Cancer Treatment Reviews 99 (2021) 102238

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Anti-tumour Treatment

NRAS mutant melanoma: Towards better therapies

Tijana Randic [a,1], Ines Kozar [a,1], Christiane Margue [a,1], Jochen Utikal [b,c,2], Stephanie Kreis [a,1,*]

[a] Department of Life Sciences and Medicine, University of Luxembourg, 6, avenue du Swing, L-4367 Belvaux, Luxembourg
[b] Skin Cancer Unit, German Cancer Research Center (DKFZ), D-69120 Heidelberg, Germany
[c] Department of Dermatology, Venereology and Allergology, University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, D-68135 Mannheim, Germany

ARTICLE INFO

Keywords:
Cutaneous melanoma
NRAS mutation
MEKi/CDK4/6i dual therapy
Resistance mechanisms
New combination therapies

ABSTRACT

Genetic alterations affecting RAS proteins are commonly found in human cancers. Roughly a fourth of melanoma patients carry activating *NRAS* mutations, rendering this malignancy particularly challenging to treat. Although the development of targeted as well as immunotherapies led to a substantial improvement in the overall survival of non-$NRAS^{mut}$ melanoma patients (e.g. $BRAF^{mut}$), patients with $NRAS^{mut}$ melanomas have an overall poorer prognosis due to the high aggressiveness of RAS^{mut} tumors, lack of efficient targeted therapies or rapidly emerging resistance to existing treatments. Understanding how *NRAS*-driven melanomas develop therapy resistance by maintaining cell cycle progression and survival is crucial to develop more effective and specific treatments for this group of melanoma patients. In this review, we provide an updated summary of currently available therapeutic options for $NRAS^{mut}$ melanoma patients with a focus on combined inhibition of MAPK signaling and CDK4/6-driven cell cycle progression and mechanisms of the inevitably developing resistance to these treatments. We conclude with an outlook on the most promising novel therapeutic approaches for melanoma patients with constitutively active NRAS.

Statement of significance: An estimated 75000 patients are affected by $NRAS^{mut}$ melanoma each year and these patients still have a shorter progression-free survival than $BRAF^{mut}$ melanomas. Both intrinsic and acquired resistance occur in *NRAS*-driven melanomas once treated with single or combined targeted therapies involving MAPK and CDK4/6 inhibitors and/or checkpoint inhibiting immunotherapy. Oncolytic viruses, mRNA-based vaccinations, as well as targeted triple-agent therapy are promising alternatives, which could soon contribute to improved progression-free survival of the $NRAS^{mut}$ melanoma patient group.

Introduction

Cutaneous melanoma is a highly aggressive malignancy with a growing incidence accounting for around 290 000 cases (1.6%) of all newly diagnosed cancers worldwide and more than 60 000 deaths in 2018 [1,2]. Exogenous and endogenous risk factors cause malignant transformation of melanocytes, which are neural crest-derived and melanin-producing cells [2-4]. Exposure to ultraviolet (UV) radiation and a history of sunburns are the main carcinogens responsible for the onset of melanoma and other skin cancers, causing the highest tumor mutational burden (TMB) in somatic cells of all cancer types (median TMB of melanoma 14.4 mutations/Mb, squamous cell carcinoma 45.2, and basal cell carcinoma 47.3 mutations/Mb) [5-7]. Ageing is an additional risk factor accounting for the cumulative mutational burden under UV exposure due to deterioration of DNA repair mechanisms and modifications of cell division [8].

Regarding genetic susceptibility, several germline mutations affecting genes such as *CDKN2A*, *CDK4*, *MITF*, *TERT*, *MC1R*, and *IDH1* have been associated with an increased risk of familial cutaneous melanoma [9-11]. The most frequent germline alterations affect the *CDKN2A* locus, and pathogenic mutations in this tumor-suppressor gene coding for p14ARF and p16INK4A are found in ~20–40% of melanoma-prone families [10,12]. Based on the somatic mutational status, cutaneous melanomas are divided into four subtypes: i) B-Raf proto-oncogene serine/threonine kinase mutant ($BRAF^{mut}$), ii) NRAS proto-oncogene GTPase mutant ($NRAS^{mut}$), iii) neurofibromin 1 mutant

* Corresponding author.
E-mail addresses: tijana.randic@uni.lu (T. Randic), ines.kozar@uni.lu (I. Kozar), christiane.margue@uni.lu (C. Margue), j.utikal@Dkfz-Heidelberg.de (J. Utikal), stephanie.kreis@uni.lu (S. Kreis).
[1] 6, avenue du Swing L-4367 Belvaux.
[2] Theodor-Kutzer-Ufer 1-3, 68125 Mannheim.

https://doi.org/10.1016/j.ctrv.2021.102238
Received 26 January 2021; Received in revised form 24 May 2021; Accepted 26 May 2021
Available online 29 May 2021

(*NF1*^{mut}), and iv) none of the three, triple BRAF/NRAS/NF1 wild-type (WT) [13-15]. Genetic mutations in RAS isoforms, namely NRAS, KRAS, and HRAS, are among the most prevalent oncogenic alterations detected in around 16–25% of all cancers [16,17]. In melanomas, *KRAS* and *HRAS* mutations are detected infrequently in approximately 5% of patients, whereas *NRAS* mutations are found in ~25% of cases making *NRAS* the second most frequent mutation type, after *BRAF,* which affects ~40–45% of all cases [17-20].

