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Published on: 01 Mar 2017 - Ejso (Elsevier)

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DOI: <https://doi.org/10.1016/j.ejso.2016.10.014>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-128596>

Journal Article

Accepted Version

Originally published at:

Amann, V C; Ramelyte, E; Thurneysen, S; Pitocco, R; Bentele-Jaberg, N; Goldinger, S M; Dummer, R; Mangana, J (2017). Developments in targeted therapy in melanoma. *European Journal of Surgical Oncology*, 43(3):581-593.

DOI: <https://doi.org/10.1016/j.ejso.2016.10.014>



Review

Developments in targeted therapy in melanoma

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Accepted 24 October 2016

Available online ■ ■ ■

Abstract

Melanomas are disease entities driven in part by the mitogen activated protein kinase (MAPK) pathway. The TCGA network recently defined four genetic subtypes based on the most prevalent significantly mutated genes, including mutant *BRAF*, mutant *RAS* (N/H/K), mutant *NFI*, and Triple *wild-type* melanoma (harboring none of the aforementioned mutations, but instead includes *KIT*, *GNA* and *GNAQ* mutations).

The successful development of kinase inhibitors marked a milestone in the treatment of metastatic melanoma. Combination treatment with a BRAF- and MEK-inhibitor is the current standard of care for inoperable stage IIIC/IV *BRAF*-mutated melanoma. Recent data demonstrate excellent long-term outcome, especially in patients with normal baseline LDH levels, and confirm that there is a subset of BRAF inhibitor-naïve patients who experience durable responses without progression on combination treatment. In the future, adding a third compound based on individual genetic alterations might further improve the outcome of targeted therapy.

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Keywords: BRAF; NRAS; MEK; Kinase inhibitors; Targeted therapy; Melanoma

Introduction

In the past decade, revolutionary insights were made in understanding and treating melanoma. The identification of the critical role of the mitogen activated protein kinase (MAPK) pathway and development of targeted therapy dramatically changed prognosis and overall survival (OS) of metastatic melanoma patients.^{1,2} Novel techniques such as next generation sequencing enable the identification of new cancer “driver” genes and their mutations. These new insights contribute to prognostication and create opportunities for development of innovative mutation-directed therapies.

Sequencing data have shown that the median mutation rate in melanoma is >10 mutations/Mb, the highest of all cancers so far analyzed by The Cancer Genome Atlas (TCGA) network.³ Nevertheless, the number of mutations differs according to the site, with the lowest rate in primary melanomas on non-ultraviolet-exposed non-glabrous skin and the highest in patients with history of chronic sun exposure.⁴ Genetic alterations in melanoma oncogenes and tumor suppressor genes commonly cause constitutive signaling through RAS-RAF-MEK-ERK, also known as the MAPK pathway.^{5,6} This cascade concludes in activation of ERK1 and ERK2, which can then translocate to the nucleus and regulate MITF, c-MYC and other transcription factors, resulting in alteration of cell proliferation and senescence (Fig. 1).⁶ Less frequently identified, yet also relevant are genetic aberrations in other cellular pathways, such as cell cycle control (*CDKN2A*), apoptosis (*PT53*) and the PI3K pathway (*TERT*, *PTEN*).^{7–10} In 2015, TCGA

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<http://dx.doi.org/10.1016/j.ejsso.2016.10.014>

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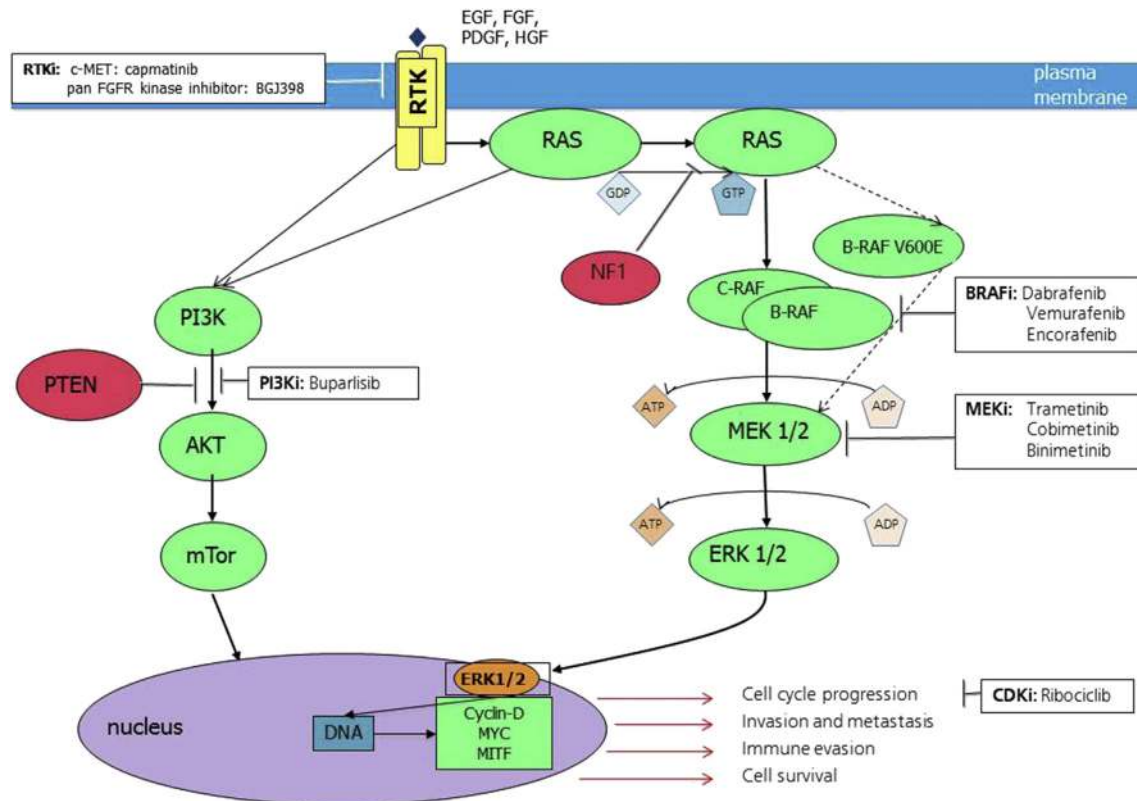


