Targeted therapy in BRAF-mutant melanoma: Advances and challenges (Review)

LU ZHANG[1], DONGLIANG SHEN[1], JIANGUO FENG[2] and LILING TANG[1]

[1]Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, P.R. China; [2]Anesthesiology and Critical Care Medicine Key Laboratory of Luzhou, Department of Anesthesiology, The Affiliated Hospital, Southwest Medical University, Luzhou, Sichuan 646000, P.R. China

Received November 18, 2025; Accepted May 19, 2026

DOI: 10.3892/ijo.2026.5901

Abstract. Over half of patients with melanoma exhibit v-Raf murine sarcoma viral oncogene homolog B (BRAF) mutations, which drive hyperactivation of the MAPK pathway and confer high proliferative potential. To target this oncogenic mutation, BRAF inhibitors, as well as MEK inhibitors (targeting the downstream effector of BRAF), have been approved for melanoma therapy. Although these inhibitors initially decrease the tumor burden, nearly all patients eventually develop drug resistance, leading to aggressive disease relapse at both the primary and metastatic sites. Understanding the mechanisms underlying tumor escape from drug lethality is crucial for the development of strategies against melanoma. The present study aimed to summarize the discovery, development and clinical evolution of the BRAF and MEK inhibitors approved for the treatment of melanoma, their notable efficacy in suppressing aggressive melanoma progression and the underlying mechanisms of acquired resistance.

Contents

1. Introduction
2. BRAF inhibitor monotherapy
3. MEK inhibitor monotherapy
4. Combination therapy with BRAF and MEK inhibitors
5. Challenges in melanoma targeted therapy
6. Therapeutic strategies to overcome acquired MAPKi resistance
7. Conclusion

Correspondence to: Professor Liling Tang, Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, 174 Shazheng Street, Chongqing 400044, P.R. China
E-mail: tangliling@cqu.edu.cn

Professor Jianguo Feng, Anesthesiology and Critical Care Medicine Key Laboratory of Luzhou, Department of Anesthesiology, The Affiliated Hospital, Southwest Medical University, Luzhou, Sichuan 646000, P.R. China
E-mail: fengjianguo@swmu.edu.cn

Key words: BRAF, melanoma, plasticity, resistant, targeted therapy

1. Introduction

During embryonic development, melanoblasts, derived from neural crest stem cells (NCSCs), migrate to the epidermis and differentiate into melanocytes. Upon stimulation by ultraviolet radiation or genetic alterations, melanocytes initiate tumorigenesis in the epidermis (1). They invade the dermis by reacquiring NCSC-like characteristics and losing well-differentiated melanocytic phenotypes (1). Mutations in driver gene are key for the carcinogenic transformation of melanocytes. Among these, the v-Raf murine sarcoma viral oncogene homolog B (BRAF) mutation is the most prevalent in melanoma and was first identified in 2002 (2). Somatic mutation screening has shown that BRAF mutations occur in 59% of melanoma (2,3), 44% of papillary thyroid carcinomas (4), 18% of colorectal cancer, 11% of glioma, 9% of sarcoma and 3% of lung cancer cases (2).

As a member of the RAF kinase family (which also includes ARAF and CRAF), BRAF serves a key role in the MAPK signaling pathway, regulating critical cellular processes including proliferation, metastasis, differentiation and survival. Serving as the primary regulator of the RAS/RAF/MAPK kinase (MEK)/ERK signaling cascade, BRAF mediates the activation of downstream targets, MEK and ERK, throughout this pathway (5,6). The kinase activity of the class I mutant BRAF is constitutively activated, leading to hyperstimulation of the MAPK pathway and excessive cell proliferation and metastasis. Class I BRAF mutations typically occur at the V600 residue, with the mutant protein exhibiting high kinase activity even in its monomeric form. Notably, the kinase activity of V600-mutant BRAF is less RAS-dependent than that of non-V600 mutations, similar to that of class II mutant BRAF (5). By contrast with class I mutations, class II BRAF mutations occur at non-V600 sites and result in moderate kinase activity in the dimeric conformation.

Class III BRAF mutations, which also involve non-V600 sites, typically form heterodimers with wild-type RAF and exhibit RAS-dependent, minimal kinase activity (5-7) (Table I). In total, >97% of BRAF mutations are class I mutations. Non-V600 mutations, such as K601, L579, G469, G464, D549, G466, and D287, occur in ~1%) of patients with melanoma (5,8). Given the high prevalence of V600-mutant BRAF in patients with melanoma, targeted small-molecule inhibitors of $BRAF^{V600}$ have been developed, such as vemurafenib, dabrafenib and encorafenib (6). While BRAF inhibitors are promising in delaying disease progression, the majority of patients with melanoma develop resistance after several treatment cycles, and ~70% of patients succumb to disease progression within 5 years of treatment (9), highlighting the importance of understanding and overcoming the relapse phase in therapeutic resistance.

The present study aimed to review the discovery, development and clinical evolution of BRAF and MEK inhibitors for targeted melanoma therapy (Table II). Tumor-intrinsic resistance mechanisms driven by dynamic phenotypic plasticity and acquired genetic mutations, as well as the potential therapeutic strategies to overcome drug resistance, are also discussed.

2. BRAF inhibitor monotherapy

Vemurafenib, the first BRAF inhibitor approved for clinical trials. Vemurafenib, known as PLX4032 during preclinical testing, is an analog and optimized derivative of PLX4720 (10), the first selective $BRAF^{V600E}$ inhibitor. PLX4720 served as the lead compound and demonstrated high selectivity for $BRAF^{V600E}$ over wild-type BRAF and a panel of 70 other kinases (11). Mechanistically, it occupies the interlobe cleft of the BRAF kinase domain and targets the hinge-proximal region to competitively block the ATP-binding site (11). PLX4032 replaced PLX4720 as the first-in-class therapeutic drug in human clinical trials because of its superior pharmacokinetic properties (12). Preclinical evaluation of PLX4032 antitumor activity demonstrated that it suppresses ERK phosphorylation, a downstream target of BRAF, and inhibits cell proliferation by inducing cell cycle arrest and apoptosis in BRAF-mutant melanoma cells (13-15). PLX4032 exhibits the opposite effect in BRAF wild-type melanoma cells. By stimulating CRAF in a RAS-independent manner, it paradoxically activates ERK signaling, thereby promoting cell proliferation and migration (16,17). This may explain PLX4032 selective inhibition of $BRAF^{V600E}$-mutant cells while maintaining minimal toxicity in normal cells. The predominant preclinical results in BRAF-mutant melanoma cells led to the initiation of PLX4032 phase I clinical trials in 2009 to evaluate its safety, pharmacokinetics and pharmacodynamics (18). Similar to most small-molecule inhibitors, PLX4032 shows dose-dependent toxicity in arthralgia, keratoacanthoma/cutaneous squamous cell carcinoma (cSCC) and rash. Notably, 89% of these adverse events are grade 1 or 2, indicating manageable toxicity (10,12). The aforementioned clinical trial established the optimal therapeutic dose of PLX4032 for melanoma therapy through dose-escalation assay, while also providing a critical foundation for subsequent clinical trials and the development of next-generation BRAF inhibitors.

PLX4032 was renamed vemurafenib in 2010 and was approved by the US Food and Drug Administration (FDA) in 2011 as the first-in-class BRAF inhibitor for unresectable or metastatic $BRAF^{V600E}$-mutant melanoma (19). This was primarily supported by the positive results of the phase 3 BRIM-3 trial (trial no. NCT01006980) of vemurafenib in melanoma (20). Despite adverse events consistent with the PLX4032 known safety profile, vemurafenib-treated patients show superior outcomes compared with dacarbazine chemotherapy, with a higher objective response rate (48 vs. 5%) (20) and improved median overall survival (13.6 vs. 9.7 months) (21). Although all patients in the aforementioned study discontinued treatment owing to disease progression, vemurafenib set a benchmark for BRAF inhibitors optimization and transformed the therapeutic landscape for melanoma.

Dabrafenib is a widely utilized BRAF inhibitor with advanced safety. Concurrently with the clinical application of vemurafenib, dabrafenib (GSK2118436), a second-generation BRAF inhibitor, was developed. Approved by the FDA in 2013, dabrafenib has become the second BRAF inhibitor for unresectable or metastatic $BRAF^{V600E}$-mutant melanoma, offering an alternative therapeutic option. Similar to vemurafenib, GSK2118436 competitively binds the ATP-binding sites of the BRAF kinase domain (22). Preclinical studies demonstrate that GSK2118436 inhibits ERK and MEK phosphorylation, suppressing cell proliferation through induction of cell cycle arrest (23,24). GSK2118436 was renamed dabrafenib in 2012 and has superior efficacy vs. dacarbazine, with higher objective response rate (50 vs. 6%), improved median progression-free survival (mPFS, 5.1 vs. 2.7 months) and lower progressive disease rate (5 vs. 37%) (25). These preclinical and clinical results collectively supported the FDA 2013 approval of dabrafenib as a $BRAF^{V600E}$-specific inhibitor for targeted melanoma therapy.

Although dabrafenib monotherapy displays tumor suppression efficacy comparable with vemurafenib in melanoma treatment, it exhibits superior performance in several other aspects. Unlike vemurafenib, which impairs lymphocyte counts (particularly central memory $CD4^+$ T cell populations) and interferon-g/interleukin-9 cytokine production, dabrafenib shows minimal immunotoxicity (26). Cutaneous toxicity of dabrafenib is lower than that of vemurafenib (skin papilloma, 15 vs. 29; cSCC/keratoacanthoma, 10 vs. 26; photosensitivity, 1 vs. 52%) (27). The markedly decreased dermatological toxicity, combined with the lower immunosuppressive risk of dabrafenib, establishes it as a clinically optimized BRAF inhibitor.

3. MEK inhibitor monotherapy

While the development of vemurafenib and dabrafenib has improved outcomes for patients with BRAF-mutant melanoma, long-term therapy has revealed paradoxical activation of the MAPK signaling pathway. Theoretically, the dimerization of RAF family members is key for the activation of their kinase activity and transduction of signals from upstream RAS. Monomeric wild-type BRAF remains inactive due to autoinhibition mediated and maintained by the 14-3-3 protein cradle (28). V600 mutations induce a conformational change

Table I. Categorization of BRAF mutations.

Property	BRAF variant			
	Wild-type	Class I	Class II	Class III
Mutation site	Not applicable	V600	Non-V600 (G464, G469, L597, K601)	Non-V600 (D287, V459, G466, S467)
Kinase activity	Regulated	High	Moderate	Low or inactive
RAS-dependency	Dependence	Independence	Partial dependence	Dependence
Dimerization activation	Yes	No	Yes	Yes
Sensitivity to BRAF inhibitors	Resistance	High sensitivity	Low sensitivity	Resistance

Table II. MAPK inhibitors approved by the US Food and Drug Administration for melanoma therapy.

Drug	Year	Phase	Trial ID	Enrolled	(Refs.)
Vemurafenib	2011	1	NCT00405587	55 patients in the dose-escalation phase; 32 metastatic melanomas with $BRAF^{V600E}$ in the extension phase	(10)
		2	NCT00949702	132 stage IV metastatic melanomas with $BRAF^{V600E}$	(170)
		3	NCT01006980	675 unresectable stage IIIC or IV melanomas with $BRAF^{V600E}$	(20)
Dabrafenib (GSK2118436)	2013	1	NCT00880321	156 metastatic melanomas with $BRAF^{V600E/K}$; 28 non-melanoma solid tumors with $BRAF^{V600E}$	(171)
		2	NCT01153763	92 stage IV metastatic melanomas with $BRAF^{V600E/K}$	(27)
		2	NCT01266967	172 brain-metastatic melanomas with $BRAF^{V600E/K}$	(172)
		3	NCT01227889	250 metastatic melanomas with $BRAF^{V600E}$	(25)
Trametinib (GSK112021)	2013	1	NCT00687622	81 melanomas; 30 NSCLCs; 28 CRCs; 26 PCs; 16UMs	(173)
		2	NCT01037127	97 metastatic melanomas with $BRAF^{V600E/K/D}$ previously treated with a BRAF inhibitor or chemotherapy/immunotherapy	(36)
		3	NCT01245062	322 unresectable stage IIIC or IV melanomas with $BRAF^{V600E/K}$	(35)
Dabrafenib and trametinib	2014	1/2	NCT01072175	247 metastatic melanomas with $BRAF^{V600E/K}$	(38)
		2	NCT02039947	125 brain-metastatic melanomas with $BRAF^{V600D/E/K/R}$	(174)
		3	NCT01584648	423 unresectable stage IIIC or IV metastatic melanomas with $BRAF^{V600E/K}$; compared to dabrafenib monotherapy	(175)
		3	NCT01597908	704 metastatic melanomas with $BRAF^{V600E/K}$; compared with vemurafenib monotherapy	(39)
		3	NCT01682083	870 completely resected, stage III melanomas with $BRAF^{V600E/K}$	(176)
Vemurafenib and cobimetinib	2015	1	NCT01271803	129 unresectable stage IIIC or IV melanoma with $BRAF^{V600E/K}$	(177)
		2	NCT02230306	101 brain metastatic melanomas with BRAF mutations	(178)
		3	NCT01689519	495 unresectable stage IIIC or IV melanoma with $BRAF^{V600E/K}$; compared with vemurafenib monotherapy	(45)
Encorafenib and binimetinib	2018	1/2	NCT01543698	126 melanomas or CRCs with BRAF V600 mutations	(179)
		2	NCT05026983	35 melanomas with brain metastases or leptomeningeal disease harboring BRAF V600 mutations	(180)
		3	NCT01909453	577 unresectable or metastatic cutaneous melanomas with $BRAF^{V600E/K}$; compared with encorafenib or vemurafenib monotherapy	(54)

CRC, colorectal cancer; NSCLC, non-small-cell lung cancer; PC, pancreatic cancer; UM, uveal melanoma.

in the kinase domain of BRAF, stimulating the kinase function of monomeric BRAF and enabling RAS-independent MEK activation (6). Owing to the development of $BRAF^{V600}$ selective inhibitors, abnormal proliferation and metastasis driven by activated BRAF monomers are no longer a notable concern. However, $BRAF^{V600E}$ and wild-type RAF form heterodimers to

enhance kinase activity. When BRAF inhibitors bind mutant BRAF, the conformation of the inhibitor-free RAF protomer changes, leading to its release and transactivation, triggering the activation of the MAPK pathway (29-31). This is known as paradoxical activation. To address this, MEK inhibitors are combined with BRAF inhibitors as a dual-targeted therapy for melanoma.