Although early diagnosis and surgical removal of cutaneous melanoma indisputably improve the patient's survival rates, once melanoma metastasizes, successful treatment becomes more challenging accompanied by a dismal prognosis for most patients [21,22]. In comparison to *BRAF*^{mut} or triple WT subtypes, *NRAS*^{mut} melanomas are more aggressive resulting in lower median overall survival [23,24]. This poor survival is also due to a delayed development of targeted therapies: while the first BRAF inhibitors received FDA/EMA approval almost a decade ago, followed by the introduction of even more successful combination therapies targeting BRAF and MEK [25], therapeutic options for *NRAS*^{mut} melanoma patients lag behind. Immunotherapies represent another leap forward and recent years have seen remarkable improvement in progression-free survival (PFS) and overall survival (OS) of melanoma patients independent of mutational status [26]. However, the frequent and rapid development of drug resistance against current treatments and clinical relapse call for novel therapeutic approaches.

Here, we provide an overview of the *NRAS*^{mut} signaling pathways and the currently available therapies for melanoma patients harboring this type of mutation, followed by a summary of known mechanisms of resistance to single and combined treatments. We conclude with an up-to-date overview of ongoing clinical trials and promising novel therapeutic approaches all aiming at markedly improving the clinical outcome for *NRAS*^{mut} melanoma patients.

Wild-type NRAS signaling

RAS proteins are small intracellular GTPases and in normal human melanocytes both GTP-bound active and GDP-bound inactive states exist [27]. Receptor tyrosine kinase (RTK) signaling induces transition towards the NRAS active state that is mediated by the recruitment of guanine nucleotide exchange-factors (GEFs; e.g. SOS1, SOS2, and RASGRF10) and adaptor molecules, like growth factor receptor-bound protein 2 (GRB2) [28,29]. GTPase-activating proteins (GAPs) catalyze hydrolysis and RAS reversion to the inactive form [28,29] (Fig. 1A).