Figure 1. (Oncogenes shown in green, tumor suppressors shown in red) Extracellular signals such as EGF (epidermal growth factor), FGF (fibroblast growth factor), PDGF (platelet derived growth factor) or HGF (hepatocyte growth factor) bind to corresponding receptors (EGFR, FGFR, PDGFR and c-Myc, respectively) and induce signal transduction, which results in activation of RAS (exchange of protein-bound GDP (inactive form) to GTP (active form), accelerated by guanine nucleotide exchange factors).¹³ NF1 negatively regulates RAS by increasing RAS GTPase activity and hence turning RAS-GTP to RAS-GDP.⁸ Active RAS leads to activation of BRAF (dimerization of non-mutated BRAF; BRAF V600E mutated protein can be active as a monomer) and subsequently MEK. This cascade concludes in activation of ERK1 and ERK2, which can then translocate to the nucleus and regulate MITF, c-MYC and other transcription factors, resulting in alteration of cell proliferation and senescence.⁹ Receptor tyrosine kinases (RTK) or RAS-GTP can activate the PI3K-AKT pathway, while PTEN is a negative regulator, mutation of which leads to constitutive activation of AKT and is followed by changes in cell growth, motility and invasion.¹²⁴

Network suggested a new genomic classification for melanoma based on the most prevalent significantly mutated genes: mutant *BRAF*, mutant *RAS*, mutant *NF1* and triple wild-type (triple *wt*) melanoma.⁷

Genetic landscape of mutations

The most common genetic alteration, accounting for around 50% of all somatic mutations in cutaneous melanoma, is activating mutation of serine–threonine kinase *BRAF* gene.^{7,8,11} This causes constitutive activation of BRAF protein, which results in increased proliferation and survival of melanoma cells.¹² In more than 90% of cases valine is substituted with glutamate at codon 600 (V600E), less frequently with lysine (V600K) or arginine (V600R).^{5,7} Interestingly, patients with *BRAF* mutations are generally younger. It is most frequent in cutaneous melanoma, while it's detected in only 10–20% of mucosal melanoma.⁵

RAS is a member of the guanosine 5'-triphosphatase (GTP)-binding protein family. Under normal conditions, RAS activation is induced by extracellular signaling and

is reversible. It's driven by guanine nucleotide exchange factor, which results in exchange of protein-bound guanosine diphosphate (GDP) to triphosphate (GTP). Mutations in the *RAS* gene impair inactivation and keep RAS protein in the activated state.¹³ The active form, RAS-GTP, initiates phosphorylation of RAF and subsequently MEK and ERK and leads to the activation of the MAPK pathway.¹³ This specific structure causes difficulties in targeting; hence, RAS was named the “undruggable” target.¹³ *RAS* mutations are found in around 30% of melanomas and usually affect *NRAS* (Q61R, Q61K, and Q61H).^{7,11,14} *RAS* not only activates the MAPK pathway, but also has activators and effectors among other cellular pathways such as phosphatidylinositol-3 kinases (PI3K), T-Cell Lymphoma Invasion and Metastasis 1 (TIAM1) and others.¹³

The third most frequently identified genetic mutation is located in the *NF1* tumor suppressor gene, which serves as a regulator of RAS through GTP-ase activating protein. Due to an inactivating mutation, the regulative properties of NF1 are lost, which results in continuous activation of RAS.¹⁵ This mutation was observed in 14% of samples analyzed by TCGA,⁷ and in 46.4% of *BRAF* and *NRAS*

wt melanomas analyzed by Krauthammer et al.⁸ Interestingly, around 60% of these melanomas harbored co-mutations in *RAS-opathy* genes (*RASA2*, *PTPN11*, *SOS1*, *RAF1* and *SPRED1*),^{8,16} which are known to be linked with Noonan, Leopard and Legius syndromes.⁸

Melanomas referred to as triple *wt* show none of the three previously mentioned mutations. This subgroup includes *GNAQ*, commonly found in uveal melanoma, or *KIT* mutations. Interestingly, only 30% of triple *wt* melanomas harbor a UV signature, compared to over 90% of *BRAF*-, *RAS*- and *NF1*-subtypes, but more copy number changes and complex structural arrangements are identified in triple *wt*.⁷

Identifying mutations open opportunities for the development of new therapeutic agents, identification of predictive biomarkers and aid in understanding of melanoma genesis. Shain et al. evaluated genetic alterations in 37 primary melanomas and their adjacent antecedent lesions.¹⁷ Interestingly, histologically benign lesions harbored V600E mutations only, while intermediate lesions harbored additional *BRAF* as well as *NRAS* mutations. Compared to benign and intermediate lesions, more Telomerase reverse transcriptase (*TERT*) promoter and copy-number alterations were identified in melanoma, whereas Phosphatase and tensin homolog (*PTEN*) and tumor protein p53 (*TP53*) mutations were only found in advanced primary melanomas.^{7,17} This suggests that *BRAF* mutations are sufficient for nevus formation, but additional oncogenic alterations are needed for malignant transformation.

Driver mutations as prognostic factors in melanoma?

One of the major questions raised in the last years is whether the identification of driver mutations has prognostic or predictive clinical significance.