Trametinib. Trametinib, originally known as GSK1120212, is the only FDA-approved MEK inhibitor for monotherapy, authorized in 2013 for the treatment of patients with unresectable or metastatic melanoma harboring $BRAF^{V600E}$ or $BRAF^{V600K}$ mutations. It directly binds unphosphorylated MEK1 and MEK2, maintaining them in an inactive, dephosphorylated state, leading to allosteric inhibition of kinase activity and suppression of cell proliferation and tumor growth (32,33). Notably, trametinib demonstrates a more favorable safety profile than BRAF inhibitors, with fewer severe adverse events, potentially due to its lower therapeutic dosage. Although rash and diarrhea are the most frequent adverse events, trametinib treatment, by contrast with BRAF inhibitors, is not associated with secondary malignancy such as cSCC (34).

In treatment-naïve patients, trametinib demonstrates superior clinical outcomes compared with that with conventional chemotherapy (intravenous dacarbazine or paclitaxel). mPFS and response rate (RR) are improved in the trametinib cohort compared with that in the chemotherapy cohort (mPFS: 4.8 vs. 1.5 months; RR: 22 vs. 8%) (35). Although the RR to trametinib is not as high as that to vemurafenib and dabrafenib, the PFS and overall survival are comparable. Patients pretreated with BRAF inhibitors show minimal response to trametinib (36), implying potential cross-resistance mechanisms between BRAF and MEK inhibitors. Trametinib avoids the toxicities associated with BRAF inhibitors, particularly in cSCC. Thus, combinations of BRAF and MEK inhibitors have emerged as strategies to optimize the therapeutic index through efficacy enhancement and toxicity reduction.

4. Combination therapy with BRAF and MEK inhibitors

Despite encouraging initial responses, clinical evidence shows that most patients relapse after responding to BRAF or MEK inhibitor monotherapy. To improve treatment efficiency, prolong response, enhance survival and reduce toxicity, a combination of BRAF and MEK inhibitors has emerged as a promising strategy for patients with melanoma (37).

Dabrafenib + trametinib. In 2014, the FDA approved a combination of dabrafenib and trametinib for patients with unresectable or metastatic melanoma carrying $BRAF^{V600E}$ or $BRAF^{V600K}$ mutations. The combination therapy demonstrates improved outcomes compared with dabrafenib monotherapy, with higher RR (76 vs. 54%), longer mPFS (9.4 vs. 5.8 months) and decreased incidence of skin-related toxicity (7 vs. 19% cSCC) (38). The dabrafenib-trametinib combination outperforms not only dabrafenib alone but also vemurafenib monotherapy, with an mPFS of 11.4 vs. 7.3 months and a RR of 64 vs. 51% (39). Baseline lactate dehydrogenase (LDH) levels influence treatment outcomes of dabrafenib and trametinib combination therapy. Additional baseline prognostic factors associated with improved clinical outcomes in patients with melanoma include older age, female sex, $BRAF^{V600E}$ mutation status and limited disease burden (<3 sites) (40).

Dabrafenib and trametinib combination therapy may be a suboptimal therapeutic choice for patients at risk of fever, given the 50% incidence of drug-induced pyrexia (38,41). These findings highlight the need to develop alternative therapeutic strategies for treating patients with distinct clinical risk profiles. Notably, the combination therapy shows limited efficacy in patients who develop acquired resistance to BRAF inhibitor monotherapy (42). This may be because the resistant cells are no longer dependent on MAPK signaling.

Vemurafenib + cobimetinib. The most frequent adverse effects of vemurafenib are cutaneous events, arthralgia, fatigue and photosensitive skin reactions, whereas pyrexia occurs in only 22% of treated patients (20). This favorable febrile profile makes vemurafenib an advantageous BRAF inhibitor option for fever-prone patients. A key therapeutic consideration is identifying the optimal MEK inhibitor partner. Cobimetinib, a selective MEK1 inhibitor developed by Exelixis, is a promising therapeutic candidate. By contrast with trametinib-induced dual inhibition of MEK1 and MEK2, cobimetinib (also known as GDC-0973 or XL518 during the developmental stage) demonstrates highly selective MEK1 inhibition, showing negligible activity against MEK2 and >100 other kinases. Although cobimetinib monotherapy elicits a RR of 50% in patients with melanoma, the FDA has not granted approval for single-agent use in melanoma therapy because of the high risk of serious adverse events, such as gastrointestinal disorder (43).

The recommended cobimetinib regimen employs a 21-day on/7-day off-dose schedule, administered once daily (43). This intermittent dosing minimizes drug accumulation risks, a key consideration given the prolonged half-life of cobimetinib, while maintaining optimal pharmacodynamic synergy with vemurafenib. Compared with vemurafenib monotherapy, the cobimetinib-vemurafenib combination extends both 5-year PFS (14 vs. 10%) and overall survival rate (31 vs. 26%), while enhancing the confirmed objective RR (70 vs. 50%) with comparable toxicity (44-46). Similar to the dabrafenib-trametinib combination regimen, treatment outcomes with the vemurafenib-cobimetinib combination therapy also vary based on baseline LDH levels. Typically, patients with elevated LDH levels have poorer survival outcomes than those with normal LDH levels (47). Based on these findings and supported by positive results, the FDA approved the vemurafenib-cobimetinib combination in 2015 for metastatic or unresectable melanoma harboring $BRAF^{V600E}$ or $BRAF^{V600K}$ mutation.

Encorafenib + binimetinib. Even in the absence of a high risk of pyrexia, the vemurafenib-cobimetinib combination leads to a high incidence of rash (73%), indicating cutaneous toxicity (45). To address this issue, Array BioPharma developed a new BRAF inhibitor, encorafenib, and a new MEK inhibitor, binimetinib. This combination strategy was approved by the FDA in 2018 for the treatment of unresectable or metastatic melanoma with BRAF V600E or V600K mutations (48).

At the 103rd Annual Meeting of the American Association for Cancer Research in 2012, encorafenib (LGX818) was

Table III. Terminal half-life and dosages of US Food and Drug Administration-approved BRAF and MEK inhibitors.

Class	Drug	Terminal half-life	Dosage	(Refs.)
BRAF inhibitors	Dabrafenib	4.0-6.8 h	150 mg twice daily	(181)
	Vemurafenib	57.0 h	960 mg twice daily	(19)
	Encorafenib	2.9-4.4 h	450 mg once daily	(57)
MEK inhibitors	Trametinib	4.0 days	2 mg once daily	(173)
	Cobimetinib	43.6 h	60 mg once daily for 21 days and 7 days off	(43)
	Binimetinib	8.7 h	45 mg twice daily	(52)

introduced as a highly potent RAF inhibitor. It demonstrates selective anti-tumor efficacy in $BRAF^{V600E}$-mutant cells, with no notable activity against wild-type BRAF or 100 other kinases (49,50). Mechanistically, encorafenib downregulates cyclin D1, induces G1-phase cell cycle arrest and promotes senescence in $BRAF^{V600E}$-mutant melanoma cells (51).

Binimetinib, a selective MEK1/2 inhibitor, was originally developed for autoimmune disease but was discontinued in clinical development owing to insufficient efficacy in trials (52). It effectively suppresses pERK levels and inhibits the proliferation of BRAF- and NRAS-mutant cells, including melanoma cells, with a low half-maximal inhibitory concentration of 5 nM (53). As both encorafenib and binimetinib were developed by the same pharmaceutical company, their combination therapy could be systematically evaluated for inherent advantages in terms of drug compatibility and synergistic development.

Compared with encorafenib or vemurafenib monotherapy, the encorafenib-binimetinib combination demonstrates improved clinical outcomes, with higher 5-year PFS rate (23 vs. 19 and 10%, respectively) and RR (64.1 vs. 51.8 and 40.8%, respectively). Moreover, the most common adverse events occurring with dabrafenib-trametinib (pyrexia, 50%) and vemurafenib-cobimetinib (rash, 73%) are decreased by the encorafenib-binimetinib combination (19 and 15%, respectively). While gastrointestinal toxicity and serous retinopathy occur frequently, these toxicities are generally managed through dose interruption or adjustment, as most are grade 1-2 in severity (54,55).

Compared with patients who receive monotherapy, those who receive combination therapy with BRAF and MEK inhibitors typically have better outcomes. Combination of BRAF and MEK inhibitors provides a double assurance to block MAPK signal transduction at the same time, thereby delaying or preventing drug resistance (6). The MAPK pathway is a linear cascade reaction involving BRAF, MEK and ERK. When only BRAF inhibitors are used, tumor cells may bypass BRAF through various mechanisms, such as NRAS mutation, BRAF alternative splicing or CRAF dimerization, thereby reactivating downstream MEK and ERK. Once this occurs, the BRAF inhibitors no longer function. However, the application of MEK inhibitors can prevent MAPK signaling activation by blocking MEK, even when BRAF inhibitors are ineffective.

To date, the FDA has approved three combined targeted therapeutic approaches: Dabrafenib + trametinib, vemurafenib + cobimetinib and encorafenib + binimetinib. The timelines for FDA-approved BRAF and MEK inhibitors are shown in Fig. 1. The doses and terminal half-lives are listed in Table III. Typically, the terminal half-life of an inhibitor is negatively associated with its administrated frequency. However, although vemurafenib has a terminal half-life of 57 h, it is administrated twice daily, whereas encorafenib, with a half-life of only 2.9-4.4 h, is dosed once daily. This is attributed to the non-linear pharmacokinetics and narrow therapeutic index of vemurafenib. Although the terminal half-life of encorafenib is short, its active metabolite, LHY746, has an extended half-life of up to 83 h (56). To the best of our knowledge, there is no study directly comparing the efficacy of these three strategies. However, these differences may be understood by examining the RR and mPFS. The latter two combination regiments are slightly, but not significantly, more effective than the former and all combination options exhibit notable improvements over single-agent therapy. Overall, the most recently approved combination therapy, encorafenib + binimetinib, is the most effective option for melanoma in terms of antitumor efficacy (Table IV). Notably, encorafenib exhibits a longer dissociation half-life (>30 h) than dabrafenib (2 h) and vemurafenib (0.5 h) (57), meaning that once bound to mutant BRAF proteins, encorafenib inhibits tumor proliferation for an extended period, thus showing a durable inhibitory effect. Paradoxical ERK activation is a notable concern associated with BRAF inhibitor administration. Encorafenib shows a higher paradox index (the time in which anti-tumor activity is exerted without ERK activation) than that of dabrafenib and vemurafenib (50 vs. 10 and 5.5, respectively), indicating it is less likely to activate ERK while exerting its antitumor effect (58). Due to its longer dissociation half-life and greater paradox index, the combination of encorafenib and binimetinib exhibits better tolerability and antitumor efficacy than the other two strategies. When considering adverse events, the encorafenib-binimetinib combination is associated with a high risk of diarrhea and vomiting, which limits its use in patients with sensitive gastrointestinal systems (54). For patients prone to fever, dabrafenib-trametinib is not the preferred choice (39). In such cases, vemurafenib and cobimetinib combination therapy may serve as alternatives.

5. Challenges in melanoma targeted therapy

MAPK inhibitor (MAPKi) therapy, particularly the combination of BRAF and MEK inhibitors, shows promise in delaying disease progression in melanoma. However, nearly all patients who initially respond to treatment develop drug insensitivity, leading to relapse not only at the primary lesion but also at

Table IV. 5-year follow-up of phase 3 clinical trials of BRAF and MEK inhibitors combination therapy for unresectable or metastatic melanomas with $BRAF^{V600E/K}$.

	Overall survival		Progression-free survival		
Therapy	Median, months	Rate, %	Median, months	Rate, %	(Refs.)
D	20.2	21	5.8	3	(182)
D + T	25.0	28	9.4	13	
V	17.4	26	7.2	10	(47)
V + C	22.5	31	12.6	14	
E	23.5	35	9.6	19	(183)
E + B	33.6	35	14.9	23	

D, dabrafenib; V, vemurafenib; E, encorafenib; T, trametinib; C, cobimetinib; B, binimetinib; AEs, adverse event.