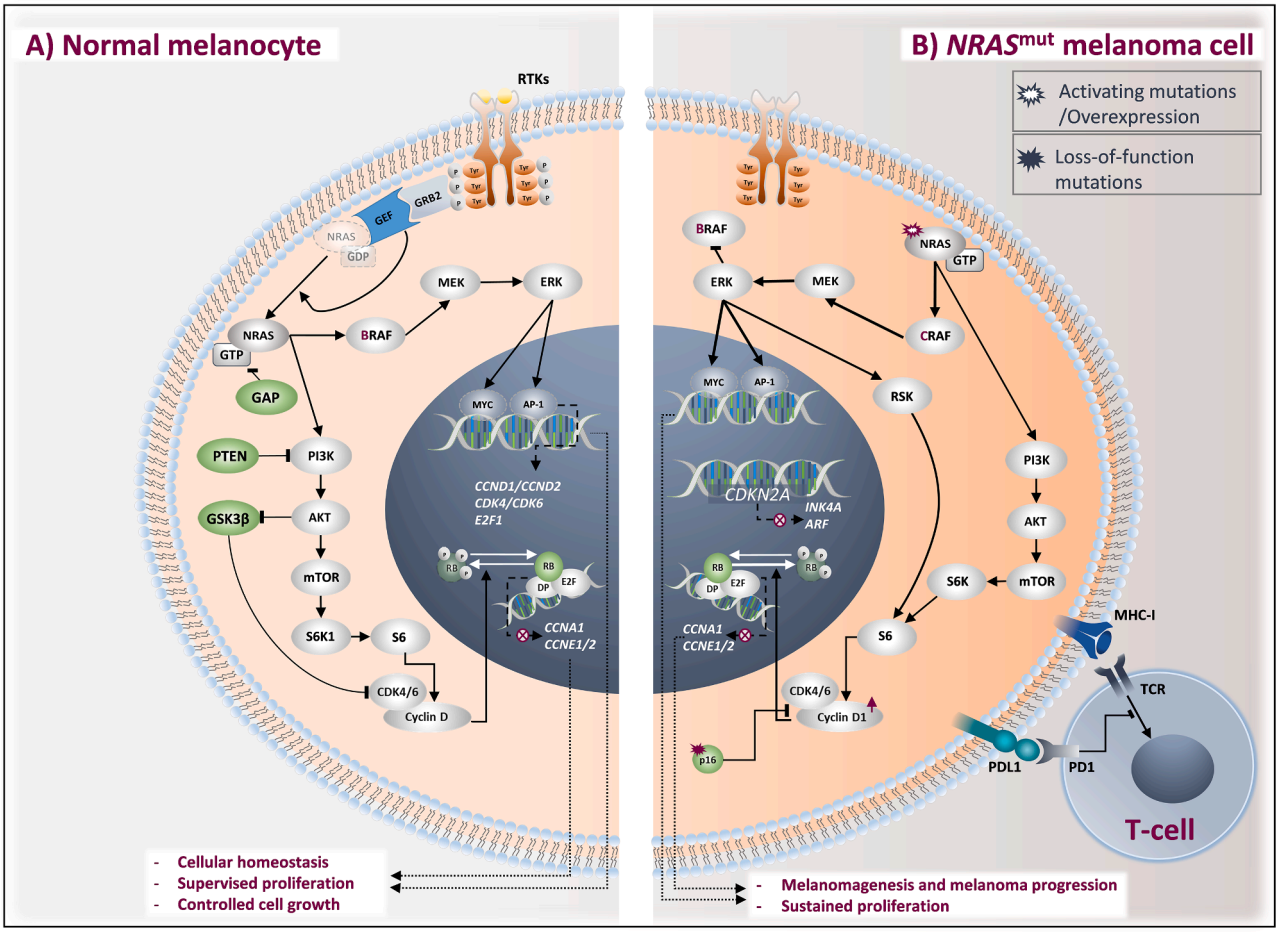

Fig. 1. Schematic representation of *NRAS*^{WT} versus *NRAS*^{mut} signaling pathways. **(A)** NRAS signaling under normal physiological conditions. Upon ligand-mediated dimerization and auto-phosphorylation of RTKs (e.g. EGFR and VEGFR, FGFR, the AXL subfamily, and c-KIT), NRAS is activated by GRB2 and GEF recruitment. Active NRAS promotes MAPK and PI3K signaling. MAPK cascade induces AP-1-mediated expression of D-type cyclins (cyclin D1, D2, and D3), supporting cell cycle progression. PI3K leads to the recruitment and activation of AKT kinase, which regulates mTOR and its downstream effector S6K, which further contributes to cell cycle progression by enhanced translation of cyclin D1. Catalytic activation of D-type cyclins and complex formation with CDK4/6 has an essential role in early G1 cell cycle commitment. **(B)** NRAS-induced constitutive activation of oncogenic signal transduction can trigger emergence and sustained melanoma progression predominantly through MAPK signaling, cyclin D1 overexpression, and p16INK4A inactivation. Additionally, melanoma cells expressing PDL1 lead to immune evasion by reducing effector T cell activity through PD1L and PD1 interaction. *** EGFR and VEGFR, epidermal and vascular endothelial growth factor receptor; FGFR, fibroblast growth factor receptor; 4E-BP1, factor 4E-binding protein 1; MHC, major histocompatibility complex; TCR, T cell receptor; PD1, programmed cell death protein 1.

Active RAS GTPases can stimulate a variety of cellular processes such as proliferation and survival, differentiation, but also apoptosis, cell–cell and cell-extracellular matrix interactions [28,30,31]. The most studied RAS-mediated downstream effector pathways are the mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) cascade [32] (Fig. 1).

Due to the high affinity of NRAS to bind to its effector BRAF in healthy melanocytes, MAPK signaling is preferably mediated by BRAF activation rather than other RAF-isoforms (e.g. CRAF) [28] (Fig. 1A). Activated RAF initiates a signaling cascade resulting in phosphorylation of extracellular signal-regulated protein kinase (ERK)1/2, which regulates approximately 500 substrates [33]. Among others, p-ERK triggers cell proliferation by stabilization of FOS and subsequent formation of activator protein 1 (AP-1) complexes, consisting of members of the FOS and JUN protein families [30,34]. AP-1 transcriptional activity induces expression of D-type cyclins and consequently supports a transition from the G1 to S phase of the cell cycle [30,34]. Cyclin D-CDK4/6 complex-mediated hyperphosphorylation and inactivation of the retinoblastoma protein (RB) releases E2F-induced transcriptional activity [35-37], which in turn leads to the expression of E-type cyclins (cyclin E1 and E2) (Fig. 1) that form complexes with CDKs (CDK1, 2, 3). CDK2 further phosphorylates RB, finally leading to G1-S cell cycle transition [35,38,39]. As mentioned above, RAS activates the PI3K pathway, which is also involved in cell cycle progression and is required for metabolic processes during the S phase of the cell cycle, while inhibition of PI3K signaling is associated with a prolonged S phase [34] (Fig. 1).

Cell signaling in NRAS^{mut} melanoma

Aberrant RAS function in *RAS*^{mut} human tumors is mainly caused by oncogenic missense mutations at codons 12, 13, or 61 [40]. *NRAS*^{Q61} occurs in 90% of all *NRAS*^{mut} melanomas and induces constitutive RAS-GTPase activity and conformational changes towards the GTP-bound active state, whereas oncogenic alterations at codons 12 or 13 impair mechanisms of GTP hydrolysis [27,32]. Interestingly, the Q61R substitution at codon 61 (*NRAS*^{Q61R}) has been shown to possess an inherently higher efficiency of tumor initiation in melanocytes than G12D at codon 12 (*NRAS*^{G12D}) [41].