Long et al. were initially able to demonstrate an association between *BRAF* mutations and inferior clinical outcome in a prospective cohort of 197 Australian melanoma patients, albeit with no influence on disease free survival (DFS).¹⁸ Similarly, *NRAS* mutant melanoma patients were also reported to have impaired survival with a higher incidence of central nervous system (CNS) involvement in a retrospective setting; however no difference in overall survival (OS) between *BRAF* mutated and *wt* melanomas was noted.¹⁹

Since then, several studies have been conducted, none of which were able to show an influence of *BRAF* or *NRAS* mutation status on OS.^{20–26}

A subsequent prospective Australian study (n = 308) was not able to confirm the previously reported impact on OS of *BRAF* mutant melanoma patients. However, one-year survival from diagnosis of metastasis was significantly longer for patients with *BRAF* mutant melanoma treated with a kinase inhibitor than those without (29%, p < 0.001), or for *BRAF wt* patients (37%, p < 0.001).²⁷ Similarly, Frauchiger et al. confirmed that *BRAF* mutant

melanoma patients treated with selective kinase inhibitors have a statistically significant longer OS compared to *wt* patients (OS 14.5 vs 10.6 months, p = 0.14).²⁴

Relating to immunotherapy, it has recently been shown that harboring an *NRAS* mutation increases the response rate (RR) to checkpoint inhibitors with no significant impact on OS and progression-free survival (PFS).^{28,29} A recent retrospective study affirmed this assumption: although mutational status had no influence on OS in anti-CTLA-4 treated patients, a non-statistically-significant trend for superior clinical outcome in *NRAS* mutant patients was observed.³⁰

As outlined previously, the third most commonly reported mutation is the inactivating mutation of *NF1*, which shows similar OS compared to *BRAF*-mutant, *NRAS*-mutant or triple *wt* melanomas.⁸ No correlation between the loss of *NF1* and response to treatment with MEK or ERK inhibitors has been noted in vitro so far.⁸ Nevertheless, further large prospective clinical trials are needed to address this issue.

Besides these most commonly observed mutations in melanoma, other features seem to have prognostic impact. *TERT* promoter mutations are associated with impaired OS in cutaneous compared to acral, mucosal and uveal melanomas³¹ and are present in approximately 70–80% of *BRAF*-, *NRAS*- and *NF1*- compared to 7% of triple *wt* melanomas.⁷ Furthermore, they were found in 61% of fast-growing compared to 32% of slow-growing melanomas (p = <0.0001).³² Thus, it was suggested that the presence of *TERT* promoter mutations can partly explain a more aggressive clinical outcome, associated with an accelerated growth rate. In contrast, *BRAF* and *NRAS* hotspot mutations have not been found to be associated with increased growth. Therefore, *TERT* promoter mutation may serve as a future biomarker to identify aggressive tumors, which could benefit from adjuvant treatment.

The prognostic significance of *PTEN* promoter methylation in clinical outcome of melanoma patients was recently elucidated.^{9,33} In a cohort of 392 melanoma patients, *PTEN* was identified as an independent predictor for impaired survival, highlighting potential therapeutic opportunities in this field.⁹

In summary, the mutation status alone does not seem to be sufficient to predict the outcome of advanced melanoma patients and cannot be used as an independent prognostic factor. However, the identification of other genetic features and their eventual prognostic significance are fundamental steps in the field of melanoma management.

Multi-kinase inhibitors

Tyrosine kinase inhibitors

Among targeted therapies, monoclonal antibodies (mAbs) and small-molecule inhibitors (SMIs) of multiple tyrosine kinase activity present as ideal candidates for

melanoma treatment.³⁴ Tyrosine kinase inhibitors (TKIs) block vital pathways in the context of cell growth and survival via binding to small intercellular molecules, which are common phosphorylation sites of kinases (most commonly tyrosine or serine–threonine kinases) that are either associated with growth factor receptors or downstream signaling molecules. Several clinical trials have been performed over the last years either with single agents or in combination with chemotherapy. However, the demonstrated clinical benefit with multi-targeted tyrosine kinase inhibitors (mTKIs) was limited compared to selective BRAF and MEK inhibitors.

Sorafenib

Sorafenib is an orally available, broad-spectrum mTKI with anti-proliferative and anti-angiogenic effects.³⁵ Sorafenib targets several RAF isoforms as well as other receptor tyrosine kinase inhibitors (RTKI) like vascular endothelial growth factor (VEGFR 2/3), platelet-derived growth factor receptor beta (PDGFR- β), fms-related tyrosine kinase 3 (FLT-3) and c-KIT.^{30,35}

In clinical trials with advanced melanoma patients, sorafenib showed no clinical benefit as a single agent.^{36,37} In the context of evaluating combination therapies, Flaherty et al. presented encouraging preliminary results of sorafenib combined with carboplatin and paclitaxel (CP).³⁸ The combination showed a 37% partial response (PR) rate in the interim analysis while another 48% demonstrated stable disease (SD).³⁸ Nevertheless, phase III clinical trials failed to show significant benefit.^{39,40}

In addition, several phase I/II studies with sorafenib in combination with dacarbazine (DTIC) or temozolomide (TMZ) showed low anti-tumor activity and mainly achieved SD.^{41–43} The beneficial 45.5% overall metabolic RR (PERCIST criteria) in a recent clinical trial did not result in lasting objective responses.⁴⁴ Sorafenib in combination with CP in uveal melanoma patients also failed to achieve clinical benefit with the study being terminated early (PFS 4 months, OS 11 months, no confirmed objective tumor responses by RECIST criteria).⁴⁵ However, the results of the STREAM study in chemo-naïve metastatic uveal melanoma patients are still pending.⁴⁶

In general, sorafenib monotherapy and combination therapies had a manageable toxicity profile, with the majority of adverse events (AEs) being mild.^{36,37,39–41,47} Severe adverse events (SAE) were reported in 51% of patients, with the most common being hematologic toxicities.⁴⁷ The most commonly observed drug-related AEs under sorafenib monotherapy were skin reactions (rash and palmar-plantar erythrodysesthesia syndrome), gastrointestinal and constitutional disorders (corresponding grade 1–2 intensity).^{36,37} Combination treatments with sorafenib led to hematotoxicity, fatigue, sensory neuropathy and skin reactions.^{39,40,43}

Based on the limited activity of sorafenib, doubts were raised concerning the potential of RAF inhibition as a

therapeutic option. Therapeutic failure of sorafenib in advanced melanoma was likely due to an inability to selectively achieve RAF inhibition at maximum tolerated doses. Subsequent trials with selective BRAF inhibitors (BRAFi) followed, changing the era of melanoma treatment.

Pazopanib

Pazopanib is another orally-bioavailable, adenosine triphosphate (ATP)-competitive TKI with selectivity for VEGFR-1, -2, and -3, FDA-approved for renal carcinoma and soft tissue sarcoma in 2009.⁴⁸ Furthermore, it blocks the PDGFR- α and - β as well as c-KIT.