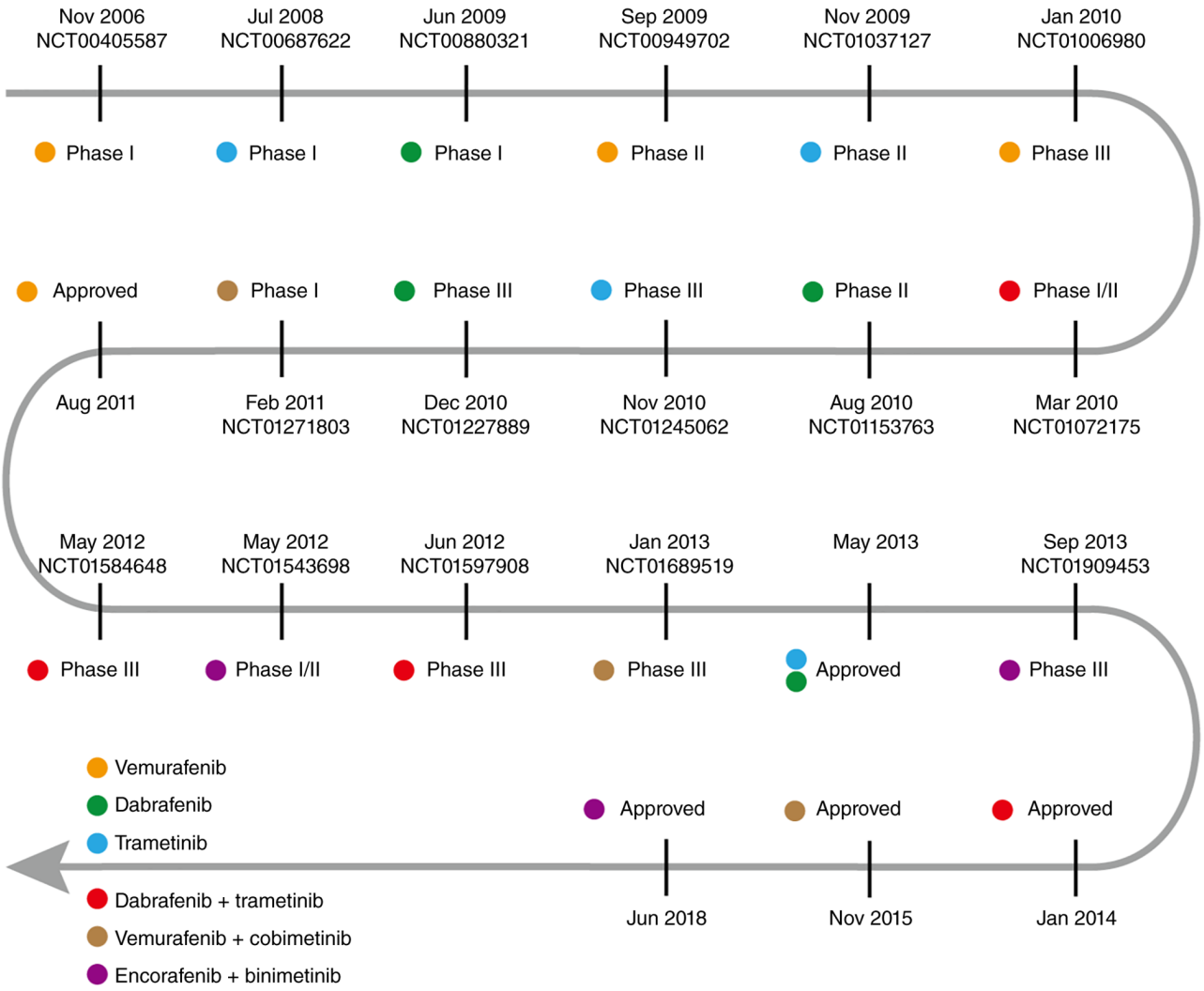

Figure 1. Timeline of clinical trials and US Food and Drug Administration approval for BRAF and MEK inhibitors in melanoma therapy. Dates indicate the study start dates. All data were sourced from ClinicalTrials.gov (clinicaltrials.gov).

previously non-tumor sites. Therefore, a challenge for melanoma-targeted therapy is the development of drug resistance. Currently, several resistance mechanisms have been identified: Tumor heterogeneity and plasticity enable melanoma cells to rapidly rewire phenotypic plasticity and adapt to therapeutic stress (59); epigenetic reprogramming renders tumor cells less sensitive to drug treatment through histone modification, chromatin remodeling and metabolic rewiring (60) and genetic mutations provide melanoma cells with additional survival signaling pathways (9). Phenotypic switching and epigenetic

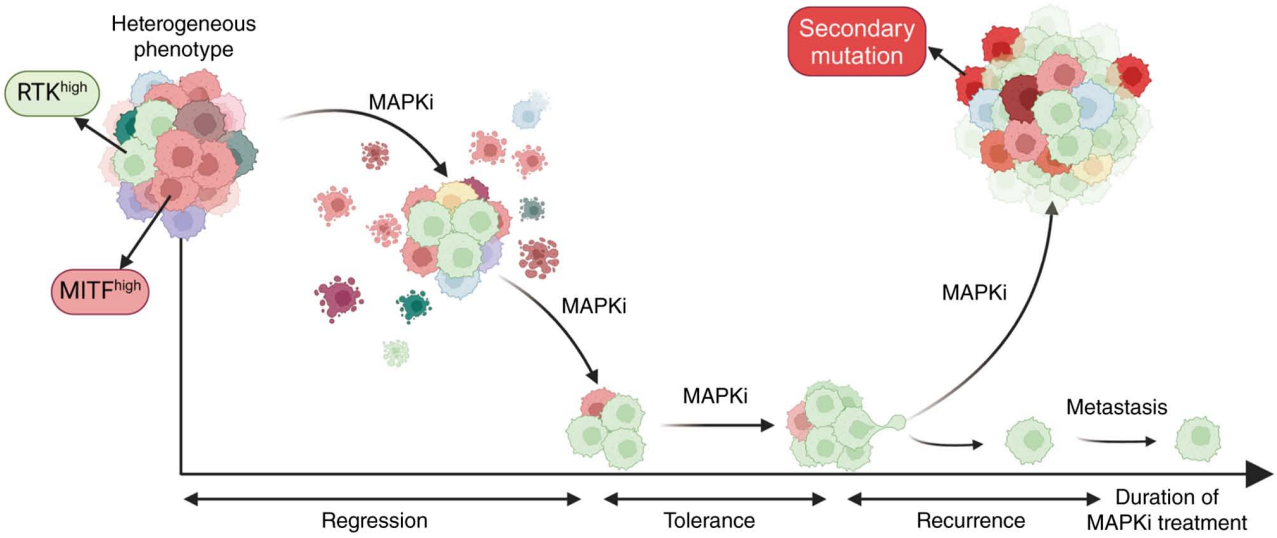

Figure 2. Acquisition of drug resistance in melanoma. Melanoma is highly heterogenous and exhibits phenotypic plasticity, enabling phenotypic switching in response to MAPKi treatment. In the early-treatment phase, MAPKi-mediated induction of cell death preferentially targets MITF-high melanoma populations, leading to tumor shrinkage within a short timeframe. Cells expressing high levels of RTKs, such as AXL receptor tyrosine kinase and nerve growth factor receptor, are intrinsically resistant to MAPKi and survive drug-induced cell death. The duration of this drug-tolerant phase varies and dormant cells typically suppress proliferative signals to evade immune surveillance. Through epigenetic reprogramming, residual tumor cells acquire an invasive phenotype, facilitating dissemination to distant organs, or a dedifferentiated phenotype, enabling them to evade continuous drug pressure until they develop secondary mutations (64,71,169). i, inhibitor; MITF, microphthalmia-associated transcription factor; RTK, receptor tyrosine kinase.

reprogramming are adaptive responses of melanoma cells to drug pressure. A subset of cells rapidly shifts their phenotype to survive MAPKi treatment-induced apoptosis, which is accompanied by epigenetic remodeling. As these changes do not involve alterations at the DNA level, they are reversible (61). During long-term treatment, melanoma genomes become unstable owing to metabolic changes, such as increased reactive oxygen species (ROS) levels. Once genetic mutations are acquired, tumor cells enter a rapid proliferation phase; changes at this stage are irreversible (62). Resistant melanoma is essentially heterogenous. Phenotypic switching, epigenetic reprogramming and genetic mutation can occur simultaneously and coexist in distinct tumor cell subpopulations.

Heterogeneity and phenotype plasticity. Microphthalmia-associated transcription factor (MITF), the master transcriptional regulator of melanocytes, drives the phenotypic switch of melanoma cells between proliferative and invasive states (63). MITF expression exhibits a dynamic bell-shaped distribution, with both high and low levels linked to therapeutic resistance. Melanoma cells with high MITF expression exhibit enhanced proliferative capacity and differentiation, as evidenced by the upregulation of cell cycle-associated and anti-apoptotic genes. This phenotype decreases dependence on the MAPK pathway for survival, thereby conferring intrinsic tolerance to MAPKi (64). By contrast, melanoma cells with low MITF levels exhibit a more invasive and stem cell-like phenotype, enhancing their adaptability to evade therapeutic pressure (64). During the initial treatment response, melanoma cells with high basal MITF levels are MAPKi-sensitive and undergo rapid cell death, whereas those with low basal MITF levels exhibit intrinsic resistance that counteracts MAPKi-induced cytotoxicity (65). However, MITF expression is significantly increased following MAPKi treatment (66-68). This reflects the dynamic fluctuations in MITF expression, wherein residual cells may transiently upregulate MITF expression as a survival mechanism under therapeutic stress. As treatment progresses to the minimal residual disease (MRD) phase, stem cell-like cells with an invasive phenotype become the most abundant population in residual tumors. These cells are characterized by a high expression of receptor tyrosine kinases (RTKs), including AXL receptor tyrosine kinase (AXL) (69), nerve growth factor receptor (NGFR) (70), epidermal growth factor receptor (EGFR) (71), and platelet-derived growth factor receptor β (PDGFRB) (72), as well as the transcriptional factor SOX9. Simultaneously, the expression of MITF and its upstream regulator SOX10 decreases (71). At this stage, a subset of the residual surviving cells remains dormant, exhibiting minimal proliferation marker Ki67 activity. Another subset undergoes transcriptional reprogramming to acquire a mesenchymal phenotype that facilitates its dissemination to distant organs (73). The increase in RTKs during the MRD stage provides alternative survival pathways, allowing melanoma cells to bypass dependency on the MAPK signals. Consequently, cells with low MITF and high RTK expression exhibit resistance to MAPKi treatment, ultimately driving relapse (65,69,74) (Fig. 2).

The shift of melanoma cells from a melanocytic/proliferative phenotype to a dedifferentiated/invasive phenotype in response to therapeutic pressure is typically considered one of the reasons for acquiring drug resistance. The switch between these two phenotypes is not saltatory, which means there is an intermediate state to mediate the transition. An increasing number of studies have demonstrated an intermediate state that can transition to an alternative state depending on microenvironmental factors (71,75,76). For example, treating cells with TGFβ and TNFα induces a shift from a melanocytic or intermediate to mesenchymal phenotype, leading to enhanced

migratory and invasive capabilities, as well as resistance to subsequent therapy (75). Tsoi *et al* (71) proposed a comprehensive two-dimensional trajectory model to describe the temporal changes in melanocyte dedifferentiation during therapy. In brief, starting from a well-differentiated melanocytic state, melanoma cells transition to a transitory state following BRAF inhibitor treatment and adopt an NCSC-like phenotype within 11-21 days. Notably, the transitory state exhibits hybrid characteristics of both the melanocytic and NCSC-like states. Over the next 60-90 days, the cells further shift to an undifferentiated state, which displays high dissemination similar to that by mesenchymal cells and increased NF-κB signaling. The inherent heterogeneity of melanoma facilitates phenotypic plasticity, allowing dynamic transitions between four broadly defined states: A melanocytic, differentiated, pigmented and proliferative state; a transitory, intermediate and starved-like state; a NCSC-like invasive state; and an undifferentiated, mesenchymal-like state. Melanomas are highly heterogeneous. Beyond therapy-induced resistant cells, a subset of pre-existing subtypes exhibits intrinsic resistance to drug treatment, characterized by increased RTKs, including EGFR and AXL (77).

Epigenetic reprogramming. Epigenetic mechanisms associated with melanoma resistance induced by targeted therapy include histone modification, chromatin remodeling and metabolic rewiring.

The mechanisms by which histone modifications and chromatin remodeling influence therapeutic resistance typically involve the regulation of gene transcriptional (60). BRAF inhibitors induce the switch from suppressive histone H3K27 methylation (H3K27me3) to active acetylation (H3K27ac) by downregulating the methyltransferase EZH2. The H3K27 methyl-to-acetyl shift increases PGC1a expression, leading to a change in oxidative metabolism that confers resistance to BRAF inhibitors. Blocking this switch re-sensitizes melanoma cells to therapy (78). Similarly, the methyltransferase SETDB1/2 (79) and demethylase KDM5B/JARID1B (80) are associated with transcriptional suppression in MAPKi-treated melanoma cells.

Under physiological conditions, melanoma cells preferentially use glycolysis rather than oxidative phosphorylation (OXPHOS) to acquire energy for accelerated proliferation (81). However, to adapt to the therapeutic pressure of MAPKi treatment, melanoma cells switch their metabolic patterns through PGC1a by increasing OXPHOS, fatty acid oxidation (FAO) and glutamine metabolism, while decreasing glycolysis (82). This metabolic reprogramming enables melanoma cells to maintain their energy balance and suppress apoptosis under MAPKi inhibition, thereby conferring resistance to targeted therapy. U2AF homology motif kinase 1 (UHMK1), a RNA processing kinase that is key for the transport and translation of metabolism-associated proteins, is involved in metabolic reprogramming induced by targeted therapy (83). UHMK1 activation reduces cell death and induces resistance to MAPKi therapy by regulating mitochondrial metabolism (83). Moreover, melanoma cells increase FAO through carnitine palmitoyltransferase 1A under MAPKi treatment to survive therapeutic pressure (84). The surviving cells with elevated FAO levels contribute to melanoma resistance.

Acquired mutations. The approach of reversing the undifferentiated melanoma cells and epigenetic reprogramming allows MAPKi to regain efficacy owing to the reversible and transient nature of non-genetic switching. Once melanoma cells acquire additional driver gene mutations, they become independent of MAPK signaling, rendering MAPKi ineffective. There are three notable mechanisms underlying MAPKi resistance: BRAF alterations that reactivate the MAPK pathway; non-BRAF alterations that also reactivate the MAPK pathway and activation of alternative survival pathways.

FDA-approved MAPK inhibitors typically target BRAF V600E or V600K mutations but are ineffective against other BRAF mutations outside the V600 position. Therefore, patients with non-V600 BRAF mutations typically exhibit resistance to MAPKi therapy. Whole-exome sequencing reveals that patients with $BRAF^{V600E}$ mutations who progress on MAPKi treatment acquire secondary BRAF mutations at L514V (85) or L505H (86,87). Mutations at these sites, located in the kinase domain, confer continuous activation of BRAF kinase activity, mimicking the V600E mutation (88). Reactivation of BRAF kinase caused by non-V600 mutations leads to continuous activation of the MAPK pathway, independent of $BRAF^{V600}$. Therefore, MAPK inhibitors that specifically target the V600 site of BRAF are no longer effective. BRAF amplification also drives melanoma drug resistance to MAPKi, as supported by whole-exome sequencing, which reveals a $BRAF^{V600E}$ copy-number gain in 20% of patients with melanoma resistant to MAPKi treatment (89,90). However, high-dose BRAF inhibitors reverse this resistance by downregulating pEKR levels due to the saturated state of ERK reactivation (89). Alternative splicing of $BRAF^{V600E}$ is another cause of drug resistance to MAPKi (91,92). MAPKi-resistant melanoma cells acquire a truncated $BRAF^{V600E}$ protein through mRNA splicing, which lacks the Ras-binding domain. The truncated $BRAF^{V600E}$ shows increased dimerization compared with the full-length version; thus, MAPKi is not sufficient to block the activation of downstream ERK signaling (93). Up to 30% of patients with melanoma resistant to MAPKi treatment have an alternative $BRAF^{V600E}$ mRNA isoform, including isoforms lacking exons 4-8, 3-9, 1-9 and 2-11 (94). Although rare, the formation of BRAF fusions (95), such as the ArfGAP with GTPase domain, ankyrin repeat and PH domain 3/BRAF fusion gene (96) and switching between the three RAF isoforms (97), are associated with the acquisition of drug tolerance. BRAF fusions typically involve the rearrangement of the 3' portion of the BRAF gene, which encodes the kinase domain, behind the 5' portion of another gene. This rearrangement results in constitutive activation of BRAF due to the loss of the 5' portion of the BRAF gene, which encodes the autoinhibitory domain (98). The fusion of BRAF with other genes is observed not only in MAPKi-resistant melanomas harboring BRAF mutations but also in EGFR tyrosine kinase inhibitor-resistant lung cancer with EGFR mutations, as evidenced by fusions involving acylglycerol kinase (AGK/BRAF) or praja ring finger ubiquitin ligase 2/BRAF (99). Such fusions are also found in FGFR inhibitor-resistant gastric cancer, as demonstrated by fusions with jumonji C domain-containing histone demethylase 1 homolog D/BRAF (100). Moreover, an AGK/BRAF fusion mutation has been reported in immunotherapy-resistant advanced acral melanoma (101). These finding highlight

the key role of BRAF rearrangements in drug resistance. In the mechanisms associated with RAF switching, melanoma cells rewire signaling pathways by using ARAF and CRAF under chronic BRAF inhibition to reactivate MAPK signaling, although the underlying mechanisms remain poorly understood (97).