In contrast to normal melanocytes, the activation of the MAPK pathway in *NRAS*^{mut} melanoma is achieved through activation of the NRAS effector CRAF rather than BRAF [28,42,43] (Fig. 1B). Although previous findings implicated both individual and concomitant activations of MAPK and PI3K pathways, a predominant signaling through the MAPK pathway has been demonstrated for *NRAS*^{mut} melanomas [28,43,44] (Fig. 1B). The downstream effector of mammalian target of rapamycin (mTOR), p70 ribosomal S6 kinase (P70S6K, also known as S6K1), as well as the downstream effector of the MAPK pathway, p90 ribosomal S6 kinase (RSK), have been shown to phosphorylate the ribosomal protein S6. Therefore, S6 represents an intersection point between the MAPK and PI3K pathways (Fig. 1). Under normal physiological conditions, S6 phosphorylation mainly depends on S6K1, whereas it is regulated by RSK in case of MAPK pathway hyperactivation [45] (Fig. 1B).

NRAS^{mut} melanomas often display a dysregulated cell cycle, which is characterized by the upregulation of cyclin D1 and loss of tumor suppressor p16INK4A [46]. Loss of p16INK4A, a specific inhibitor of the CDK4/6-cyclin D1 complex, may support *NRAS*^{mut} melanoma progression due to its dependency on CDK4/6 [41,47]. Indeed, deficiency of *INK4A* in transgenic mice expressing oncogenic *NRAS*^{Q61K} resulted in frequent formation of melanoma with short latency [48]. Inactivation or loss of p14ARF and p16INK4A compromises MDM2 inhibition resulting in elevated MDM2-mediated p53 ubiquitination [49,50]. Overall, these cell cycle-related alterations enhance CDK4/6 activity and subsequently lead to early G1-S cell cycle transition [51]. Moreover, *TP53* is commonly mutated gene in *NRAS*^{mut} melanoma, occurring in up to 17% of cases [52]. Mutated *TP53* maximizes RAS activity through hnRNPK-mediated splicing that induces the cytosine-rich exons inclusion within GAPs. Therefore, p53-mediated recruitment of the RNA-binding protein hnRNPK has been implicated in sustained GTP-binding and *RAS*^{mut} tumor growth [53].

Pharmacological approaches for treatment of *NRAS*^{mut} melanoma

Current clinical treatments

To date, the first-line treatment for surgically incurable *NRAS*^{mut} melanoma at stage III/IV is immunotherapy with checkpoint inhibitors of programmed cell death protein (anti-PD-1), nivolumab, or pembrolizumab [54]. The potency of immunotherapies in *NRAS*^{mut} melanoma treatment remains controversial. The immunosuppressive microenvironment in *NRAS*^{mut} melanoma has been shown to limit the effects of immunotherapy [55]. Still, Johnson et al. reported an overall better response to immunotherapies in patients harboring *NRAS* mutations (n = 60) versus those with *NRAS*^{wt} melanomas [56]. The recent study also demonstrated the association of longer PFS and the presence of *NRAS* mutations in melanoma patients who received anti-PD1 as a first-line monotherapy [57]. Combined anti-PD-1 and anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) blockade with ipilimumab has also been assessed in first-line administration in *NRAS*^{mut} melanoma [54] with conflicting results [55,58,59]. In another recent retrospective study, Rose and colleagues showed significantly improved PFS and OS in *NRAS*^{mut} melanoma patients treated with anti-PD1 + anti-CTLA4 co-therapy (HR 0.34, 95% CI 0.16 to 0.71 and HR 0.24, 95% CI 0.10 to 0.62) in comparison to anti-PD1 monotherapy. Therefore, the *NRAS* mutational status has been suggested as a biomarker for an immunotherapeutic regimen [60].

Second-line *NRAS*^{mut} melanoma treatment may involve MEK inhibition, yet with modest responses compared to the traditional cytostatic dacarbazine treatment [54,61]. Furthermore, for selected *NRAS*^{mut} melanoma patients, oncolytic viral therapy (talimogene laherparepvec, T-VEC) may serve as an additional treatment option in patients with injectable tumor lesions [54]. In this context, a combination of anti-PD-1 immunotherapies with oncolytic viruses yielded encouraging results for melanoma patients [62].

In both preclinical and clinical settings, different therapies targeting oncogenic signaling downstream of NRAS have been extensively researched as alternative first- or second-line treatments and are summarized below. Altogether, the therapeutic success of available drugs for *NRAS*^{mut} melanoma is limited, emphasizing the need to identify new targeted combinations that are at least as efficacious as available treatments for *BRAF*^{mut} patients.