Clinical trials in melanoma patients with pazopanib are limited. None of the studies published so far reported significant clinical benefit in advanced *BRAF wt* melanoma patients.^{49,50} A recent single-center pilot study investigating the metabolic response, the early cytokine and chemokine profile and the histological findings of metastatic tissue under pazopanib and paclitaxel in the second line setting showed moderate efficacy.⁵¹ 17 patients with stage III or IV melanoma were included. 5 out of 14 evaluable patients showed partial metabolic response (using PRECIST 1.0 criteria) after 10 days of pazopanib monotherapy. No response was achieved at day 70 of combination treatment. The median progression-free survival (mPFS) was 70 days, the median overall survival (mOS) 208 days.⁵¹

The drug was generally well tolerated with 87% of all AEs being mild to moderate, such as loss of appetite, weakness, skin reactions, bone marrow function impairment and neurological symptoms. 13% of AEs were grade 3 or grade 4.

Other multi-kinase inhibitors

Axitinib, another tyrosine kinase inhibitor against *VEGFR-1, -2, -3, PDGFR- β* and *c-KIT*, showed promising activity in combination with carboplatin and paclitaxel in *wt* metastatic melanoma patients in a phase II clinical study (RR 18.8%, 6-month-PFS of 33.9%).⁵² However, these results warrant further testing in randomized phase III trials. On the other hand, the multi-kinase inhibitor Lenvatinib (E7080) achieved limited responses both in monotherapy and in combination with TMZ in the phase I setting.⁵³

KIT inhibitors

The KIT receptor protein tyrosine kinase is a transmembrane protein consisting of extra- and intracellular binding domains and a signal transmembrane region. Most *KIT* mutations are located in exon 11, which codes for the juxta-membrane domain, and in exon 13, which codes for a kinase domain.

Although amplifications or activating mutations of *KIT* are generally rare in melanoma, they are more commonly found in mucosal, acral and in melanomas arising from chronically sun damaged-skin.⁵⁴ As the number of

advanced melanoma patients harboring *KIT* mutations is low, the clinical experience of *KIT* inhibitors is limited.

The most widely investigated *KIT* inhibitor is imatinib, which is FDA-approved for gastrointestinal stromal tumors (GIST) and dermatofibrosarcoma protuberans.

Out of 51 *KIT* mutant melanoma patients in a phase II clinical trial, 28 patients were treated with imatinib 400 mg orally bid. The overall response rate (ORR) was 16% with a median OS of 46.3 weeks.⁵⁵ In a phase II clinical trial of 43 *KIT* mutant melanoma patients, 23 had an ORR (10 patients achieved PR and 13 SD).⁵⁶ 1-year OS was 51%, 6-month PFS rate was 36.6%. The best predictor of treatment response in both trials was the presence of mutation in *c-KIT* exons 11 and 13. Most frequently observed adverse events were hematologic toxicities (leukopenia and anemia), fatigue, nausea, rash and periorbital edema.

Nilotinib is a BCR-ABL1 TKI that was rationally designed to have increased potency and selectivity for the oncogene BCR-ABL1. It also inhibits *c-KIT* with greater potency than imatinib and is effective against several known *c-KIT* mutations in vitro.^{57,58} Nilotinib achieved a satisfactory disease control rate (DCR) in a phase II clinical study of twenty-seven melanoma patients that had progressed on imatinib or in patients with brain metastases (4-month DCR 27% and 12.5%, respectively).⁵⁹

The effect of nilotinib (400 mg bid) was investigated in another open-label single-arm clinical trial (TEAM Trial, submitted for publication).⁶⁰ Similarly, among 42 patients in the nilotinib arm, the ORR was 26.2% (95% CI, 13.9%–42.0%; PRs, $n = 11$; complete response (CR), $n = 0$). The median PFS was 4.2 months (95% CI, 2.1–5.8 months). At 6 months, the estimated PFS rate was 34.6% (95% CI, 20.2%–49.3%).

Monoclonal antibodies

Bevacizumab

The prognostic implications of overexpression of VEGF in clinical outcome and disease progression in melanoma remain controversial.⁶¹ VEGF is assumed to be the dominant growth factor in angiogenesis.⁶² The relevance of angiogenesis/neoangiogenesis in tumor metabolism, proliferation and the tumor microenvironment is unquestioned,⁶³ thus the inhibition of the VEGF pathway offers a promising therapeutic approach.

Bevacizumab, a monoclonal antibody against VEGF-A, was the first anti-angiogenic agent on the market. It has been approved for breast neoplasms, non-small cell lung cancer, renal, ovarian, cervix and colorectal cancer.⁶⁴ According to a recently published phase II clinical study, nab-paclitaxel in combination with bevacizumab showed an ORR of 36% in unresectable stage III and IV melanoma patients ($n = 50$) in a first line setting.⁶⁵ The following multicenter phase II clinical trial combining temozolomide (TMZ) with bevacizumab showed limited efficacy.⁶⁶ On the

other hand, current phase I/II clinical trials of bevacizumab in combination with ipilimumab, erlotinib or imatinib demonstrated no synergistic effect.^{67–69} Nevertheless, well-controlled phase III clinical trials are warranted to further investigate these results.

Tendency in clinical research opts in favor of combination therapies, since a certain immunomodulatory effect of TKIs seems to be existent.^{70,71} Summarizing the literature, multi-targeted TKI are not established as standard treatment of advanced melanoma but are still discussed as potential second line options in *BRAF*- and *NRAS* *wt* melanoma patients.⁹³

Etaracizumab

Etaracizumab (MEDI-522), a monoclonal antibody against Integrin $\alpha_5\beta_3$, resulted in similar OS and PFS rates when compared to combination with DTIC (12.6 versus 9.4 months, respectively)⁷² and was not further investigated in a phase III setting.

