients receiving BRAFi/MEKi until disease progression as their first treatment.

It is important to note that the initial advantageous early infiltration of T cells observed with BRAFi is no longer present at the time of disease progression, with a reduced CD8 + T cell population and a more pronounced protumor, anti-inflammatory TAM gene expression profile [49,70,86]. In order to use BRAFi/MEKi to prime the TME for ICI treatment, a shorter priming phase, such as in the third arm of SECOMBIT, might be included in trials. In this way, it may be possible to capture the effects of MAPKi at an optimum timepoint for activating the patient’s immune response and thus augmenting ICI treatment, but before the onset of resistance.

6.2. Targeting immune cells that contribute to BRAFi resistance: myeloid cells

The importance of the infiltrating myeloid cell compartment in the development of BRAFi resistance has been demonstrated in studies which show how CCL2/CCR2 signaling is restored as BRAFi resistance develops, increasing the numbers of MDCs and the frequency of TAMs in the TME [87]. Aside from CCL2/CCR2, signaling through colony-stimulating factor 1 receptor (CSF1R), expressed on myeloid cells, is essential for their recruitment and maintenance in the TME. Small-molecule inhibitors against CSF1R in combination with
BRAFi have demonstrated a reduction in tumor size in preclinical studies [88]. However, anti-CSF1R is less efficacious than BRAFi when used alone and is also expressed on other myeloid cells that may play beneficial roles in anti-tumor immunity [67]. Despite this, there are a two ongoing early-phase clinical trials exploring the role of CSF1R inhibitors as monotherapy (NCT02071940, NCT02975700) and two in combination with BRAFi and MEKi (NCT03455764, NCT03101254) (Table 2).

### 6.3. Angiogenesis and BRAF resistance in melanoma

Angiogenesis is a recognized hallmark of cancer, and within melanoma it is associated with increased aggressiveness and worse prognosis [89]. The importance of VEGF signaling within tumorigenesis has been demonstrated across multiple cancer types including colorectal, ovarian, and uterine cancers [89]. Antiangiogenic drugs bind either angiogenic factors (e.g., VEGF-A) or their receptors (e.g., VEGFR1/2). Antiangiogenic agents not only remodel irregular and leaky vessels in cancer lesions, resulting in improved tumor penetration of chemotherapy agents, but may also impact on the TME through relieving tissue hypoxia, which can alter immune cell composition [89]. The first trials involving antiangiogenic drugs within melanoma date back to almost two decades ago, when chemotherapy was the only available treatment for advanced stage disease; these trials often involved small population sizes [89]. Prior to the introduction of targeted and ICIs, the FDA, but not the EMA or the MHRA, had approved Bevacizumab, a monoclonal antibody recognizing VEGF, plus cytotoxic chemotherapy as a first-line treatment for unresectable melanoma [90,91]. There is significant interest in reexamining the role of VEGF in optimizing current regimens with BRAFi/MEKi and ICIs. In the setting of BRAFi resistance, it is recognized that BRAFi can lead to the paradoxical activation of the MAPK pathway in non-mutant cells in the TME, including TAMs [67], driving these cells toward immunosuppressive phenotypes and, in the case of TAMs, increasing the secretion of VEGF, which in turn, can reactivate MAPK in melanoma cells [67,92]. Blocking VEGF and its interaction with VEGF-R-expressing cells, in conjunction with BRAFi, delayed the time to treatment resistance in a mouse model of melanoma [92].

A number of clinical trials are studying this further (NCT01495988, NCT03175432) [89]. There have also been initial promising results within clinical trials combining immunotherapy and antiangiogenic agents which are now progressing to phase III studies (NCT04356729, NCT02681549, and NCT03239145) [93,94] (Table 2). Exploring combinations of inhibitors such as anti-VEGF may still reveal novel mechanisms in the future that may help understand BRAFi resistance.

### 6.4. Enhancing Immune Effector Function: Adoptive Cell Therapies

Personalized cell therapy targeting tumor-associated antigens with expanded tumor-infiltrating lymphocytes (TILs) has shown some promise in metastatic melanoma since the early 1990s [95]. Adoptive cell therapy (ACT) of T cells, involving the allogenic transplant of TILs, and CAR-T therapy, whereby genetically modified T cells expressing novel T cell receptors, chimeric antigen receptors (CARs) are expanded and reinfused into the patient, have both shown promise in treating hematological malignancies but efficacy is yet to be realized in solid tumors, including melanoma [96].