RAS inhibitors

Attempts to develop efficient GTP-competitive drugs that directly target RAS proteins have been largely unsuccessful, due to their picomolar affinity to bind GTP and the lack of druggable pockets outside of the nucleotide-binding site [31]. Previous attempts to develop RAS inhibitors focused on restricting RAS activation and translocation to the plasma membrane by inhibiting farnesyltransferase activity, that is crucial for the post-translational modification on the CAAX motif at the RAS C-terminus, or disabling RAS interaction with prenyl-binding protein phosphodiesterase-δ (PDEδ) [31]. While some farnesyltransferase inhibitors (FTIs) showed effects when targeting *HRAS*^{mut} tumors in preclinical settings, FTIs could not effectively block NRAS- and KRAS-cell membrane association due to the NRAS/KRAS alternative prenylation by geranylgeranyl transferase type 1 [31,63]. Although *RAS* mutations have been notoriously challenging to target, recent success has been achieved in pharmacological targeting of the *KRAS*^{G12C} oncoprotein with the specific compound ARS-1620 and similar agents [31,40,64]. Moreover, regulating the upstream NRAS activity by ATP-

competitive focal adhesion kinase (FAK) inhibition is currently being evaluated in a phase I clinical trial (Table 1) (no data available yet).

Inhibitors of MAPK signaling

Despite the evident anti-tumor effects of ATP-competitive BRAFi (e.g. dabrafenib, vemurafenib, or encorafenib) in *BRAF*^{mut} melanoma, these agents paradoxically activate MAPK signaling in *RAS*^{mut} cancer cells [18,65-69]. A signaling switch from BRAF to CRAF and RAF dimerization in *RAS*^{mut} tumors have all been described as mechanisms underlying BRAFi insensitivity [42,70]. In *NRAS*^{mut} melanoma, 20% partial response (PR) and 3.7 months PFS were scored in a phase II clinical trial when using MEK targeted therapy (binimetinib) [71]. In a subsequent open-label phase III clinical study, monotherapy of advanced *NRAS*^{mut} melanoma with binimetinib resulted in 15% OR and 2.8 months median PFS, compared to 1.5 months median PFS in the dacarbazine single treatment group [61]. Patients previously treated with immunotherapy benefit more from MEK inhibition, with 5.5 months PFS compared to 1.6 months PFS in the dacarbazine chemotherapy arm [61]. Currently, clinical efficacy and safety of two novel MEK inhibitors (HL-085 and FCN-159), with an allegedly 10x higher selectivity for MEK1/2 are assessed in ongoing phase I trials (Table 1). Additionally, several clinical trials are ongoing to test ERK1/2 inhibitors in the treatment of *NRAS*^{mut} melanoma (Table 1).

Combinatorial treatments for NRAS^{mut} melanoma

It is now well established that MEK inhibition works in melanoma patients who have constitutively active MAPK signaling due to either *BRAF* or *NRAS* mutations. However, as outlined above, MEKi alone is not sufficient to provide sustained responses and significant PFS. Rapid resistance to single agent therapies hampers successful long-term treatment. Therefore, many drug combinations are currently being evaluated to enhance the therapeutic efficacy of MEKi.

Co-targeting MEK and NRAS downstream signaling pathways

CRAF-mediated RAS signaling significantly reduces the effectiveness of MEKi, suggesting that CRAF silencing in *KRAS*^{mut} and *NRAS*^{mut} tumors could improve the effects of MEKi [72]. However, Dorard *et al.* have demonstrated that *CRAF* ablation does not affect tumor progression in *NRAS*^{mut} melanoma due to a rapid switch to BRAF-driven signaling. Strikingly, upon simultaneous *CRAF* and *BRAF* ablation, resistant cells emerged, which exhibited strong ARAF-mediated ERK activation [73]. Indeed, the RAF inhibitory compound LXH254 prevents BRAF and CRAF dimerization and highlights the therapeutic option for *NRAS*^{mut} tumors but failed to inhibit ARAF [74]. Subsequently, the novel and well-tolerated pan-RAFi belvarafenib demonstrated improved anti-tumor effects in patients with advanced *RAS*^{mut}/*RAF*^{mut} solid tumors [75] (Fig. 2). Therefore, dual therapies involving pan-RAF and MEK inhibitory compounds hold promise for *NRAS*^{mut} melanoma (Fig. 2), and are currently in clinical trials [75,76] (Table 2). Indeed, combining a pan-RAFi and trametinib (MEKi) had anti-proliferative properties *in vitro*, where sensitive cells showed higher MAPK pathway dependency and down-regulation of cyclin D1 after treatment [77].

As ERK1/2-mediated MAPK pathway reactivation often occurs in MEKi-treated *NRAS*^{mut} melanoma, co-targeting MEK and ERK is a worthwhile clinical approach to support sensitivity to MAPK pathway inhibition [78,79]. Combined MEKi (AZD6244) and ERKi (VTX-11e) suppressed cyclin D1 reactivation in a synergistic manner resulting in increased apoptosis and delayed resistance compared to MEKi in combination with CDK4/6i or PI3Ki [79]. Despite these promising results, concomitant MEKi/ERKi treatment has considerable side effects and toxicities [80]. The novel ATP-competitive ERK1/2i ulixertinib was better tolerated and demonstrated anti-proliferative capacity in cancers resistant to therapies targeting the MAPK pathway [81] (Fig. 2). In addition, co-administration of MEKi/ERK5i effectively reduced the growth of *NRAS*^{mut} melanoma cells *in vitro* and *in vivo* [82].