Intetumumab

The anti- α_v -integrin monoclonal antibody showed only a nonsignificant trend towards an improved OS in a randomized phase II trial compared to DTIC.⁷³

mTOR-inhibitors

Since the mammalian target of rapamycin (mTOR) signaling is upregulated in metastatic melanoma, drugs targeting mTOR seem to represent promising therapeutic targets. Everolimus (RAD-001), an orally administered inhibitor of mTOR, achieved only SD as best ORR in a cohort of 20 metastatic melanoma patients with a PFS of 3 months.⁷⁴ It failed to show significant objective responses in combination with TMZ over TMZ alone.⁷⁵ A phase II clinical study of Everolimus in combination with pasireotide didn't meet its primary endpoint in uveal metastatic melanoma.⁷⁶

Kinase inhibitors

Early developments

The successful development of kinase inhibitors marked a milestone in the treatment of metastatic melanoma. Until 2010, no systemic treatment had demonstrated any improvement of overall survival in metastatic melanoma. Tsai et al. first discovered a potent, selective inhibitor of *BRAF* V600E, using a scaffold-based drug design approach.⁷⁷ In preclinical studies, this compound showed impressive antitumor activity in *BRAF* V600E mutated cell lines by inducing cell cycle arrest and apoptosis, with no such effect on *BRAF* *wt* cell lines. Oral administration in xenograft models harboring the V600E mutation resulted

in a substantial block of tumor growth and clinical regression without any apparent toxicity.^{77,78} Early clinical trials confirmed that BRAF inhibition did in fact cause complete or partial tumor regression in a large portion of patients harboring the *BRAF* V600 mutation.^{79,80}

BRAF inhibition

BRAF inhibition rapidly became standard of care in *BRAF* mutated melanoma patients. Vemurafenib is an orally bioavailable, ATP-competitive, small-molecule (489.92 Da) inhibitor of BRAF. Dabrafenib (519.56 Da) and encorafenib (540.01 Da) are similar molecules. The change in paradigm of treatment of advanced melanoma is mainly based on the results of the following two pivotal trials.

The international, multicenter phase III randomized clinical trial comparing vemurafenib to the reference chemotherapy dacarbazine (BRIM-3) showed a significantly longer mOS in the vemurafenib group (13.6 months [95% CI 12.0–15.2] vs 9.7 months [7.9–12.8]; hazard ratio [HR] 0.70 [95% CI 0.57–0.87]; $p = 0.0008$), as well as a significantly increased mPFS (6.9 months [95% CI 6.1–7.0] vs 1.6 months [1.6–2.1]; HR 0.38 [95% CI 0.32–0.46]; $p < 0.0001$). OS and PFS were significantly shorter in patients with increased LDH levels at baseline in both groups. The RR amounted to 57% in the vemurafenib group vs. 9% in the dacarbazine group.^{81,82} Following this phase III trial, vemurafenib was approved by the FDA in 2011 for treatment of Stage IIIC and IV metastatic melanoma patients harboring a *BRAF* V600E mutation.

Moreover, the multicenter phase III randomized clinical trial evaluating the BRAFi dabrafenib vs. dacarbazine (BREAK-3) showed comparable results, with a mPFS of 6.9 months for dabrafenib vs. 2.7 months for dacarbazine, hazard ratio (HR) 0.37 (95% CI 0.23–0.57; $p < 0.0001$). The mOS in this study at last update was at 18.2 months vs. 15.6 months, HR 0.76 (95% CI 0.48–1.21). The ORR was 50% in the dabrafenib group and 6% in the dacarbazine group.^{83,84} Dabrafenib received FDA approval in 2013.

Common AEs of BRAFi monotherapy with vemurafenib include arthralgia (56% of patients), rash (41%), fatigue (46%) and UVA-dependent photosensitivity (41%).⁸⁵ The most frequent grade 3–4 side effects include cutaneous squamous cell carcinoma (19%) and keratoacanthoma (10%), rash (9%) as well as abnormal liver function tests (11%). Interestingly, 2.4% of patients developed a new primary melanoma during treatment.⁸⁶ Treatment discontinuation due to SAEs occurred in 7% of patients on vemurafenib.^{81,82} Common side effects of dabrafenib monotherapy comprise cutaneous AEs (hyperkeratosis 36%, alopecia 27%, skin papilloma 22%, palmar-plantar hyperkeratosis 19%, rash 30%), pyrexia (16%), fatigue (18%), headache (18%) and arthralgia (19%). 10% of patients developed cutaneous squamous cell carcinoma or keratoacanthoma. Treatment discontinuation due to SAEs

occurred in 3% of dabrafenib patients.^{83,84} The most common grade 3–4 AEs for dabrafenib include cutaneous squamous cell carcinoma (7%) and pyrexia (3%). Direct comparison of AEs in these phase III trials is difficult. In general, dabrafenib monotherapy exhibited a much lower rate of photosensitivity (2%) compared to vemurafenib (41%), as it has UVA-absorbing properties, while other BRAF inhibitors do not.⁸⁵ Further, dabrafenib showed a lower rate of cutaneous malignancies, while exhibiting a higher frequency of pyrexia.

In summary, BRAF inhibition proved to be very efficacious in *BRAF*-mutated patients, with a high response rate and a rapid onset of response, but BRAFi monotherapy is almost invariably followed by relapse due to acquired drug resistance, most likely as a result of reactivation of MEK and ERK.^{87–89}

MEK inhibition

After observing that *BRAF* mutation is associated with an increased and selective sensitivity to MEK inhibition compared to *BRAF*-wt cells, Solit et al. suggested that *BRAF* mutant tumors were dependent on MEK activity and thus proposed MEK inhibition as a possible treatment for metastatic melanoma.⁹⁰ Initial trials with MEK inhibitors (MEKi) confirmed this observation.⁹¹ MEKi are orally bioavailable, non-ATP competitive, allosteric binding inhibitors of MEK. While trametinib (615.39 Da) and binimetinib (441.23 Da) inhibit MEK 1 and 2, cobimetinib (531.31 Da) inhibits MEK1 only.