In addition to BRAF alterations, mutations in other genes involved in MAPK signaling activate the MAPK pathway, enabling melanoma cells to evade suppression by BRAFi, such as NRAS mutations at Q61K (72), Q61R (102), G13R and P185S (91), MEK2 mutations at F57C (91), Q60P (90,103) and C125S (104) and MEK1 mutations at Q56P and P124L (105). The aforementioned mutations in NRAS result in its constitutive activation, which reactivates the MAPK pathway by inducing the dimerization of BRAF and CRAF. The kinase function of this RAF dimer is not affected by BRAF inhibitors that target only monomeric BRAF (31). Normally, MEK is activated by its upstream regulator, BRAF. However, MEK mutations induce conformational changes that render MEK activation independent of BRAF. Consequently, the MAPK pathway evades the inhibitory effects of MAPK inhibitors and remains active (106).

Alternative pathways involved in proliferation and survival protect melanoma cells from MAPKi-induced cell death, independent of the MAPK pathway. These pathways enable melanoma cells to escape therapeutic pressure and promote the survival and proliferation of residual cells, leading to relapse. Numerous studies have illustrated the involvement of the PI3K/AKT/mTOR (92,107-109) and Rho-kinase (ROCK)-mediated pathway (110,111) and p21-activated kinase signaling (112-115) in melanoma resistance to targeted therapy.

6. Therapeutic strategies to overcome acquired MAPKi resistance

After acquiring resistance to MAPKi, tumors no longer respond to MAPKi treatment and may progress aggressively within a short time. Several strategies have been developed to address.

Strategies based on phenotype switching. Guiding intermediate cells towards the melanocytic state rather than the undifferentiated state may be a strategy for overcoming therapy resistance. The corepressor for element 1-silencing transcription factor (CoREST) has been identified as a promising therapeutic target to achieve. Mechanistically, CoREST drives melanoma cell plasticity by decreasing dual-specificity phosphatase levels (116). Treatment of BRAF inhibitor-resistant cell lines with the CoREST inhibitor corin not only enriches the intermediate cell population but also re-establishes BRAF inhibitor responsiveness (116). Proactive intervention during the MRD phase before relapse is optimal for prolonging the treatment response until the complete tumor disappears. However, selective targeting and elimination of dedifferentiated resistant cells at the recurrence stage are viable secondary strategies. For example, dedifferentiated melanoma cells are sensitive to ferroptosis. Consequently, erastin, a small-molecule ferroptosis inducer, has been proposed as a combination therapy with MAPKi to counteract dedifferentiation-mediated resistance (71,117).

Strategies based on genetic mutation. Once melanoma cells acquire additional mutations, it is impossible to re-sensitize them to MAPKi treatment. The available strategies either inhibit the newly activated pathways or induce cell death in the resistant cells. For example, combining MAPKi with inhibitors targeting activated pathways, such as LY294002 (a PI3K inhibitor) (118) and blebbistatin (a ROCK inhibitor) (110), is sufficient to delay tumor relapse. Next-generation sequencing is commonly used to identify new mutations that arise during MAPKi therapy. Another agent targeting newly acquired mutations can be administered following tumor progression. Although combining infigratinib (an FGFR inhibitor), capmatinib (a MET inhibitor), bupailisib (a PI3K inhibitor) or ribociclib (a CDK4/6 inhibitor) with encorafenib and binimetinib is insufficient for patients with progressive disease following encrofenib and binimetinib treatment (119), the use of niraparib, a poly (ADP-ribose) polymerase inhibitor targeting genes involved in the homologous recombination pathway, and uprosertib, an Akt inhibitor to block Akt1/2/3, are promising for patients with melanoma who progress on MAPKi therapy (120,121). Furthermore, numerous mutant genes that contribute to MAPKi resistance in melanoma encode client proteins of heat shock protein 90 (HSP90). The HSP90 inhibitor XL888 can overcome acquired MAPKi resistance in melanoma (122). Although a phase 1 clinical trial of XL888 combined with vemurafenib and cobimetinib in patients with melanoma harboring $BRAF^{V600E/K}$ mutations show non-negligible toxicity (123), the improved clinical outcomes facilitate further optimization of XL888 dosing strategies.

Strategies based on activation of immune system. Immune checkpoint inhibitors have also been used following MAPKi therapy in patients with melanoma who develop resistance to MAPKi. Ascierto *et al* (124) found that treating patients with metastatic $BRAF^{V600}$-mutant melanoma with encorafenib + binimetinib until disease progression, followed by ipilimumab-nivolumab combination therapy, results in slightly improved outcomes, as evidenced by a 29% 4-year total PFS. Similar outcomes are also observed in patients treated with dabrafenib + trametinib until disease progression, followed by nivolumab + ipilimumab therapy, with a 51.5% 2-year overall survival and a 29.6% objective RR (125). Notable, while patients who receive immunotherapy after progression on MAPKi therapy show extended survival, their outcomes are not as favorable as those of patients who receive first-line immunotherapy until disease progression followed by MAPKi therapy (125).

Preclinical studies have demonstrated that macrophages may be involved in the paradoxical activation of MAPK pathways, leading to resistance (126,127). Consequently, the inhibitors LY3022855 [blocking the RTK colony stimulating factor 1 receptor (CSF-1R expressed on macrophages) or MCS110 (targeting CSF-1, the ligand of CSF-1R) have been evaluated in combination with vemurafenib and cobimetinib in patients with metastatic melanoma harboring mutant $BRAF^{V600}$ (128,129). Although the phase I clinical trial was discontinued owing to strategic drug development considerations, the 20% RR in this small melanoma cohort supports the potential feasibility of these two drugs, although further optimization may be necessary.

Additionally, Samarkina *et al* (130) found that the androgen receptor (AR) is increased in BRAF inhibitor-resistant melanoma; thus, inhibiting AR activity with AZD3514 overcomes BRAF inhibitor resistance by promoting $CD8^+$ T cell infiltration. Although AZD3514 has only been evaluated in patients with castration-resistant prostate cancer (131,132), its low toxicity in humans and potent anti-tumor efficacy against MAPKi-resistant melanoma suggest potential for melanoma therapy.

Strategies based on metabolic reprogramming. One hallmark of MAPKi-resistant melanoma is increased metabolic reprogramming (81,133). Therefore, inhibitors targeting metabolic shifting are promising for re-sensitizing melanoma resistant to MAPKi. For example, IACS-010759, a mitochondrial oxidative phosphorylation complex I inhibitor, suppresses the growth of MAPKi-resistant BRAF-mutant melanoma by disrupting the tricarboxylic acid cycle and glycolysis (134). FK866 and GMX1778, inhibitors that decrease nicotinamide adenine dinucleotide levels, extend survival in mice bearing MAPKi-resistant melanoma (135). Inhibitors targeting glutamine (136,137) and FAO (84) suppress the proliferation and metastasis of MAPKi-resistant melanoma. Additionally, MAPKi-resistant melanoma cells show elevated ROS levels. Vorinostat, a histone deacetylase inhibitor, exceeds the tolerable ROS threshold in these cells, triggering lethal ROS accumulation and selective toxicity, which kills the resistant cells. Vorinostat has no cytotoxic effects on normal cells because the low basal ROS levels in these cells prevent the drug from inducing a lethal dose of ROS (138). Although most of these metabolism-associated inhibitors remain in preclinical studies, the aforementioned results support their further clinical investigation for melanoma therapy.

7. Conclusion

With the development of MAPK inhibitors, the American Cancer Society predicts that the mortality of patients with melanoma will decrease (139). The immune system helps patients combat progressive disease. In addition to BRAF and MEK inhibitors, the FDA has approved the use of immune checkpoint inhibitors in sequence with MAPKi to enhance antitumor efficacy (6). Generally, immunotherapy shows superior long-term outcomes compared with targeted therapy in patients with treatment-naïve $BRAF^{V600}$-mutant metastatic melanoma, as supported by a higher 2-year overall survival rate (71.8 vs. 51.5%) and longer PFS (11.8 vs. 8.5 months) (125). In addition, induction with MAPKi (encorafenib + binimetinib) for 12 weeks, followed by immune checkpoint inhibitors (ipilimumab + nivolumab), exhibits antitumor efficacy comparable to that of immunotherapy alone (140). The sequence of targeted therapy and immunotherapy affects patient outcomes; however, the effects of the sequence on anti-tumor outcomes vary between patients with different conditions (141,142). The efficiency of three strategies based on the sequencing of targeted therapy and immunotherapy have been evaluated in patients with untreated, metastatic $BRAF^{V600}$-mutant melanoma (143). The strategies included A (targeted therapy with encorafenib + binimetinib first until disease progression, followed by immunotherapy with ipilimumab + nivolumab), B (immunotherapy until disease progression, followed by targeted therapy) and C (targeted therapy for 8 weeks, followed by immunotherapy until disease progression, followed by targeted therapy). Immunotherapy followed by targeted therapy is the preferred treatment approach for patients with melanoma with stable disease. This is supported by better 5-year outcomes: Total PFS rates for strategies A, B, and C are 27, 50 and 50%, respectively, and the overall survival rates are 45, 52 and 57%, respectively (125,144). Biomarker analyses performed on baseline tumor tissues using next-generation sequencing have demonstrated that patients with higher serum IFN-γ levels, tumor mutation burden and LDH levels typically show poor overall survival and PFS (124,144).

Different sequences of targeted therapy and immunotherapy in patients with stable disease lead to differences in antitumor efficacy, which are mainly dependent on changes in the tumor immune microenvironment. Targeted therapy following immunotherapy triggers an immunostimulatory environment, as indicated by the increased infiltration of $CD8^+$ T effector memory cells and $CD8^+$ stem-like progenitor T cells, which are necessary for generating tumor-killing effector cells to maintain a long-term immune response (145). However, the reverse sequence (immunotherapy following targeted therapy) shows limited changes in the tumor immune microenvironment (145). Although immunotherapy shows better long-term outcomes, targeted therapy exhibits a superior objective RR compared with immunotherapy (72 vs. 51%) (146), demonstrating that targeted therapy as a first-line therapy is suitable for rapidly decreasing tumor burden, especially in patients with aggressive diseases, such as those with brain metastases or organ dysfunction (147). In general, the sequence of targeted therapy and immunotherapy depends on treatment goal. If there is an urgent need to eliminate large tumor lesions, it is better to administer targeted therapy first, followed by immunotherapy. However, immunotherapy is preferably used as a first-line treatment to achieve more durable efficacy. Notably, targeted therapy and immunotherapies partially share resistance mechanisms. This cross-resistance is attributed to a lack of functional dendritic cells, which results in an immunosuppressive tumor microenvironment characterized by a non-effective T cell response (148).

In addition to the sequential application of targeted therapy and immunotherapy, triplet combination therapy with BRAF, MEK and PD-1 inhibitors has been clinically evaluated for its anti-tumor efficacy in patients with $BRAF^{V600}$-mutant melanoma. Patients receiving triplet therapy consistently exhibit better outcomes than those receiving targeted therapy alone, not only when they are treatment-naïve (149,150), but also following progression to immunotherapy or targeted therapy (151). However, triplet combination therapy is not used as a first-line clinical strategy for patients with melanoma owing to severe adverse events, including diarrhea, fever, rash and elevated liver transaminase, which are caused by increased toxicity (152). Therefore, the triplet regimens combining targeted therapy with immunotherapy are under clinical investigation.

Although the therapeutic methods differ, the underlying mechanisms by which tumor cells acquire resistance to targeted therapy and immunotherapy are similar. Phenotypic switching from a melanocytic to an undifferentiated state

has been observed in immunotherapy-resistant melanoma cells (153,154). This highlights the plasticity of melanoma cells in response to drug treatment. However, epigenetic reprogramming in immune cells contribute to impaired anti-tumor function, affecting macrophage polarization, dendritic cell maturation and T cell infiltration, ultimately leading to immunotherapy resistance (155). Although challenges remain, such as the difficulty in completely eliminating tumor cells and preventing recurrence, advances in targeted therapy and immunotherapy offer hope for patients with melanoma. Therapeutic strategies for patients with melanoma are no longer limited to single-agent approaches, ≥2 treatment methods are typically combined for clinical treatment. For example, neoadjuvant concurrent therapy with immunotherapy drugs and MAPKi achieves 80.0-86.7% pathological response (156,157) and 71% 2-year event-free survival (158). Similarly, a combination of encorafenib, binimetinib and pembrolizumab has 65% overall response in patients with unresectable advanced or metastatic $BRAF^{V600E/K}$-mutant melanoma (159). Moreover, phenformin, an inhibitor that blocks the mitochondrial electron transport chain to suppress oxidative phosphorylation, has been introduced in combination with dabrafenib and trametinib to enhance the anti-tumor efficacy of MAPKi (160,161). MAPKis exhibit limited efficacy in patients with melanoma with brain metastases owing to poor blood-brain barrier (BBB) penetration. Ren *et al* (162) developed PF-07284890 (ARRY-461), a novel $BRAF^{V600E}$ inhibitor with high BBB permeability and notable tumor suppression, in a $BRAF^{V600E}$ melanoma xenograft mouse model. Currently, ARRY-461 is being evaluated in a phase 1 clinical trial (trial no. NCT04543188).