To avoid crosstalk between the MAPK and PI3K pathways, inhibition of PI3K signaling is another promising option to prevent or delay acquired resistance to MAPKi in melanoma [44,45,83]. In both *BRAF*^{mut} and *NRAS*^{mut} melanoma, combined MAPK and PI3K/mTOR inhibition had synergistic effects *in vitro* and *in vivo* [44,45,84] (Fig. 2), and targeting the mTOR-mediated activation of S6K1 has been shown to decrease cell proliferation [45].

However, drug combinations based on horizontal inhibition of

Table 1
Targeted monotherapies for *NRAS*^{mut} melanoma in clinical trials.

Identifier	Target	Agent	Study designPatient Population	Efficacy	Time frame
NCT01320085 [71]	MEK1/2	Binimetinib (MEK162)	Untreated and pretreated AJCC disease stage advanced or patients; Non-Randomized; metastatic (IIIB to IV) *NRAS*^{mut} (n = 30) and Phase II study*BRAF*^{V600} melanoma	mPFS: 3.7 months (95% CI 2·5–5·4); PR: 20%	March 2011Ongoing
NCT01763164 [61]	MEK1/2	Binimetinib (MEK162)	Untreated and pretreated AJCC stage advanced (IIIC) or metastatic patients; Randomized; (IV) *NRAS*^{mut} melanoma (n = 269) Phase III study	mPFS: 2.8 months (95% CI 2.8–3.6); OS: 11 months	July 2013-June 2019
NCT01693068 [139]	MEK1/2	Pimasertib	Untreated patients; Locally advanced or metastatic malignant Randomized; Phase II study *NRAS*^{mut} melanoma (n = 191 (pimasertib n = 130, dacarbazine n = 61))	mPFS: 13 wks; ORR: odds ratio 2.24 (95% CI 1.00–4.98); PR: 9%; OS: 11 months	December 2012October 2016
NCT03932253 [140]	MEK1/2	FCN-159	Pretreated patients; NonRandomized; Phase Ia/Ib study	AJCC stage advanced (III or IV) melanoma harboring *NRAS* aberration (n = 37)	NA	March 2019Ongoing
NCT03973151 [141]	MEK	HL-085	Phase I/II study	AJCC stage advanced (III or IV) melanoma harboring *NRAS*^{mut} (n = 54)	PFS: 17.4 wks; ORR: 33.3% with DCR 83.3%	September 2017Ongoing
NCT00866177	MEK1/2	Selumetinib	Phase II study	Stage advanced (III or IV) *BRAF*^{mut} or *NRAS*^{mut} (n = 9) melanoma	mPFS: 2.2 months in the high pAKT cohort and 7.1 months in the low pAKT cohort	March 2009September 2013
NCT04109456	Focal adhesion kinase (FAK)	IN10018	Non-Randomized; Phase Ib study	Metastatic stage uveal melanoma and *NRAS*^{mut} melanoma (n = 52)	NA	March 2020Ongoing
NCT01781429 [78]	ERK1/2	Ulixertinib (BVD-523)	Untreated and pretreated patients; Phase I study	Advanced or metastatic malignant tumors, including *NRAS*^{mut} melanoma (n = 24)	PR: 18%	March 2013-September 2018

AJCC, American Joint Committee on Cancer; PFS, progression-free survival; PR, partial response; OS, overall survival; ORR, overall response rate; NA, not available.