After positive phase I and II trials, monotherapy with trametinib in *BRAF* mutant melanoma was investigated in a phase III multicenter open-label trial. Chemotherapy with dacarbazine or paclitaxel served as a comparison. The mPFS was significantly prolonged compared to chemotherapy (4.8 months vs. 1.5 months, HR 0.45 [95% CI 0.33 to 0.63; $p < 0.001$]). The rate of OS at 6 months improved as well, with 81% in the trametinib group and 67% in the chemotherapy group despite crossover (HR 0.54) [95% CI 0.32 to 0.92; $p = 0.01$].⁹²

In a phase II trial, MEKi monotherapy with binimetinib was evaluated in patients with *NRAS* as well as in patients with *BRAF* mutation, with a similar RR of 20% in *BRAF*- and *NRAS*-mutated patients. Thus, MEK inhibition was the first targeted therapy to show activity in patients harboring an *NRAS* mutation. The median PFS was 3.7 months in patients with *NRAS*-mutated melanoma [95% CI 2.5–5.4] and 3.6 months [95% CI 2.0–3.8] in patients with *BRAF*-mutated melanoma. The difference in PFS between this and the aforementioned study might be explained by the fact that patients previously treated with ipilimumab or BRAF inhibitors were included in this trial, while being excluded from the aforementioned one. Of note, this study also showed evidence of MEKi activity in brain metastases.⁹³ An ongoing phase III clinical trial of binimetinib in patients with advanced *NRAS*-mutant melanoma (NEMO)

recently met its primary endpoint of improving progression-free survival compared to dacarbazine, with a median PFS of 2.8 months for binimetinib versus 1.5 months for dacarbazine; HR 0.62, [95% CI 0.47–0.80], $p < 0.001$.⁹⁴

While MEK inhibition did show improvement in both PFS and OS, the RRs seem to be inferior to those shown for BRAF inhibitors. The molecular basis for this phenomenon remains unclear.

The side effect profile of MEKi differs greatly from BRAFi. The most common AEs with trametinib were rash (57%), diarrhea (43%) and peripheral edema (26%). Frequent grade 3–4 AEs include hypertension (12%), rash (8%) and fatigue (4%). Further side effects were fatigue, acneiform dermatitis, nausea, alopecia, hypertension, constipation and vomiting. Moreover, asymptomatic and reversible reduction in left ventricular ejection fraction (LVEF) and ocular toxic effects (blurred vision, reversible chorioretinopathy) were observed.⁹⁵ Dose interruptions due to AEs occurred in 35% of patients. However, MEK inhibition did not seem to cause any cutaneous squamous-cell carcinomas or hyperproliferative skin lesions.⁹² While both classes have been known to cause a rash, MEKi seem to cause a papulopustular rash, while BRAFi cause a hyperkeratotic maculopapular rash.⁹⁶

In addition, MEK inhibition was evaluated in combination with chemotherapy.⁹⁷ However, there was no evidence for an increase in efficacy compared to MEKi monotherapy; hence, this was not evaluated in any further trials.

Combined BRAF- and MEK inhibition

With BRAF and MEK inhibitors both showing efficacy in melanoma, preclinical studies suggested an enhanced anti-tumor effect and a reduction of BRAFi-induced cutaneous SCC when combining the two classes. Due to emerging resistance to BRAFi as a result of MEK-ERK signaling reactivation, patients inevitably experience relapse.^{87,88} The effect of MEKi treatment after treatment resistance to BRAFi in patients harboring a *BRAF* mutation was evaluated in a small cohort of patients, with no significant response.^{93,98}

In contrast, early trials evaluating the combination of BRAFi and MEKi were very promising (Table 1). A randomized phase I/II clinical trial comparing the combination

of dabrafenib and trametinib vs. dabrafenib alone (COMBI-d) showed a marked increase in PFS in the combination group (9.4 months vs. 5.8 months, HR 0.39 [95% CI 0.25–0.62; $p < 0.001$]) and a higher RR (76% complete or partial response vs. 54% in the monotherapy group ($p = 0.03$)).⁹⁹

Subsequently, phase III of the abovementioned trial (COMBI-d) confirmed the superiority of BRAF and MEKi combination, with a significantly prolonged mPFS of 11.0 months (95% CI 8.0–13.9) in the dabrafenib and trametinib group vs. 8.8 months (5.9–9.3) in the dabrafenib monotherapy group (HR 0.67, 95% CI 0.53–0.84; $p = 0.0004$). The ORR were at 69% for combination vs 53% for monotherapy ($p = 0.0014$), while the mOS was 25.1 months (95% CI 19.2–not reached) for the combination and 18.7 months (15.2–23.7) for monotherapy (hazard ratio [HR] 0.71, 95% CI 0.55–0.92; $p = 0.0107$).¹⁰⁰ A recent survival update showed landmark OS rates of 52% at 2 years and 44% at 3 years.¹⁰¹ The best outcome was seen in patients with normal LDH levels and less than three disease sites.

Another open-label, phase III trial comparing the combination of dabrafenib and trametinib vs. vemurafenib monotherapy (COMBI-v) was able to produce comparable results. The mPFS amounted to 11.4 months in the combination group and 7.3 months in the vemurafenib group (HR 0.56; 95% CI, 0.46 to 0.69; $p < 0.001$). The ORR was 64% in the combination and 51% in the vemurafenib group ($p < 0.001$).¹⁰² Most important, quality of life was rated significantly better in the combination group using three standardized questionnaires measuring health-related quality of life during treatment and at disease progression.¹⁰³

Finally, a phase III clinical trial investigating the combination of vemurafenib and cobimetinib vs. vemurafenib and placebo (coBRIM) found a significant difference in mPFS (9.9 months in the combination group vs. 6.2 months in the control group (HR 0.51; [95% CI 0.39 to 0.68; $p < 0.001$])) and a significantly higher ORR of 68% in the combination group vs. 45% in the BRAF monotherapy group ($p < 0.001$).¹⁰⁴ The vemurafenib and cobimetinib combination reached a mOS of 22.3 months [95% CI: 20.3-not reached], compared to 17.4 months for vemurafenib alone [95% CI: 15.0–19.8], HR: 0.70; 95% CI: 0.55–0.90, $p = 0.005$. The OS benefit was seen in all groups, including patients with high LDH at baseline.¹⁰⁵

Table 1
Outcome of combined BRAF and MEK inhibition of several landmark studies.