In the latest Phase 2 study examining ACT using TILs in patients with treatment refractory melanoma, 36% patients had a partial response (22 of 66), with two patients having a complete response [97]. The safety profile was comparable to lymphodepleting chemotherapy, and this has led to studies evaluating combinations of ACT with other treatments. MAPKi have been suggested to enhance the clinical efficacy of ACT. In a preclinical study, BRAFi was shown to induce the upregulation of the mannose 6 phosphate receptor (M6PR) in a dose-dependent fashion in both BRAFi-sensitive and -resistant melanoma cells. M6PR increases cancer cell uptake of granzyme B, a main component of the cytotoxic activity of CTLs. Thus, it has been suggested that it may be beneficial to treat with BRAFi prior to TIL therapy, as a means of enhancing ACT-mediated elimination of tumor cells [98]. A clinical study of 13 patients with checkpoint inhibitor-resistant melanoma evaluated priming treatment with Vemurafenib prior to ACT with TILs. This study demonstrated significant clinical responses (one complete, eight partial responses, three patients with stable disease) and no unexpected toxicity [99]. While this may serve as proof of principle, larger trials with longer follow-up times are required in order to identify patients most likely to benefit, identify the most appropriate point of intervention, and to establish durability of responses.

There has been significant interest in examining the role of CTL populations in anti-cancer therapy, however other cell types within the TME, including NK cells, can also engender tumor cell destruction. NK cells are of particular interest since their effector functions are not impaired as BRAFi resistance develops, and indeed their ability to lyse melanoma cells can increase in models of BRAFi-resistant melanoma [100,101]. Boosting the effector function of NK cells may delay BRAFi resistance and increase an effective anti-tumor response. The TLR7 agonist, imiquimod, already used for the topical treatment of non-melanoma skin cancers, has been shown to increase the activity of both NK cells and T cells in a murine model of melanoma [50], and this is currently being tested in clinical trials as an adjuvant to melanoma vaccine therapies and checkpoint inhibitors (NCT0436423, NCT04401995).

Aside from ACT, several large-scale clinical trials using CAR-T cells for melanoma are ongoing as a second-line therapy after treatment failure with ICI or BRAFi/MEKi; however, the use of CAR-T in BRAFi-resistant melanoma remains underexplored [102].

### 7. Conclusion

Although designed to impact the growth, proliferation, and survival of melanoma cells, MAPK pathway small-molecule inhibitors such as BRAFi in clinical use for the treatment of
BRAF mutant melanoma can influence the immune composition of the TME. With immunotherapy dominating the landscape of melanoma therapeutics development, it is worth understanding how BRAFi can manipulate the immune response and how new and established treatments may be used sequentially or in combination to take advantage of cancer vulnerabilities and improve outcomes for patients.

8. Expert opinion

The past two decades have witnessed an unprecedented expansion in our understanding of the biology of melanoma, underpinning the development of new therapeutic classes: targeted oncogene inhibitors and ICIs. These developments have significantly improved the historically low five-year survival rates in advanced melanoma. Within cancer therapeutics, however, the development of resistance is a frustrating, yet unsurprising, phenomenon, given the selection pressure that therapies apply on cancer cells.

The success of ICIs has highlighted the importance of understanding the dynamic interactions between tumor cells and the immune component of the TME. Research has largely focused on the role of BRAF as an oncogenic target; however, there is evidence that MAPK inhibition significantly impacts on the TME by its effects in both immune and nonimmune cells. In the next decade, research will reveal previously unappreciated immune mechanisms which accompany response and resistance to BRAFi. These will enable us to better select treatment combinations or refine the sequence and timing of administering therapeutic agents to unlock more durable responses for our patients.

BRAFi initially leads to profound tumor regression; however, responses are short lived. This contrasts with ICI, where responses are less predictable but more durable. The approach within current clinical practice suggests that these treatments are mutually exclusive as first-line therapy, with debate over which treatments should be used first. New evidence suggests that ICIs may prolong survival in patients with BRAF mutant melanoma over MAPK inhibition [16]; however, it does not follow that BRAFi and MEKi should be relegated to second-line therapies, especially given that there are still significant limitations to ICI therapy. There remains a degree of unpredictability about which patients will respond to ICIs and whether responses to treatment differ between patient cohorts. For example, patients of female sex and increasing age appear to derive less benefit from ICI than their younger and male counterparts [103,104], while at the same time BRAFi appear to have comparable efficacy across age groups [105].

An arsenal of multiple first-line therapies and patient stratification may help ensure more patients, from multiple cohorts, respond to treatment. In addition to this, it is important to understand how MAPKi can modulate the immune system, and with the correct timing, could be successfully used as an adjuvant not only to ICIs, but to other therapies too.