Identification of a universal biomarker would be beneficial to predict responses to combination therapy with BRAF and MEK inhibitors in most patients with melanoma. These biomarkers do not exist. However, several promising biomarkers have emerged. $BRAF^{V600}$ mutation levels, or the variant allele frequency (VAF) of $BRAF^{V600}$, which indicates the proportion of mutant alleles to total alleles, is a potential marker for reflecting clinical outcomes and response to melanoma-targeted therapy (163). Specifically, patients with metastatic melanoma with $BRAF^{V600}$-VAF >45% show shorter median PFS (10 months) and overall survival (26 months) than those with $BRAF^{V600}$-VAF <45% (13 and 29 months, respectively) (164). Tumors with high $BRAF^{V600}$-VAF are typically associated with strong heterogeneity, invasion and a high dependence on the MAPK signaling pathway for proliferation and survival, leading to poor patient outcomes and high risk of recurrence. Circulating tumor DNA (ctDNA) from liquid biopsy is another biomarker for predicting the treatment response and prognosis of patients with melanoma, with the advantages of being dynamic, non-invasive and real-time (165). ctDNA directly reflects the therapeutic effects during treatment. Elevated ctDNA levels indicate progressive disease with high accuracy (166). In patients with resected $BRAF^{V600}$-mutant melanoma treated with dabrafenib and trametinib combination therapy, the presence of ctDNA in plasma samples is associated with worse median recurrence-free (16.59 vs. 68.11 months) and overall survival (40.31 months vs. not reached) compared with patients without detectable ctDNA (167). Even when not used to predict whether patients will respond to therapy, LDH is a strong prognostic biomarker. High baseline LDH levels are typically associated with poor outcomes (47,168). Therefore, appropriate therapeutic strategies may be selected based on the levels of $BRAF^{V600}$-VAF, plasma ctDNA and baseline LDH before treatment.

Acknowledgements

Not applicable.

Funding

The present study was supported by Luzhou Science and Technology Program (grant no. 2025RCX002), the Natural Science Foundation of China (grant no. 32271363) and the Natural Science Foundation of Chongqing (grant no. cstc2021jcyj-cxttX0002).

Availability of data and materials

Not applicable.

Authors' contributions

LZ and LT conceived the study. LZ wrote the manuscript. DS, JF and LT revised the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Centeno PP, Pavet V, and Marais R: The journey from melanocytes to melanoma. Nat Rev Cancer 23: 372-390, 2023.
2. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, *et al*: Mutations of the BRAF gene in human cancer. Nature 417: 949-954, 2002.
3. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, Cho KH, Aiba S, Bröcker EB, LeBoit PE, *et al*: Distinct sets of genetic alterations in melanoma. N Engl J Med 353: 2135-2147, 2005.
4. Zoghlami A, Roussel F, Sabourin JC, Kuhn JM, Marie JP, Dehesdin D and Choussy O: BRAF mutation in papillary thyroid carcinoma: predictive value for long-term prognosis and radioiodine sensitivity. Eur Ann Otorhinolaryngol Head Neck Dis 131: 7-13, 2014.
5. Yao Z, Torres NM, Tao A, Gao Y, Luo L, Li Q, de Stanchina E, Abdel-Wahab O, Solit DB, Poulikakos PI and Rosen N: BRAF Mutants Evade ERK-dependent feedback by different mechanisms that determine their sensitivity to pharmacologic inhibition. Cancer Cell 28: 370-383, 2015.
6. Hanrahan AJ, Chen Z, Rosen N and Solit DB: BRAF-a tumour-agnostic drug target with lineage-specific dependencies. Nat Rev Clin Oncol 21: 224-247, 2024.

7. Holderfield M, Deuker MM, McCormick F and McMahon M: Targeting RAF kinases for cancer therapy: BRAF-mutated melanoma and beyond. Nat Rev Cancer 14: 455-467, 2014.
8. Cheng L, Lopez-Beltran A, Massari F, MacLennan GT and Montironi R: Molecular testing for BRAF mutations to inform melanoma treatment decisions: A move toward precision medicine. Mod Pathol 31: 24-38, 2018.
9. Robertson BM, Fane ME, Weeraratna AT and Rebecca VW: Determinants of resistance and response to melanoma therapy. Nat Cancer 5: 964-982, 2024.
10. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K and Chapman PB: Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 363: 809-819, 2010.
11. Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S, Bremer R, Gillette S, Kong J, Haass NK, *et al*: Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc Natl Acad Sci USA 105: 3041-3046, 2008.
12. Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, Spevak W, Zhang C, Zhang Y, Habets G, *et al*: Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. Nature 467: 596-599, 2010.
13. Sondergaard JN, Nazarian R, Wang Q, Guo D, Hsueh T, Mok S, Sazegar H, MacConaill LE, Barretina JG, Kehoe SM, *et al*: Differential sensitivity of melanoma cell lines with BRAFV600E mutation to the specific Raf inhibitor PLX4032. J Transl Med 8: 39, 2010.
14. Tap WD, Gong KW, Dering J, Tseng Y, Ginther C, Pauletti G, Glaspy JA, Essner R, Bollag G, Hirth P, *et al*: Pharmacodynamic characterization of the efficacy signals due to selective BRAF inhibition with PLX4032 in malignant melanoma. Neoplasia 12: 637-649, 2010.
15. Lee JT, Li L, Brafford PA, van den Eijnden M, Halloran MB, Sproesser K, Haass NK, Smalley KS, Tsai J, Bollag G and Herlyn M: PLX4032, a potent inhibitor of the B-Raf V600E oncogene, selectively inhibits V600E-positive melanomas. Pigment Cell Melanoma Res 23: 820-827, 2010.
16. Halaban R, Zhang W, Bacchiocchi A, Cheng E, Parisi F, Ariyan S, Krauthammer M, McCusker JP, Kluger Y and Sznol M: PLX4032, a selective BRAF(V600E) kinase inhibitor, activates the ERK pathway and enhances cell migration and proliferation of BRAF melanoma cells. Pigment Cell Melanoma Res 23: 190-200, 2010.
17. Joseph EW, Pratilas CA, Poulikakos PI, Tadi M, Wang W, Taylor BS, Halilovic E, Persaud Y, Xing F, Viale A, *et al*: The RAF inhibitor PLX4032 inhibits ERK signaling and tumor cell proliferation in a V600E BRAF-selective manner. Proc Natl Acad Sci USA 107: 14903-14908, 2010.
18. Flaherty K, Puzanov I, Sosman J, Kim K, Ribas A, McArthur G, Lee RJ, Grippo JF, Nolop K and Chapman P: Phase I study of PLX4032: Proof of concept for V600E BRAF mutation as a therapeutic target in human cancer. J Clin Oncol 27 (15_suppl): S9000, 2009.
19. Kim G, McKee AE, Ning YM, Hazarika M, Theoret M, Johnson JR, Xu QC, Tang S, Sridhara R, Jiang X, *et al*: FDA approval summary: Vemurafenib for treatment of unresectable or metastatic melanoma with the BRAFV600E mutation. Clin Cancer Res 20: 4994-5000, 2014.
20. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, *et al*: Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 364: 2507-2516, 2011.
21. Chapman PB, Robert C, Larkin J, Haanen JB, Ribas A, Hogg D, Hamid O, Ascierto PA, Testori A, Lorigan PC, *et al*: Vemurafenib in patients with BRAFV600 mutation-positive metastatic melanoma: Final overall survival results of the randomized BRIM-3 study. Ann Oncol 28: 2581-2587, 2017.
22. Stellwagen JC, Adjabeng GM, Arnone MR, Dickerson SH, Han C, Hornberger KR, King AJ, Mook RA Jr, Petrov KG, Rheault TR, *et al*: Development of potent B-RafV600E inhibitors containing an arylsulfonamide headgroup. Bioorg Med Chem Lett 21: 4436-4440, 2011.
23. King AJ, Arnone MR, Bleam MR, Moss KG, Yang J, Fedorowicz KE, Smitheman KN, Erhardt JA, Hughes-Earle A, Kane-Carson LS, *et al*: Dabrafenib; preclinical characterization, increased efficacy when combined with trametinib, while BRAF/MEK tool combination reduced skin lesions. PLoS One 8: e67583, 2013.
24. Laquerre S, Arnone M, Moss K, Yang J, Fisher K, Kane-Carson LS, Smitheman K, Ward J, Heidrich B, Rheault T, *et al*: Abstract B88: A selective Raf kinase inhibitor induces cell death and tumor regression of human cancer cell lines encoding B-RafV600E mutation. Mol Cancer Ther 8 (12_Suppl): B88, 2009.
25. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, Rutkowski P, Blank CU, Miller WH Jr, Kaempgen E, *et al*: Dabrafenib in BRAF-mutated metastatic melanoma: A multicentre, open-label, phase 3 randomised controlled trial. Lancet 380: 358-365, 2012.
26. Schilling B, Sondermann W, Zhao F, Griewank KG, Livingstone E, Sucker A, Zelba H, Weide B, Trefzer U, Wilhelm T, *et al*: Differential influence of vemurafenib and dabrafenib on patients' lymphocytes despite similar clinical efficacy in melanoma. Ann Oncol 25: 747-753, 2014.
27. Ascierto PA, Minor D, Ribas A, Lebbe C, O'Hagan A, Arya N, Guckert M, Schadendorf D, Kefford RF, Grob JJ, *et al*: Phase II trial (BREAK-2) of the BRAF inhibitor dabrafenib (GSK2118436) in patients with metastatic melanoma. J Clin Oncol 31: 3205-3211, 2013.
28. Park E, Rawson S, Li K, Kim BW, Ficarro SB, Pino GG, Sharif H, Marto JA, Jeon H and Eck MJ: Architecture of autoinhibited and active BRAF-MEK1-14-3-3 complexes. Nature 575: 545-550, 2019.
29. Hatzivassiliou G, Song K, Yen I, Brandhuber BJ, Anderson DJ, Alvarado R, Ludlam MJ, Stokoe D, Gloor SL, Vigers G, *et al*: RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. Nature 464: 431-435, 2010.
30. Poulikakos PI, Zhang C, Bollag G, Shokat KM and Rosen N: RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. Nature 464: 427-430, 2010.
31. Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvas I, Dhomen N, Hussain J, Reis-Filho JS, Springer CJ, Pritchard C, Marais R: Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell 140: 209-221, 2010.
32. Gilmartin AG, Bleam MR, Groy A, Moss KG, Minthorn EA, Kulkarni SG, Rominger CM, Erskine S, Fisher KE, Yang J, *et al*: GSK1120212 (JTP-74057) is an inhibitor of MEK activity and activation with favorable pharmacokinetic properties for sustained in vivo pathway inhibition. Clin Cancer Res 17: 989-1000, 2011.
33. Yoshida T, Kakegawa J, Yamaguchi T, Hantani Y, Okajima N, Sakai T, Watanabe Y and Nakamura M: Identification and characterization of a novel chemotype MEK inhibitor able to alter the phosphorylation state of MEK1/2. Oncotarget 3: 1533-1545, 2012.
34. Falchook GS, Lewis KD, Infante JR, Gordon MS, Vogelzang NJ, DeMarini DJ, Sun P, Moy C, Szabo SA, Roadcap LT, *et al*: Activity of the oral MEK inhibitor trametinib in patients with advanced melanoma: A phase 1 dose-escalation trial. Lancet Oncol 13: 782-789, 2012.
35. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, Demidov LV, Hassel JC, Rutkowski P, Mohr P, *et al*: Improved survival with MEK inhibition in BRAF-mutated melanoma. N Engl J Med 367: 107-114, 2012.
36. Kim KB, Kefford R, Pavlick AC, Infante JR, Ribas A, Sosman JA, Fecher LA, Millward M, McArthur GA, Hwu P, *et al*: Phase II study of the MEK1/MEK2 inhibitor Trametinib in patients with metastatic BRAF-mutant cutaneous melanoma previously treated with or without a BRAF inhibitor. J Clin Oncol 31: 482-489, 2013.
37. Imani S, Roozitalab G, Emadi M, Moradi A, Behzadi P and Jabbarzadeh Kaboli P: The evolution of BRAF-targeted therapies in melanoma: Overcoming hurdles and unleashing novel strategies. Front Oncol 14: 1504142, 2024.
38. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, Hamid O, Schuchter L, Cebon J, Ibrahim N, *et al*: Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med 367: 1694-1703, 2012.
39. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, Stroiakovski D, Lichinitser M, Dummer R, Grange F, Mortier L, *et al*: Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med 372: 30-39, 2015.
40. Robert C, Grob JJ, Stroyakovskiy D, Karaszewska B, Hauschild A, Levchenko E, Chiarion Sileni V, Schachter J, Garbe C, Bondarenko I, *et al*: Five-Year outcomes with dabrafenib plus trametinib in metastatic melanoma. N Engl J Med 381: 626-636, 2019.
41. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, Garbe C, Jouary T, Hauschild A, Grob JJ, *et al*: Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. N Engl J Med 371: 1877-1888, 2014.
42. Chen G, McQuade JL, Panka DJ, Hudgens CW, Amin-Mansour A, Mu XJ, Bahl S, Jané-Valbuena J, Wani KM, Reuben A, *et al*: Clinical, molecular, and immune analysis of dabrafenib-trametinib combination treatment for BRAF inhibitor-refractory metastatic melanoma: A phase 2 clinical trial. JAMA Oncol 2: 1056-1064, 2016.