Study	Phase	Experimental (combination) arm	mPFS (months)	mOS (months)	1-yr OS (%)	2-yr OS (%)	3-yr OS (%)	Ref.
BRIM7	I	Vemurafenib, Cobimetinib	13.8	31.2	83	64	37	106
coBRIM	III	Vemurafenib, Cobimetinib	9.9	22.3	n/a	n/a	n/a	105
Combi D	II	Dabrafenib, Trametinib	9.4	n/a	72	60	47	99,107
Combi D	III	Dabrafenib, Trametinib	11.0	25.1	74	51	44	100,101
Combi V	III	Dabrafenib, Trametinib	11.4	26.1	72	53	45	102,134
COLUMBUS	III	Encorafenib, Binimetinib	14.9	n/a	n/a	n/a	n/a	111

These data are supported by recent extended follow-up results of a phase Ib trial of vemurafenib and cobimetinib (BRIM7), with landmark survival for BRAF naïve patients of 82%, 64%, and 37%, respectively at 1, 2, and 3 years, and a mOS of 31.2 months.¹⁰⁶

Addressing the question of long-term benefit, a recent update on the randomized, phase II COMBI-d trial demonstrated that there is, in fact, a subset of BRAFi-naïve patients who experience long-term responses without progression on combination treatment. Normal baseline LDH levels were associated with a continued long term response. Prolonged survival was associated with normal baseline LDH levels and with fewer than 3 affected organ sites. The OS at 1, 2, and 3 years for BRAFi-naïve patients receiving dabrafenib at standard dose 150 mg bid and trametinib 2 mg qd was 72%, 60%, and 47%, respectively. In the population with normal baseline LDH levels, OS at 1, 2, and 3 years was 88%, 75%, and 62%, respectively, with a HR of 0.25 (0.12–0.53).¹⁰⁷ Thus, long term OS rates for BRAF/MEKi combination are similar to OS rates for first line anti-PD1 treatment.¹⁰⁸ The OS may have been impacted by subsequent immunotherapy after progression on kinase inhibitors.¹⁰⁷

Further approaches to BRAF/MEKi combination include trials with encorafenib and binimetinib. Encorafenib is a BRAFi with increased affinity to BRAF and thus a longer binding time. Results from a phase I/II clinical trial confirm a RR and PFS consistent with other BRAF/MEKi combinations (mPFS 11.3 months [95% CI 7.4–14.6]), with a considerably higher PFS in the baseline low LDH group, going along with previous reports.¹⁰⁹ A phase II trial evaluating encorafenib and binimetinib alone and in combination with a third agent after progression (LOGIC-2), reported an ORR of 68% for BRAF- and MEK-inhibitor naïve patients, and 20% for non-naïve patients, which is in accordance to results from other combinations.¹¹⁰ Recent results from a phase III trial demonstrated a mPFS of 14.9 months vs. 7.3 months for vemurafenib monotherapy with a HR of 0.54 [95% CI 0.41–0.71, $p < 0.001$] vs. 9.6 months for encorafenib monotherapy with a HR of 0.75 [95% CI 0.56–1.00, $p = 0.051$].¹¹¹

In general, similar percentages of grade 3–4 adverse events were seen in both monotherapy and combination treatment. Dabrafenib and trametinib combination treatment was associated with a higher frequency of pyrexia than BRAFi monotherapy (up to 71% of patients). Furthermore, gastrointestinal toxic effects (diarrhea, nausea, vomiting) were seen more frequently with combination.^{99,100,102} Acneiform dermatitis, a common dose-limiting effect of trametinib, was reduced in combination treatment.⁹⁹ Toxic events related to paradoxical MAPK pathway activation like cutaneous SCCs and hyperkeratosis were significantly lower in all combination treatments.^{99,100,102,104,112}

The vemurafenib/cobimetinib combination is associated with a higher frequency of central serous retinopathy, gastrointestinal events (diarrhea, nausea, vomiting),

photosensitivity (due to the UVA-absorbing property of the molecule),⁸⁵ elevated aminotransferase levels, and an increased creatine kinase level, with the majority of events being grade 1–2. Keratoacanthomas, cutaneous SCC, alopecia and arthralgias were observed in a lower frequency with the combination. The frequency of clinically significant cardiac events (QT-interval prolongation and decreased ejection fraction) was low and similar in mono- and combination therapy, as was pyrexia.¹⁰⁴ Encorafenib and binimetinib exhibited lower rates of pyrexia and photosensitivity than other combinations.¹⁰⁹

Class effects of MEK blockade include reversible, asymptomatic elevated CK levels, observed in 30% of patients in cobimetinib/vemurafenib,¹⁰⁴ as well as transient drug-induced retinopathy, which is reversible, and can be managed with dose reduction or withdrawal of MEKi.

Overall, combination of BRAF and MEK inhibition is well tolerated and markedly delays the onset of resistance compared to BRAF monotherapy. Combination treatment consistently exhibited a lower rate of secondary cutaneous cancers compared to single drug BRAF inhibition.^{99,100,102,104} Long term follow-up confirms the safety, response and tolerability and suggests long-term benefit without progression for a subset of patients (approx. 20%).¹⁰⁷ Thus, combination of a BRAF- and MEKi is considered the current standard of care for patients harboring *BRAF* mutations.

Perspective

As mentioned above, the combination of a selective BRAF- and MEKi is the current standard of care. However, about 80% of patients eventually develop resistance, most notably patients with high LDH at baseline.¹⁰⁷ Prolonged responses may be achieved by adding additional molecules (triple therapy). The choice of the third molecule could potentially be determined by individual genetic alterations.⁷ Possible candidates include inhibitors of cell cycle control, the PI3K-AKT pathway, and the surface receptors MET and fibroblast growth factor receptor (FGFR).

Cyclin-dependent kinases (CDKs) are serine/threonine kinases regulating cell division by promoting transitions through the cell cycle (CDK 4, 6, 2 and 1) and modulating transcription in response to several intra- and extracellular signals.¹¹³ A number of alterations concerning the p16^{INK4A}:cyclin D-CDK4/6:RB pathway have been reported in melanoma.^{7,114} Several different orally bioavailable, specific small molecule inhibitors of CDK 4 and 6 are currently available. By targeting CDK4/6, they inhibit phosphorylation of retinoblastoma protein and thus prevent CDK-mediated G1-S phase transition. Thus, the cell cycle is arrested in the G1 phase, thereby suppressing DNA synthesis and inhibiting growth of cancer cells.