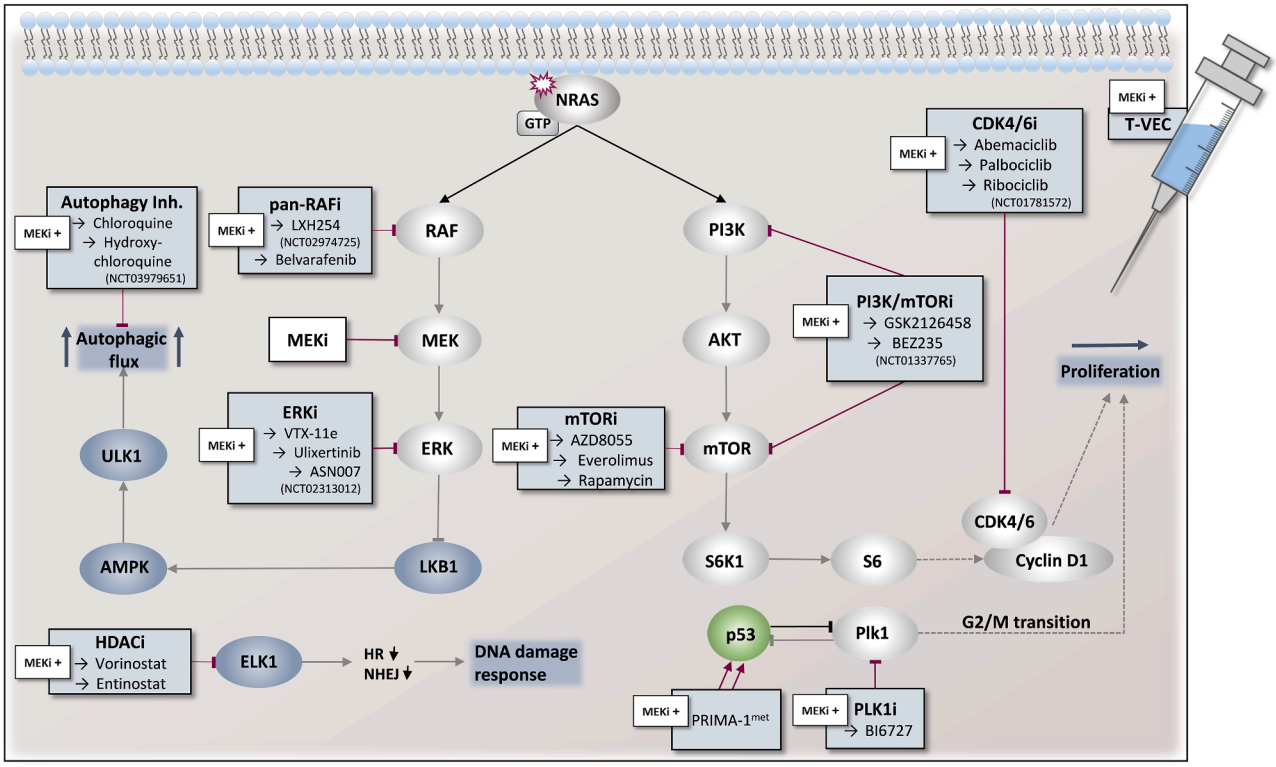

Fig. 2. Combinatorial treatments involving MEKi in *NRAS*^{mut} melanoma**.** Different compounds have been tested in pre-clinical and clinical settings to improve the effect of MEKi monotherapy. Next to the dual MAPK inhibition (MEKi/pan-RAFi), agents hindering autophagy (e.g. chloroquine), proliferation and survival (e.g. CDK4/6i; mTORi), as well as DNA repair mechanisms (e.g. HDACi), hold potential in a dual therapeutic regimen with MEKi. *** LKB1, liver kinase B1; AMPK, AMP-activated protein kinase; ULK1, unc-51-like kinase 1; ELK1, ETS Like-1 protein Elk-1; HR, homologous recombination; NHEJ, nonhomologous end-joining; **Color code:** Purple lines, Inhibitory effect of agents; Light gray, affected pathway signaling under single/dual therapy; Dashed line, indirect effect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Combined therapies with MEKi for *NRAS*^{mut} melanoma in clinical trials.

Identifier	Targets	Agents	Study design	Patient Population	Efficacy	Time frame
NCT01781572 [100]	MEK1/2 + CDK4/6	Binimetinib + LEE011 (Ribociclib)	Non-Randomized; Phase Ib/II study	Locally advanced or metastatic *NRAS*^{mut} melanoma	PR: 19.5% SD: 51.2%;	June 2013-February 2018
NCT02065063 [142]	MEK1/2 + CDK4/6	Trametinib + Palbociclib	Non-Randomized; Phase I/II study	Advanced or metastatic solid tumors including *BRAF*^{WT} cutaneous melanoma that are either *NRAS*^{WT} or *NRAS*^{mut}	NA	February 2014June 2016
NCT04109456	MEK1/2 + Focal adhesion kinase (FAK)	Cobimetinib + IN10018	Non-Randomized; Phase Ib study	Metastatic uveal melanoma or *NRAS*^{mut} (n = 52) metastatic melanoma	NA	March 2020Ongoing
NCT02974725	MEK1/2 + pan-RAF	Trametinib + LXH254	Non-Randomized; Phase Ib study	Advanced or metastatic *KRAS*^{mut}/*BRAF*^{mut} NSCLC or *NRAS*^{mut} cutaneous melanoma (n = 315)	NA	February 2017Ongoing
NCT03284502	MEK1/2 + pan-RAF	Cobimetinib + Belvarafenib (HM95573)	Non-Randomized; Phase Ib study	Locally advanced or metastatic stage solid tumors, including *NRAS*^{mut} melanoma (n = 9)	ORR: 44%	May 2017Ongoing
NCT03979651	MEK/2 + Autophagy	Trametinib + Hydroxychloroquine	Recruiting	Locally advanced or metastatic *NRAS*^{mut} melanoma	NA	October 2019Ongoing

PR, partial response; SD, stable disease; ORR, overall response rate; NA, not available.

proliferative signaling pathways in solid tumors are marked by dose-limiting toxicities that eventually influence the establishment of optimal drug concentrations [83].