43. Rosen LS, LoRusso P, Ma WW, Goldman JW, Weise A, Colevas AD, Adjei A, Yazji S, Shen A, Johnston S, *et al*: A first-in-human phase I study to evaluate the MEK1/2 inhibitor, cobimetinib, administered daily in patients with advanced solid tumors. Invest New Drugs 34: 604-613, 2016.
44. Larkin J, Ascierto PA, Dreno B, Atkinson V, Liszkay G, Maio M, Mandalà M, Demidov L, Stroyakovskiy D, Thomas L, *et al*: Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. N Engl J Med 371: 1867-1876, 2014.
45. Ascierto PA, McArthur GA, Dreno B, Atkinson V, Liszkay G, Di Giacomo AM, Mandalà M, Demidov L, Stroyakovskiy D, Thomas L, *et al*: Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. Lancet Oncol 17: 1248-1260, 2016.
46. de la Cruz-Merino L, Di Guardo L, Grob JJ, Venosa A, Larkin J, McArthur GA, Ribas A, Ascierto PA, Evans JTR, Gomez-Escobar A, *et al*: Clinical features of serous retinopathy observed with cobimetinib in patients with BRAF-mutated melanoma treated in the randomized coBRIM study. J Transl Med 15: 146, 2017.
47. Ascierto PA, Dreno B, Larkin J, Ribas A, Liszkay G, Maio M, Mandalà M, Demidov L, Stroyakovskiy D, Thomas L, *et al*: 5-year outcomes with cobimetinib plus vemurafenib in BRAFV600 mutation-positive advanced melanoma: Extended follow-up of the coBRIM study. Clin Cancer Res 27: 5225-5235, 2021.
48. Shirley M: Encorafenib and binimetinib: First global approvals. Drugs 78: 1277-1284, 2018.
49. Stuart DD, Li N, Poon DJ, Aardalen K, Kaufman S, Merritt H, Salangsang F, Lorenzana E, Li A, Ghoddusi M, *et al*: Abstract 3790: Preclinical profile of LGX818: A potent and selective RAF kinase inhibitor. Cancer Res 72 (8_Suppl): S3790, 2012.
50. Huang T, Karsy M, Zhuge J, Zhong M and Liu D: B-Raf and the inhibitors: From bench to bedside. J Hematol Oncol 6: 30, 2013.
51. Li Z, Jiang K, Zhu X, Lin G, Song F, Zhao Y, Piao Y, Liu J, Cheng W, Bi X, *et al*: Encorafenib (LGX818), a potent BRAF inhibitor, induces senescence accompanied by autophagy in BRAFV600E melanoma cells. Cancer Lett 370: 332-344, 2016.
52. Tran B and Cohen MS: The discovery and development of binimetinib for the treatment of melanoma. Expert Opin Drug Discov 15: 745-754, 2020.
53. Lee PA, Wallace E, Marlow A, Yeh T, Marsh V, Anderson D, Woessner R, Hurley B, Lyssikatos J, Poch G, *et al*: Abstract 2515: Preclinical Development of ARRY-162, A Potent and Selective MEK 1/2 Inhibitor. Cancer Res 70 (8_suppl): 2515, 2010.
54. Dummer R, Ascierto PA, Gogas HJ, Arance A, Mandala M, Liszkay G, Garbe C, Schadendorf D, Krajsova I, Gutzmer R, *et al*: Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): A multicentre, open-label, randomised phase 3 trial. Lancet Oncol 19: 603-615, 2018.
55. Gogas HJ, Flaherty KT, Dummer R, Ascierto PA, Arance A, Mandala M, Liszkay G, Garbe C, Schadendorf D, Krajsova I, *et al*: Adverse events associated with encorafenib plus binimetinib in the COLUMBUS study: incidence, course and management. Eur J Cancer 119: 97-106, 2019.
56. Piscitelli J, Reddy MB, Wollenberg L, Del Frari L, Gong J, Matschke K and Williams JH: Evaluation of the effect of modafinil on the pharmacokinetics of encorafenib and binimetinib in patients with BRAF V600-mutant advanced solid tumors. Cancer Chemother Pharmacol 94: 337-347, 2024.
57. Delord JP, Robert C, Nyakas M, McArthur GA, Kudchakar R, Mahipal A, Yamada Y, Sullivan R, Arance A, Kefford RF, *et al*: Phase I dose-escalation and -expansion study of the BRAF inhibitor encorafenib (LGX818) in metastatic BRAF-Mutant melanoma. Clin Cancer Res 23: 5339-5348, 2017.
58. Adelmann CH, Ching G, Du L, Saporito RC, Bansal V, Pence LJ, Liang R, Lee W and Tsai KY: Comparative profiles of BRAF inhibitors: The paradox index as a predictor of clinical toxicity. Oncotarget 7: 30453-30460, 2016.
59. Rambow F, Marine JC and Goding CR: Melanoma plasticity and phenotypic diversity: Therapeutic barriers and opportunities. Genes Dev 33: 1295-1318, 2019.
60. Rubanov A, Berico P and Hernando E: Epigenetic mechanisms underlying melanoma resistance to immune and targeted therapies. Cancers (Basel) 14: 5858, 2022.
61. Alkaraki A, McArthur GA, Sheppard KE and Smith LK: Metabolic plasticity in melanoma progression and response to oncogene targeted therapies. Cancers (Basel) 13: 5810, 2021.
62. Kakadia S, Yarlagadda N, Awad R, Kundranda M, Niu J, Naraev B, Mina L, Dragovich T, Gimbel M and Mahmoud F: Mechanisms of resistance to BRAF and MEK inhibitors and clinical update of US Food and Drug Administration-approved targeted therapy in advanced melanoma. Onco Targets Ther 11: 7095-7107, 2018.
63. Kemper K, de Goeje PL, Peeper DS and van Amerongen R: Phenotype switching: Tumor cell plasticity as a resistance mechanism and target for therapy. Cancer Res 74: 5937-5941, 2014.
64. Bai X, Fisher DE and Flaherty KT: Cell-state dynamics and therapeutic resistance in melanoma from the perspective of MITF and IFNγ pathways. Nat Rev Clin Oncol 16: 549-562, 2019.
65. Konieczkowski DJ, Johannessen CM, Abudayyeh O, Kim JW, Cooper ZA, Piris A, Frederick DT, Barzily-Rokni M, Straussman R, Haq R, *et al*: A melanoma cell state distinction influences sensitivity to MAPK pathway inhibitors. Cancer Discov 4: 816-827, 2014.
66. Smith MP, Ferguson J, Arozarena I, Hayward R, Marais R, Chapman A, Hurlstone A and Wellbrock C: Effect of SMURF2 targeting on susceptibility to MEK inhibitors in melanoma. J Natl Cancer Inst 105: 33-46, 2013.
67. Smith MP, Brunton H, Rowling EJ, Ferguson J, Arozarena I, Miskolczi Z, Lee JL, Girotti MR, Marais R, Levesque MP, *et al*: Inhibiting drivers of non-mutational drug tolerance is a salvage strategy for targeted melanoma therapy. Cancer Cell 29: 270-284, 2016.
68. Rambow F, Rogiers A, Marin-Bejar O, Aibar S, Femel J, Dewaele M, Karras P, Brown D, Chang YH, Debiec-Rychter M, *et al*: Toward minimal residual disease-directed therapy in melanoma. Cell 174: 843-855.e19, 2018.
69. Muller J, Krijgsman O, Tsoi J, Robert L, Hugo W, Song C, Kong X, Possik PA, Cornelissen-Steijger PD, Geukes Foppen MH, *et al*: Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma. Nat Commun 5: 5712, 2014.
70. Marin-Bejar O, Rogiers A, Dewaele M, Femel J, Karras P, Pozniak J, Bervoets G, Van Raemdonck N, Pedri D, Swings T, *et al*: Evolutionary predictability of genetic versus nongenetic resistance to anticancer drugs in melanoma. Cancer Cell 39: 1135-1149.e8, 2021.
71. Tsoi J, Robert L, Paraiso K, Galvan C, Sheu KM, Lay J, Wong DJL, Atefi M, Shirazi R, Wang X, *et al*: Multi-stage differentiation defines melanoma subtypes with differential vulnerability to drug-induced iron-dependent oxidative stress. Cancer Cell 33: 890-904.e5, 2018.
72. Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, Chen Z, Lee MK, Attar N, Sazegar H, *et al*: Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature 468: 973-977, 2010.
73. Karras P, Bordeu I, Pozniak J, Nowosad A, Pazzi C, Van Raemdonck N, Landeloos E, Van Herck Y, Pedri D, Bervoets G, *et al*: A cellular hierarchy in melanoma uncouples growth and metastasis. Nature 610: 190-198, 2022.
74. Boshuizen J, Koopman LA, Krijgsman O, Shahrabi A, van den Heuvel EG, Ligtenberg MA, Vredevoogd DW, Kemper K, Kuilman T, Song JY, *et al*: Cooperative targeting of melanoma heterogeneity with an AXL antibody-drug conjugate and BRAF/MEK inhibitors. Nat Med 24: 203-212, 2018.
75. Wouters J, Kalender-Atak Z, Minnoye L, Spanier KI, De Waegeneer M, Bravo Gonzalez-Blas C, Mauduit D, Davie K, Hulselmans G, Najem A, *et al*: Robust gene expression programs underlie recurrent cell states and phenotype switching in melanoma. Nat Cell Biol 22: 986-998, 2020.
76. Aiello-Couzo NM and Kang Y: A bridge between melanoma cell states. Nat Cell Biol 22: 913-914, 2020.
77. Kim S, Carvajal R, Kim M and Yang HW: Kinetics of RTK activation determine ERK reactivation and resistance to dual BRAF/MEK inhibition in melanoma. Cell Rep 42: 112570, 2023.
78. Zhou J, Chai X, Zhu Y, Huang Z, Lin T, Hu Z, Chen G, Luo C, Cui R and Sheng J: A methyl-to-acetyl switch in H3K27 drives metabolic reprogramming and resistance to BRAF(V600E) inhibition in melanoma. Neoplasia 68: 101223, 2025.
79. Torrano J, Al Emran A, Hammerlindl H and Schaider H: Emerging roles of H3K9me3, SETDB1 and SETDB2 in therapy-induced cellular reprogramming. Clin Epigenetics 11: 43, 2019.
80. Roesch A, Vultur A, Bogeski I, Wang H, Zimmermann KM, Speicher D, Körbel C, Laschke MW, Gimotty PA, Philipp SE, *et al*: Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B(high) cells. Cancer Cell 23: 811-825, 2013.