It is possible that different regimens of existing drugs, or better stratification of patients receiving them, may lead to better outcomes quicker than the development of novel therapies. For example, since BRAFi resistance in melanoma can lead to the upregulation of VEGF pathways, and VEGF inhibitors have shown efficacy in a range of solid tumors including renal, urological and ovarian cancers, it may be timely to reconsider the use of VEGF inhibitors alongside BRAFi. Early trials in melanoma were disappointing, a better understanding of why these therapies failed and how new treatments interact with them, may reveal how best to use them in the future. To take the first point, since the early initial trials of VEGF inhibitors, it has been demonstrated that the expression of VEGF decreases with aging [106]. This has been shown to reduce responses to anti-VEGF treatments in older patients, while the age-related proangiogenic factor secreted frizzled-related protein 2 (sFRP2) may be responsible for driving angiogenesis in older aged groups. Thus, better stratification of patients may lead to more successful implementation of tailored anti-angiogenic therapies and combinations. Alongside, since BRAFi resistance is characterized by the paradoxical increased secretion of VEGF, there is an opportunity for VEGF or other targeted anti-angiogenic inhibitors to work synergistically with a treatment which had not been discovered when VEGF inhibitors were initially trialed.

The success of ICIs has invariably led a focus on how to improve and augment the clinical efficacy of this class of drug, including how MAPKi can be used alongside ICIs. Increased understanding in how both MAPKi and ICIs alter the TME may provide answers to the current clinical problems arising from combination treatment, namely: how can toxicity be overcome and how can sequencing be optimized to prolong patient survival? New trial design, as seen in SECOMBIT and DREAMseq, whereby different sequences of therapy are compared, will be invaluable in answering some of these questions. As well as clinical outcomes, the examination of biomarkers and biopsies throughout these studies will enable a better insight into the synergistic immune mechanisms that could underpin successful combinations of the two most revolutionary melanoma treatments of the decade.

The success of ICIs may represent the end of the beginning for drug discovery in melanoma rather than the beginning of the end. In cancers where ICI have failed, clinical trials reevaluating old drugs, as well as those testing new therapies abound. In contrast, the focus within melanoma treatment appears to be optimizing ICI regimens when we know that responses are unpredictable and not universal. However, refining and augmenting established treatment strategies, such as MAPK pathway inhibition hold significant merit.

Importantly, there must be continued investment and research into new druggable targets for melanoma. The discovery of new drugs will contribute to the transformation of therapeutic strategies for melanoma patients we have witnessed over the past decade. Revisiting old therapies in the context of recent conceptual and translational advances, may unearth combinations or sequential therapeutic protocols that can improve patient care and outcomes immeasurably.
Nomenclature

BRAFi | BRAF inhibitors
MEKi | MEK inhibitors
ICI | immune checkpoint inhibitors
MAPK | mitogen-activated signalling pathway
MAPKi | mitogen-activated signalling pathway inhibitors
PFS | Progression free survival
OS | overall survival
MDSC | myeloid derived suppressor cells
irAEs | immune related adverse events
treatment-related adverse events
T-reg | regulatory T cells
TAMs | tumour associated macrophages
VEGF | vascular endothelial growth factor
APCs | antigen presenting cells
CTLs | cytotoxic T cells
NK cells | natural killer cells
DCs | dendritic cells
MHC-I | major histocompatibility complex
TILs | tumour infiltrating lymphocytes
ACT | adoptive cell therapy
CAR-T | chimeric antigen T-cell therapy

Declaration of interest

S Karagiannis is founder and shareholder of Epsilogen Ltd. and declares patents on antibody technologies. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosure

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Funding

The authors acknowledge support by the Medical Research Council (MR/ L023091/1; MR/V049445/1); Cancer Research UK King’s Health Partners Centre at King’s College London (C604/A25133); CR UK/NIHR in England/DoH for Scotland, Wales and Northern Ireland Experimental Cancer Medicine Centre (C10355/A15587); Breast Cancer Now (147; KCL-BCN-Q3); the Guy’s and St Thomas’ Foundation Trust Charity, Melanoma Special Fund (SPF573); Cancer Research UK (C30122/A11527; C30122/A15774). The research was supported by the National Institute for Health Research Biomedical Research Centre based at Guy’s and St Thomas’ NHS Foundation Trust and King’s College London (IS-BRC-1215-2006). The authors are solely responsible for study design, data collection, analysis, decision to publish, and preparation of the manuscript. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, or the Department of Health.

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