PI3Ks are intracellular signaling proteins important for inhibition of apoptosis. The PI3K-AKT pathway was found to be activated in human cancers through multiple

mechanisms, including activating *PI3K* mutations, decreased expression or function of *PI3K* suppressors (e.g. *PTEN*), *PI3K* amplifications and activation of upstream oncogenes (e.g. *NRAS*) or receptors.^{7,115,116} Thus, multiple classes of inhibitors are available, including *PI3K* inhibitors (pan-isoform and isoform-specific), dual *PI3K*-*mTOR* inhibitors, *AKT* inhibitors and *mTOR* (*mTORC1* and dual *mTORC1/2*) inhibitors. Several molecules are available for each class, many of which are currently evaluated in clinical trials.¹¹⁶ Initial trials combining Pan-*PI3K*-inhibitors with *BRAF*- and *MEK*-inhibitors have shown exceptionally high toxicity.

Most melanoma patients harboring a *BRAF* mutation seem to have some degree of innate resistance to kinase inhibitors. One major cause of innate resistance is stroma-mediated resistance. Secretion of hepatocyte growth factor (HGF) by stromal cells leads to activation of the HGF-receptor *MET*, reactivation of the *MAPK* and the *PI3K*-*AKT* signaling pathways and immediate resistance to *RAF* inhibition.¹¹⁷ Consequently, dual inhibition of *RAF* and either *MET* or HGF serves as a potential strategy to counteract this mechanism of resistance. Several small molecule, highly specific inhibitors of *MET* and HGF are currently being tested.

Human fibroblast growth factors (FGF) are polypeptide growth factors that transduce signals by binding to transmembrane receptor tyrosine kinases, the *FGFR*,^{118,119} which then activate important cellular pathways including the *MAPK* and *PI3K* pathway.¹²⁰ Amongst others, FGFs control cell proliferation, migration, angiogenesis, apoptosis and differentiation, and further play a role in neoangiogenesis, thus aiding tumor vascularization.¹²¹ Hyperactivation of *FGFR* signaling seems to be associated with growth and progression in several different types of cancers.¹²² *FGF2* is overexpressed on melanoma cells, but not on normal melanocytes,¹²³ and has been linked to tumor progression in multiple malignancies, including melanoma.¹²² *BGJ398* is an orally bioavailable, potent and selective inhibitor of *FGFRs* that selectively suppresses *FGFR* signaling and proliferation in tumor cells with *FGFR* dependency and has an effect on endothelial cells by blocking FGF-induced angiogenesis, hence inhibiting tumor growth.^{124,125} It is currently being tested in clinical trials.

Two ongoing trials are investigating the addition of a third molecule to encorafenib and binimetinib. A phase Ib/II trial is comparing the efficacy and safety of the triple therapy with encorafenib, binimetinib and ribociclib (a *CDK4/6* inhibitor) versus the dual combination of encorafenib and binimetinib in *BRAF*-mutant metastatic melanoma (NCT01543698). A phase II clinical trial (LOGIC 2, NCT02159066) adds a third molecule based on an individual profile of molecular alterations once patients progress on encorafenib and binimetinib.

Moreover, the combination of *MAPK* inhibitors with immunotherapy represents an interesting therapy approach.¹²⁶ Upregulation of melanocyte differentiation antigen expression by *BRAF*-mutant melanoma cells upon

exposure to *BRAF* inhibitors has been described in several studies in human melanoma cell lines and melanoma biopsies.^{127–129} Similarly, *MEKi* also seem to increase expression of melanocyte differentiation antigens,^{127,130} improving antigen-specific T-cell recognition.^{127,131} This might result in increased lymphocyte homing to tumor cells, especially *CD8+* cells, and improved lymphocyte function.^{126,128} Thus, efficacy of immunotherapy may be augmented by combination with kinase inhibitors. However, long-term effects are uncertain and initial clinical trials showed increased toxicity: a phase I study combining vemurafenib and ipilimumab was stopped due to liver toxicity,¹³² while a phase I dose-finding trial investigating the safety of the combination of dabrafenib and ipilimumab and triple therapy with dabrafenib, trametinib, and ipilimumab had to close the triple arm as intestinal perforation (following colitis) was seen in 2 out of 7 total patients.¹³³

Targeted- and immunotherapy have both demonstrated impressive efficacy with profound impact on survival. To date, there are no convincing clinical data available that would justify one of the two as an established first line. Since this question is highly relevant for daily clinical care, careful investigation in further clinical trials is needed.

Targeted therapy with a *BRAF*- and *MEKi* combination is a reasonable first- and second line treatment option in *BRAF*-mutated melanoma. Furthermore, *MEK* inhibition appears promising in *NRAS* mutated patients, especially after failure of immunotherapy. New combination trials are ongoing. There is justified optimism that these combinations will further improve the outcome in *BRAF*- and *NRAS*-mutated melanoma, but possibly also in other populations such as *NFI*-, *GNA*- and *GNAQ*-mutated melanoma. Additional benefit might be seen in the adjuvant and neoadjuvant setting.

Conflict of interest statement

RD receives research funding from Novartis, Merck Sharp & Dohme (MSD), Bristol-Myers Squibb (BMS), Roche, GlaxoSmithKline (GSK) and has a consultant or advisory board relationship with Novartis, Merck Sharp & Dohme, Bristol-Myers Squibb, Roche, GlaxoSmithKline and Amgen, all of which are not relevant to this manuscript. SMG serves as an intermittent consultant for BMS, MSD, Roche and Novartis; and has received travel grant support from BMS, MSD, Roche and Novartis. VCA has received honoraria from Merck Sharp & Dohme (MSD), and funding from the Euronco Foundation (Zurich, Switzerland) and the Louis Widmer AG (Schlieren, Switzerland). No other disclosures are reported.

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