81. Shen D, Zhang L, Li S and Tang L: Metabolic reprogramming in melanoma therapy. Cell Death Discov 11: 308, 2025.
82. Zhao X, Chen F and Li H: Metabolic reprogramming-a breakthrough point in overcoming resistance to BRAF mutant melanoma targeted therapy (Review). Oncol Lett 31: 184, 2026.
83. Smith LK, Parmenter T, Kleinschmidt M, Kusnadi EP, Kang J, Martin CA, Lau P, Patel R, Lorent J, Papadopoli D, *et al*: Adaptive translational reprogramming of metabolism limits the response to targeted therapy in BRAF(V600) melanoma. Nat Commun 13: 1100, 2022.
84. Aloia A, Müllhaupt D, Chabbert CD, Eberhart T, Flückiger-Mangual S, Vukolic A, Eichhoff O, Irmisch A, Alexander LT, Scibona E, *et al*: A fatty acid oxidation-dependent metabolic shift regulates the adaptation of BRAF-mutated melanoma to MAPK inhibitors. Clin Cancer Res 25: 6852-6867, 2019.
85. Wang J, Yao Z, Jonsson P, Allen AN, Qin ACR, Uddin S, Dunkel IJ, Petriccione M, Manova K, Haque S, *et al*: A secondary mutation in BRAF confers resistance to RAF inhibition in a BRAF(V600E)-Mutant brain tumor. Cancer Discov 8: 1130-1141, 2018.
86. Hoogstraat M, Gadellaa-van Hooijdonk CG, Ubink I, Besselink NJ, Pieterse M, Veldhuis W, van Stralen M, Meijer EF, Willems SM, Hadders MA, *et al*: Detailed imaging and genetic analysis reveal a secondary 505H resistance mutation and extensive intrapatient heterogeneity in metastatic mutant melanoma patients treated with vemurafenib. Pigment Cell Melanoma Res 28: 318-323, 2015.
87. Wagenaar TR, Ma L, Roscoe B, Park SM, Bolon DN and Green MR: Resistance to vemurafenib resulting from a novel mutation in the BRAFV600E kinase domain. Pigment Cell Melanoma Res 27: 124-133, 2014.
88. Arkenau HT, Kefford R and Long GV: Targeting BRAF for patients with melanoma. Br J Cancer 104: 392-398, 2011.
89. Shi H, Moriceau G, Kong X, Lee MK, Lee H, Koya RC, Ng C, Chodon T, Scolyer RA, Dahlman KB, *et al*: Melanoma whole-exome sequencing identifies (V600E)B-RAF amplification-mediated acquired B-RAF inhibitor resistance. Nat Commun 3: 724, 2012.
90. Villanueva J, Infante JR, Krepler C, Reyes-Uribe P, Samanta M, Chen HY, Li B, Swoboda RK, Wilson M, Vultur A, *et al*: Concurrent MEK2 mutation and BRAF amplification confer resistance to BRAF and MEK inhibitors in melanoma. Cell Rep 4: 1090-1099, 2013.
91. Rizos H, Menzies AM, Pupo GM, Carlino MS, Fung C, Hyman J, Haydu LE, Mijatov B, Becker TM, Boyd SC, *et al*: BRAF inhibitor resistance mechanisms in metastatic melanoma: Spectrum and clinical impact. Clin Cancer Res 20: 1965-1977, 2014.
92. Shi H, Hugo W, Kong X, Hong A, Koya RC, Moriceau G, Chodon T, Guo R, Johnson DB, Dahlman KB, *et al*: Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. Cancer Discov 4: 80-93, 2014.
93. Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, Shi H, Atefi M, Titz B, Gabay MT, *et al*: RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). Nature 480: 387-390, 2011.
94. Aya F, Lanuza-Gracia P, González-Pérez A, Bonnal S, Mancini E, López-Bigas N, Arance A and Valcárcel J: Genomic deletions explain the generation of alternative BRAF isoforms conferring resistance to MAPK inhibitors in melanoma. Cell Rep 43: 114048, 2024.
95. Botton T, Talevich E, Mishra VK, Zhang T, Shain AH, Berquet C, Gagnon A, Judson RL, Ballotti R, Ribas A, *et al*: Genetic heterogeneity of BRAF fusion kinases in melanoma affects drug responses. Cell Rep 29: 573-588.e7, 2019.
96. Kulkarni A, Al-Hraishawi H, Simhadri S, Hirshfield KM, Chen S, Pine S, Jeyamohan C, Sokol L, Ali S, Teo ML, *et al*: BRAF fusion as a novel mechanism of acquired resistance to vemurafenib in BRAF(V600E) mutant melanoma. Clin Cancer Res 23: 5631-5638, 2017.
97. Villanueva J, Vultur A, Lee JT, Somasundaram R, Fukunaga-Kalabis M, Cipolla AK, Wubbenhorst B, Xu X, Gimotty PA, Kee D, *et al*: Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. Cancer Cell 18: 683-695, 2010.
98. Lu H, Villafane N, Dogruluk T, Grzeskowiak CL, Kong K, Tsang YH, Zagorodna O, Pantazi A, Yang L, Neill NJ, *et al*: Engineering and functional characterization of fusion genes identifies novel oncogenic drivers of cancer. Cancer Res 77: 3502-3512, 2017.
99. Vojnic M, Kubota D, Kurzatkowski C, Offin M, Suzawa K, Benayed R, Schoenfeld AJ, Plodkowski AJ, Poirier JT, Rudin CM, *et al*: Acquired BRAF rearrangements induce secondary resistance to EGFR therapy in EGFR-Mutated lung cancers. J Thorac Oncol 14: 802-815, 2019.
100. Sase H, Nakanishi Y, Aida S, Horiguchi-Takei K, Akiyama N, Fujii T, Sakata K, Mio T, Aoki M and Ishii N: Acquired JHDM1D-BRAF fusion confers resistance to FGFR inhibition in FGFR2-amplified gastric cancer. Mol Cancer Ther 17: 2217-2225, 2018.
101. Zhang Y, Zhang X, Shao W, Gao J, Xiang M, Wang Y, Liu M, Zhang W and Liang X: MEK inhibitors for the treatment of immunotherapy-resistant, AGK-BRAF fusion advanced acral melanoma: A case report and literature review. J Cancer Res Clin Oncol 151: 133, 2025.
102. Wheler J, Yelensky R, Falchook G, Kim KB, Hwu P, Tsimberidou AM, Stephens PJ, Hong D, Cronin MT and Kurzrock R: Next generation sequencing of exceptional responders with BRAF-mutant melanoma: Implications for sensitivity and resistance. BMC Cancer 15: 61, 2015.
103. Wagle N, Van Allen EM, Treacy DJ, Frederick DT, Cooper ZA, Taylor-Weiner A, Rosenberg M, Goetz EM, Sullivan RJ, Farlow DN, *et al*: MAP kinase pathway alterations in BRAF-mutant melanoma patients with acquired resistance to combined RAF/MEK inhibition. Cancer Discov 4: 61-68, 2014.
104. Long GV, Fung C, Menzies AM, Pupo GM, Carlino MS, Hyman J, Shahheydari H, Tembe V, Thompson JF, Saw RP, *et al*: Increased MAPK reactivation in early resistance to dabrafenib/trametinib combination therapy of BRAF-mutant metastatic melanoma. Nat Commun 5: 5694, 2014.
105. Emery CM, Vijayendran KG, Zipser MC, Sawyer AM, Niu L, Kim JJ, Hatton C, Chopra R, Oberholzer PA, Karpova MB, *et al*: MEK1 mutations confer resistance to MEK and B-RAF inhibition. Proc Natl Acad Sci USA 106: 20411-20416, 2009.
106. Nikolaev SI, Rimoldi D, Iseli C, Valsesia A, Robyr D, Gehrig C, Harshman K, Guipponi M, Bukach O, Zoete V, *et al*: Exome sequencing identifies recurrent somatic MAP2K1 and MAP2K2 mutations in melanoma. Nat Genet 44: 133-139, 2011.
107. Irvine M, Stewart A, Pedersen B, Boyd S, Kefford R and Rizos H: Oncogenic PI3K/AKT promotes the step-wise evolution of combination BRAF/MEK inhibitor resistance in melanoma. Oncogenesis 7: 72-83, 2018.
108. Candido S, Salemi R, Piccinin S, Falzone L and Libra M: The PIK3CA H1047R mutation confers resistance to BRAF and MEK inhibitors in A375 melanoma cells through the cross-activation of MAPK and PI3K-Akt pathways. Pharmaceutics 14: 590, 2022.
109. Jazirehi AR, Wenn PB and Damavand M: Therapeutic implications of targeting the PI3Kinase/AKT/mTOR signaling module in melanoma therapy. Am J Cancer Res 2: 178-191, 2012.
110. Orgaz JL, Crosas-Molist E, Sadok A, Perdrix-Rosell A, Maiques O, Rodriguez-Hernandez I, Monger J, Mele S, Georgouli M, Bridgeman V, *et al*: Myosin II reactivation and cytoskeletal remodeling as a hallmark and a vulnerability in melanoma therapy resistance. Cancer Cell 37: 85-103. e9, 2020.
111. Misek SA, Appleton KM, Dexheimer TS, Lisabeth EM, Lo RS, Larsen SD, Gallo KA and Neubig RR: Rho-mediated signaling promotes BRAF inhibitor resistance in de-differentiated melanoma cells. Oncogene 39: 1466-1483, 2020.
112. Babagana M, Johnson S, Slabodkin H, Bshara W, Morrison C and Kandel ES: P21-activated kinase 1 regulates resistance to BRAF inhibition in human cancer cells. Mol Carcinog 56: 1515-1525, 2017.
113. Yun CY, You ST, Kim JH, Chung JH, Han SB, Shin EY and Kim EG: p21-activated kinase 4 critically regulates melanogenesis via activation of the CREB/MITF and β-catenin/MITF pathways. J Invest Dermatol 135: 1385-1394, 2015.
114. Lu H, Liu S, Zhang G, Bin Wu, Zhu Y, Frederick DT, Hu Y, Zhong W, Randell S, Sadek N, *et al*: PAK signalling drives acquired drug resistance to MAPK inhibitors in BRAF-mutant melanomas. Nature 550: 133-136, 2017.
115. Sankaran D, Amjesh R, Paul AM, George B, Kala R, Saini S and Kumar R: Hyperactivation of p21-activated kinases in human cancer and therapeutic sensitivity. Biomedicines 11: 462, 2023.
116. Wu M, Hanly A, Gibson F, Fisher R, Rogers S, Park K, Zuger A, Kuang K, Kalin JH, Nocco S, *et al*: The CoREST repressor complex mediates phenotype switching and therapy resistance in melanoma. J Clin Invest 134: e171603, 2024.

117. Hong X, Roh W, Sullivan RJ, Wong KHK, Wittner BS, Guo H, Dubash TD, Sade-Feldman M, Wesley B, Horwitz E, *et al*: The lipogenic regulator SREBP2 induces transferrin in circulating melanoma cells and suppresses ferroptosis. Cancer Discov 11: 678-695, 2021.
118. Sanchez-Hernandez I, Baquero P, Calleros L and Chiloeches A: Dual inhibition of (V600E)BRAF and the PI3K/AKT/mTOR pathway cooperates to induce apoptosis in melanoma cells through a MEK-independent mechanism. Cancer Lett 314: 244-255, 2012.
119. Dummer R, Sandhu S, Miller WH Jr, Butler MO, Taylor MH, Heinzerling L, Blank CU, Muñoz-Couselo E, Burris HA III, Postow MA, *et al*: Longitudinal genomic analysis to fine-tune targeted therapy: Results of the phase II LOGIC 2 trial in patients with BRAFV600-Mutant metastatic melanoma. Clin Cancer Res 31: 2097-2107, 2025.
120. Kim KB, Desprez PY, de Semir D, Woo RWL, Sharma A, Jones R, Caressi C, Nosrati M, Janiczek E, Rivera Penafiel J and Kashani-Sabet M: Phase II study of niraparib in patients with advanced melanoma with homologous recombination pathway gene mutations. JCO Precis Oncol 9: e2400658, 2025.
121. Algazi AP, Moon J, Lao CD, Chmielowski B, Kendra KL, Lewis KD, Gonzalez R, Kim K, Godwin JE, Curti BD, *et al*: A phase 1 study of triple-targeted therapy with BRAF, MEK, and AKT inhibitors for patients with BRAF-mutated cancers. Cancer 130: 1784-1796, 2024.
122. Paraiso KH, Haarberg HE, Wood E, Rebecca VW, Chen YA, Xiang Y, Ribas A, Lo RS, Weber JS, Sondak VK, *et al*: The HSP90 inhibitor XL888 overcomes BRAF inhibitor resistance mediated through diverse mechanisms. Clin Cancer Res 18: 2502-2514, 2012.
123. Eroglu Z, Chen YA, Smalley I, Li J, Markowitz JK, Brohl AS, Tetteh L, Taylor H, Sondak VK, Khushalani NI and Smalley KSM: Combined BRAF, MEK, and heat-shock protein 90 inhibition in advanced BRAF V600-mutant melanoma. Cancer 130: 232-243, 2024.
124. Ascierto PA, Casula M, Bulgarelli J, Pisano M, Piccinini C, Piccin L, Cossu A, Mandalà M, Ferrucci PF, Guidoboni M, *et al*: Sequential immunotherapy and targeted therapy for metastatic BRAF V600 mutated melanoma: 4-year survival and biomarkers evaluation from the phase II SECOMBIT trial. Nat Commun 15: 146, 2024.
125. Atkins MB, Lee SJ, Chmielowski B, Tarhini AA, Cohen GI, Truong TG, Moon HH, Davar D, O'Rourke M, Stephenson JJ, *et al*: Combination dabrafenib and trametinib versus combination nivolumab and ipilimumab for patients with advanced BRAF-Mutant melanoma: The DREAMseq Trial-ECOG-ACRIN EA6134. J Clin Oncol 41: 186-197, 2023.
126. Wang T, Xiao M, Ge Y, Krepler C, Belser E, Lopez-Coral A, Xu X, Zhang G, Azuma R, Liu Q, *et al*: BRAF inhibition stimulates melanoma-associated macrophages to drive tumor growth. Clin Cancer Res 21: 1652-1664, 2015.
127. Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Goedert L, Pinheiro EM, Koya RC, Graeber TG, Comin-Anduix B and Ribas A: Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma. Sci Transl Med 7: 279ra41, 2015.
128. Buchbinder EI, Giobbie-Hurder A, Haq R and Ott PA: A phase I/II study of LY3022855 with BRAF/MEK inhibition in patients with Melanoma. Invest New Drugs 41: 551-555, 2023.
129. Buchbinder EI, Giobbie-Hurder A and Ott PA: A phase I/II study of MCS110 with BRAF/MEK inhibition in patients with melanoma after progression on BRAF/MEK inhibition. Invest New Drugs 41: 365-370, 2023.
130. Samarkina A, Youssef MK, Ostano P, Ghosh S, Ma M, Tassone B, Proust T, Chiorino G, Levesque MP, Goruppi S and Dotto GP: Androgen receptor is a determinant of melanoma targeted drug resistance. Nat Commun 14: 6498, 2023.
131. Grant C, Ewart L, Muthas D, Deavall D, Smith SA, Clack G and Newham P: The value of integrating pre-clinical data to predict nausea and vomiting risk in humans as illustrated by AZD3514, a novel androgen receptor modulator. Toxicol Appl Pharmacol 296: 10-18, 2016.
132. Omlin A, Jones RJ, van der Noll R, Satoh T, Niwakawa M, Smith SA, Graham J, Ong M, Finkelman RD, Schellens JH, *et al*: AZD3514, an oral selective androgen receptor down-regulator in patients with castration-resistant prostate cancer-results of two parallel first-in-human phase I studies. Invest New Drugs 33: 679-690, 2015.
133. Loftus AW, Zarei M, Kakish H, Hajihassani O, Hue JJ, Boutros C, Graor HJ, Nakazzi F, Bahlibi T, Winter JM and Rothermel LD: Therapeutic implications of the metabolic changes associated with BRAF inhibition in melanoma. Cancer Treat Rev 129: 102795, 2024.
134. Vashisht Gopal YN, Gammon S, Prasad R, Knighton B, Pisaneschi F, Roszik J, Feng N, Johnson S, Pramanik S, Sudderth J, *et al*: A novel mitochondrial inhibitor blocks MAPK pathway and overcomes MAPK inhibitor resistance in melanoma. Clin Cancer Res 25: 6429-6442, 2019.
135. Audrito V, Managò A, La Vecchia S, Zamporlini F, Vitale N, Baroni G, Cignetto S, Serra S, Bologna C, Stingi A, *et al*: Nicotinamide phosphoribosyltransferase (NAMPT) as a therapeutic target in BRAF-Mutated metastatic melanoma. J Natl Cancer Inst 110 2018.
136. Baenke F, Chaneton B, Smith M, Van Den Broek N, Hogan K, Tang H, Viros A, Martin M, Galbraith L, Girotti MR, *et al*: Resistance to BRAF inhibitors induces glutamine dependency in melanoma cells. Mol Oncol 10: 73-84, 2016.
137. Hernandez-Davies JE, Tran TQ, Reid MA, Rosales KR, Lowman XH, Pan M, Moriceau G, Yang Y, Wu J, Lo RS and Kong M: Vemurafenib resistance reprograms melanoma cells towards glutamine dependence. J Transl Med 13: 210, 2015.
138. Wang L, Leite de Oliveira R, Huijberts S, Bosdriesz E, Pencheva N, Brunen D, Bosma A, Song JY, Zevenhoven J, Los-de Vries GT, *et al*: An acquired vulnerability of drug-resistant melanoma with therapeutic potential. Cell 173: 1413-1425. e14, 2018.
139. Siegel RL, Kratzer TB, Giaquinto AN, Sung H and Jemal A: Cancer statistics, 2025. CA Cancer J Clin 75: 10-45, 2025.
140. Robert C, Kicinski M, Dutriaux C, Routier É, Govaerts AS, Bührer E, Neidhardt EM, Durando X, Baroudjian B, Saiag P, *et al*: Combination of encorafenib and binimetinib followed by ipilimumab and nivolumab versus ipilimumab and nivolumab in patients with advanced melanoma with BRAF(V600E) or BRAF(V600K) mutations (EBIN): An international, open-label, randomised, controlled, phase 2 study. Lancet Oncol 26: 781-794, 2025.
141. Trojaniello C, Sparano F, Cioli E and Ascierto PA: Sequencing targeted and immune therapy in BRAF-Mutant melanoma: Lessons learned. Curr Oncol Rep 25: 623-634, 2023.
142. Dummer R, Welti M and Ramelyte E: The role of triple therapy and therapy sequence in treatment of BRAF-mutant metastatic melanoma. Response to overall survival with first-line atezolizumab in combination with vemurafenib and cobimetinib in BRAFV600 mutation-positive advanced melanoma (IMspire150): Second interim analysis of a multicentre, randomised, phase 3 study. J Transl Med 21: 529, 2023.
143. Ascierto PA, Mandalà M, Ferrucci PF, Guidoboni M, Rutkowski P, Ferraresi V, Arance A, Guida M, Maiello E, Gogas H, *et al*: Sequencing of ipilimumab plus nivolumab and encorafenib plus binimetinib for untreated BRAF-Mutated metastatic melanoma (SECOMBIT): A Randomized, three-arm, open-label phase II trial. J Clin Oncol 41: 212-221, 2023.
144. Ascierto PA, Mandalà M, Ferrucci PF, Guidoboni M, Rutkowski P, Ferraresi V, Arance A, Guida M, Maiello E, Gogas H, *et al*: Sequencing of checkpoint or BRAF/MEK inhibitors on brain metastases in melanoma. NEJM Evid 3: EVIDoa2400087, 2024.
145. Patel RP, Saleh R, Lim LRJ, Rao AD, Smith L, Trigos A, Haynes NM, McArthur GA and Sheppard KE: BRAF/MEK inhibition following immune checkpoint inhibitors promotes antitumor immunity associated with expansion of progenitor-exhausted $CD8^+$ T cells. EJC Skin Cancer 3: 100737, 2025.
146. Kopecký J, Pásek M, Lakomý R, Melichar B, Mrazová I, Kubeček O, Arenbergerová M, Lemstrová R, Švancarová A, Tretera V, *et al*: The outcome in patients with BRAF-mutated metastatic melanoma treated with anti-programmed death receptor-1 monotherapy or targeted therapy in the real-world setting. Cancer Med 13: e6982, 2024.
147. Boutros A, Croce E, Ferrari M, Gili R, Massaro G, Marconcini R, Arecco L, Tanda ET and Spagnolo F: The treatment of advanced melanoma: Current approaches and new challenges. Crit Rev Oncol Hematol 196: 104276, 2024.

148. Haas L, Elewaut A, Gerard CL, Umkehrer C, Leiendecker L, Pedersen M, Krecioch I, Hoffmann D, Novatchkova M, Kuttke M, *et al*: Acquired resistance to anti-MAPK targeted therapy confers an immune-evasive tumor microenvironment and cross-resistance to immunotherapy in melanoma. Nat Cancer 2: 693-708, 2021.
149. Ferrucci PF, Di Giacomo AM, Del Vecchio M, Atkinson V, Schmidt H, Schachter J, Queirolo P, Long GV, Stephens R, Svane IM, *et al*: KEYNOTE-022 part 3: A randomized, double-blind, phase 2 study of pembrolizumab, dabrafenib, and trametinib in BRAF-mutant melanoma. J Immunother Cancer 8: e001806, 2020.
150. Ascierto PA, Ferrucci PF, Fisher R, Del Vecchio M, Atkinson V, Schmidt H, Schachter J, Queirolo P, Long GV, Di Giacomo AM, *et al*: Dabrafenib, trametinib and pembrolizumab or placebo in BRAF-mutant melanoma. Nat Med 25: 941-946, 2019.
151. Albrecht LJ, Dimitriou F, Grover P, Hassel JC, Erdmann M, Forschner A, Johnson DB, Váraljai R, Lodde G, Placke JM, *et al*: Anti-PD-(L)1 plus BRAF/MEK inhibitors (triplet therapy) after failure of immune checkpoint inhibition and targeted therapy in patients with advanced melanoma. Eur J Cancer 202: 113976, 2024.
152. Dixon-Douglas JR, Patel RP, Somasundram PM and McArthur GA: Triplet therapy in melanoma-combined BRAF/MEK inhibitors and anti-PD-(L)1 antibodies. Curr Oncol Rep 24: 1071-1079, 2022.
153. Pozniak J, Pedri D, Landeloos E, Van Herck Y, Antoranz A, Vanwynsberghe L, Nowosad A, Roda N, Makhzami S, Bervoets G, *et al*: A TCF4-dependent gene regulatory network confers resistance to immunotherapy in melanoma. Cell 187: 166-183.e25, 2024.
154. Lim SY, Shklovskaya E, Lee JH, Pedersen B, Stewart A, Ming Z, Irvine M, Shivalingam B, Saw RPM, Menzies AM, *et al*: The molecular and functional landscape of resistance to immune checkpoint blockade in melanoma. Nat Commun 14: 1516, 2023.
155. Thornton J, Chhabra G, Singh CK, Guzman-Perez G, Shirley CA and Ahmad N: Mechanisms of immunotherapy resistance in cutaneous melanoma: Recognizing a shapeshifter. Front Oncol 12: 880876, 2022.
156. Hieken TJ, Nelson GD, Flotte TJ, Grewal EP, Chen J, McWilliams RR, Kottschade LA, Yang L, Domingo-Musibay E, Dronca RS, *et al*: Neoadjuvant cobimetinib and atezolizumab with or without vemurafenib for high-risk operable stage III melanoma: The phase II NeoACTIVATE trial. Nat Commun 15: 1430, 2024.
157. Block MS, Nelson GD, Chen J, Johnson S, Yang L, Flotte TJ, Grewal EP, McWilliams RR, Kottschade LA, Domingo-Musibay E, *et al*: Neoadjuvant cobimetinib and atezolizumab with or without vemurafenib for stage III melanoma: Outcomes and the impact of the microbiome from the NeoACTIVATE trial. J Immunother Cancer 13: e011706, 2025.
158. Long GV, Carlino MS, Au-Yeung G, Spillane AJ, Shannon KF, Gyorki DE, Hsiao E, Kapoor R, Thompson JR, Batula I, *et al*: Neoadjuvant pembrolizumab, dabrafenib and trametinib in BRAF(V600)-mutant resectable melanoma: The randomized phase 2 NeoTrio trial. Nat Med 30: 2540-2548, 2024.
159. Dudnichenko O, Penkov K, McKean M, Mandalà M, Kukushkina M, Panella T, Csőszi T, Gerletti P, Thakur M, Polli A, *et al*: First-line encorafenib plus binimetinib and pembrolizumab for advanced BRAF V600-mutant melanoma: Safety lead-in results from the randomized phase III STARBOARD study. Eur J Cancer 213: 115070, 2024.
160. Chapman PB, Klang M, Postow MA, Shoushtari AN, Sullivan RJ, Wolchok JD, Merghoub T, Budhu S, Wong P, Callahan MK, *et al*: Phase Ib trial of phenformin in patients with V600-mutated melanoma receiving dabrafenib and trametinib. Cancer Res Commun 3: 2447-2454, 2023.
161. Yuan P, Ito K, Perez-Lorenzo R, Del Guzzo C, Lee JH, Shen CH, Bosenberg MW, McMahon M, Cantley LC and Zheng B: Phenformin enhances the therapeutic benefit of BRAF(V600E) inhibition in melanoma. Proc Natl Acad Sci USA 110: 18226-18231, 2013.
162. Ren L, Moreno D, Baer BR, Barbour P, Bettendorf T, Bouhana K, Brown K, Brown SA, Fell JB, Hartley DP, *et al*: Identification of the clinical candidate PF-07284890 (ARRY-461), a highly potent and brain penetrant BRAF inhibitor for the treatment of cancer. J Med Chem 67: 13019-13032, 2024.
163. Mandalà M, Palmieri G, Ludovini V, Baglivo S, Marasciulo F, Castiglione F, Gili A, Osella Abate S, Rubatto M, Senetta R, *et al*: BRAFV600 variant allele frequency predicts outcome in metastatic melanoma patients treated with BRAF and MEK inhibitors. J Eur Acad Dermatol Venereol 37: 1991-1998, 2023.
164. Guida M, Apollonio B, Romano L, Spagnolo F, Quaglino P, Depenni R, Pinto R, Squicciarini T, Fucci L, Di Tullio P, *et al*: High BRAF variant allele frequency predicts poor outcomes in metastatic melanoma patients treated with BRAF/MEK inhibitors. J Transl Med 23: 1407, 2025.
165. Mattila KE, Mäkelä S, Kytölä S, Andersson E, Vihinen P, Ramadan S, Skyttä T, Tiainen L, Vuoristo MS, Tyynelä-Korhonen K, *et al*: Circulating tumor DNA is a prognostic biomarker in metastatic melanoma patients treated with chemoimmunotherapy and BRAF inhibitor. Acta Oncol 61: 1263-1267, 2022.
166. Stadler JC, Belloum Y, Deitert B, Sementsov M, Heidrich I, Gebhardt C, Keller L and Pantel K: Current and future clinical applications of ctDNA in immuno-oncology. Cancer Res 82: 349-358, 2022.
167. Syeda MM, Long GV, Garrett J, Atkinson V, Santinami M, Schadendorf D, Hauschild A, Millward M, Mandala M, Chiarion-Sileni V, *et al*: Clinical validation of droplet digital PCR assays in detecting BRAF(V600)-mutant circulating tumour DNA as a prognostic biomarker in patients with resected stage III melanoma receiving adjuvant therapy (COMBI-AD): A biomarker analysis from a double-blind, randomised phase 3 trial. Lancet Oncol 26: 641-653, 2025.
168. Gassenmaier M, Lenders MM, Forschner A, Leiter U, Weide B, Garbe C, Eigentler TK and Wagner NB: Serum S100B and LDH at baseline and during therapy predict the outcome of metastatic melanoma patients treated with BRAF inhibitors. Target Oncol 16: 197-205, 2021.
169. Arozarena I and Wellbrock C: Phenotype plasticity as enabler of melanoma progression and therapy resistance. Nat Rev Cancer 19: 377-391, 2019.
170. Ribas A, Kim KB, Schuchter LM, Gonzalez R, Pavlick AC, Weber JS, McArthur GA, Hutson TE, Flaherty KT, Moschos SJ: BRIM-2: An open-label, multicenter phase II study of vemurafenib in previously treated patients with BRAFV600E mutation-positive metastatic melanoma. J Clin Oncol 29 (15_suppl): 8509, 2011.
171. Falchook GS, Long GV, Kurzrock R, Kim KB, Arkenau TH, Brown MP, Hamid O, Infante JR, Millward M, Pavlick AC, *et al*: Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: A phase 1 dose-escalation trial. Lancet 379: 1893-1901, 2012.
172. Long GV, Trefzer U, Davies MA, Kefford RF, Ascierto PA, Chapman PB, Puzanov I, Hauschild A, Robert C, Algazi A, *et al*: Dabrafenib in patients with Val600Glu or Val600Lys BRAF-mutant melanoma metastatic to the brain (BREAK-MB): A multicentre, open-label, phase 2 trial. Lancet Oncol 13: 1087-1095, 2012.
173. Infante JR, Fecher LA, Falchook GS, Nallapareddy S, Gordon MS, Becerra C, DeMarini DJ, Cox DS, Xu Y, Morris SR, *et al*: Safety, pharmacokinetic, pharmacodynamic, and efficacy data for the oral MEK inhibitor trametinib: A phase 1 dose-escalation trial. Lancet Oncol 13: 773-781, 2012.
174. Davies MA, Saiag P, Robert C, Grob JJ, Flaherty KT, Arance A, Chiarion-Sileni V, Thomas L, Lesimple T, Mortier L, *et al*: Dabrafenib plus trametinib in patients with BRAF(V600)-mutant melanoma brain metastases (COMBI-MB): A multicentre, multicohort, open-label, phase 2 trial. Lancet Oncol 18: 863-873, 2017.
175. Long GV, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, Larkin J, Garbe C, Jouary T, Hauschild A, Grob JJ, *et al*: Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: A multicentre, double-blind, phase 3 randomised controlled trial. Lancet 386: 444-451, 2015.
176. Long GV, Hauschild A, Santinami M, Atkinson V, Mandala M, Chiarion-Sileni V, Larkin J, Nyakas M, Dutriaux C, Haydon A, *et al*: Adjuvant dabrafenib plus trametinib in stage III BRAF-Mutated melanoma. N Engl J Med 377: 1813-1823, 2017.
177. Ribas A, Gonzalez R, Pavlick A, Hamid O, Gajewski TF, Daud A, Flaherty L, Logan T, Chmielowski B, Lewis K, *et al*: Combination of vemurafenib and cobimetinib in patients with advanced BRAF(V600)-mutated melanoma: A phase 1b study. Lancet Oncol 15: 954-965, 2014.

178. Yee MK, Lin Y, Gorantla VC, Butterfield LH, Kluger HM, Chapman PB, Gangadhar TC, Milhem MM, Pavlick AC, Amaravadi RK, *et al*: Phase 2 study of cobimetinib in combination with vemurafenib in active melanoma brain metastases (coBRIM-B). J Clin Oncol 33 (15_suppl): TPS9088, 2015.
179. Sullivan RJ, Weber J, Patel S, Dummer R, Carlino MS, Tan DSW, Lebbé C, Siena S, Elez E, Wollenberg L, *et al*: A Phase Ib/II study of the BRAF inhibitor encorafenib plus the MEK inhibitor binimetinib in patients with BRAF(V600E/K) -mutant solid tumors. Clin Cancer Res 26: 5102-5112, 2020.
180. Corradi G, De Martino S, Rinaldi A, Siepe G, Marchese PV, Melotti B and Comito F: Rapid radiological response of leptomeningeal carcinosis and prolonged survival to encorafenib and binimetinib in BRAF-mutated melanoma. Anticancer Drugs 36: 691-693, 2025.
181. Falchook GS, Long GV, Kurzrock R, Kim KB, Arkenau HT, Brown MP, Hamid O, Infante JR, Millward M, Pavlick A, *et al*: Dose selection, pharmacokinetics, and pharmacodynamics of BRAF inhibitor dabrafenib (GSK2118436). Clin Cancer Res 20: 4449-4458, 2014.
182. Long GV, Eroglu Z, Infante J, Patel S, Daud A, Johnson DB, Gonzalez R, Kefford R, Hamid O, Schuchter L, *et al*: Long-term outcomes in patients with BRAF V600-Mutant metastatic melanoma who received dabrafenib combined with trametinib. J Clin Oncol 36: 667-673, 2018.
183. Dummer R, Flaherty KT, Robert C, Arance A, B de Groot JW, Garbe C, Gogas HJ, Gutzmer R, Krajsová I, Liszkay G, *et al*: COLUMBUS 5-year update: A randomized, open-label, phase III trial of encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF. Future Oncol 19: 1091-1098, 2023.