Combining MEK with cell cycle inhibitors

Aberrant cell cycle regulation is a hallmark of many cancers and targeting the cyclin D-CDK4/6-RB signaling pathway and the subsequent disruption of cell cycle progression has been extensively

researched and reviewed before [38,85-89]. The frequent cell cycle alterations and the upregulation of cyclin D1 in drug-naïve and MEKi-treated *RAS*^{mut} tumors have spurred the targeting of cyclin D-CDK4/6-RB signaling as a promising anti-proliferative therapy [90-93]. Interestingly, a clinical phase II trial investigating the efficacy of the MEKi binimetinib in patients with advanced *NRAS*^{mut} melanoma showed shorter PFS (≤3.6 months) in patients who had CCND1 or CCND3 amplifications, confirming that constitutive cyclin D-CDK4/6-RB pathway activation may affect MEKi efficacy [94]. Indeed, combined MEKi/CDK4/6i treatment has been described as an effective approach across a variety of cancers, including *NRAS*^{mut} melanoma [38,92-95] (Fig. 2). Highly ATP-selective CDK4/6i, namely PD0332991 (palbociclib), LEE011 (ribociclib), and LY2835219 (abemaciclib) obtained FDA approval for anti-tumor treatment, and have been extensively investigated in melanoma therapy [38,90,93,96,97].

Concomitant MEKi/CDK4/6i promotes cell cycle arrest in G1 in drug-naïve and CDK4/6i resistant cancer cells, suggesting a role for MAPK signaling in inducing a CDK4/6i resistant phenotype [90,95]. Different preclinical studies substantiated the benefit of dual MEKi/CDK4/6i therapy versus single MEKi by monitoring its anti-tumor effects across different patient-derived *NRAS*^{mut} melanoma cell lines *in vitro*, as well as in melanoma xenografts [98]. Yet, cooperative therapy did not enhance growth regression in all *NRAS*^{mut} melanoma cell lines [98]. In this context, Hayes et al. found that, when investigating the vulnerabilities of *NRAS*^{mut} melanoma to combined MEKi (trametinib) plus CDK4/6i (palbociclib), *NRAS*-dependent cells showed a heterogeneous response to dual therapy with subpopulations undergoing both cell cycle arrest and apoptosis [99].

Encouraging results of dual MEKi/CDK4/6i in the treatment of *NRAS*^{mut} melanoma were reported in an ongoing clinical trial investigating concurrent binimetinib plus ribociclib therapy [100] (Table 2). Noteworthy, continuous single CDK4/6i therapy in clinical trials has been associated with dose-dependent toxicities, and applying additional inhibitory compounds may worsen CDK4/6i-induced side effects [101,102]. Subsequent efforts have focused on dose optimization and titration with minimal side effects and maximal therapeutic potential. Accordingly, scheduled treatment comprising continuous MEKi plus intermittent CDK4/6i has been prioritized as the best therapeutic arm when compared to continuous CDK4/6i plus intermittent MEKi or intermittent MEKi plus CDK4/6i combination therapies, leading to a complete melanoma regression in 3 out of 8 xenograft *in vivo* models [102].

Alternative ways to enhance MEKi effects

The quest for alternative compounds to accompany MEKi in combined therapies attracted great interest in recent years. Activation of the Rho/MRTF-pathway has been associated with intrinsic resistance of *NRAS*^{mut} melanoma to MEKi [103]. Cooperative engagement of MEKi and a Rho-associated coiled-coil-containing protein kinase 1 (ROCK, a serine/threonine kinase that is activated when bound to the GTP-bound form of Rho) inhibitor (GSK269962A) triggered apoptosis by increasing the expression of BIM-extra long (Bim_{EL}), poly (ADP-ribose) polymerase (PARP), and p53 upregulated modulator of apoptosis (PUMA) [104]. Synergetic effects of Rho/MRTF pathway inhibitor CCG-222740 and MEKi have been recently demonstrated in *NRAS*^{mut} melanoma cells [103].

Co-administration of MEKi with the autophagy inhibitor chloroquine (Fig. 2) has been shown to have synergistic anti-proliferative effects in treatment of *RAS*^{mut} cancers [105]. Chloroquine provokes apoptosis of melanoma cells by BH3-dependent PUMA modulation [106]. Combined trametinib and hydroxychloroquine therapy is currently in a clinical trial for *NRAS*^{mut} melanoma patients (Table 2). Furthermore, combinations of trametinib with the pan-histone deacetylase (HDAC) inhibitor vorinostat or the class I-HDACi entinostat have promising effects in preclinical settings [107] (Fig. 2).

Activation of apoptotic signaling and MEKi resistance evasion in *NRAS*^{mut} melanoma have also been achieved by direct p53 reactivation with PRIMA-1^{met} (APR-246) regardless of *TP53* mutational status and by knockdown of the anti-apoptotic protein Bcl-2 [108]. Next, the overexpression of polo-like kinase 1 (PLK1), which is required for mitotic entry and centrosome maturation in late G2 phase/early prophase, was observed in *NRAS*^{mut} melanoma and thereby points to another promising combinatorial therapy involving MEKi and PLK1i [109,110]. Indeed, MEKi/PLK1i co-therapy synergistically enhanced p53 signaling, both G1 and G2/M phase arrest of cell cycle progression, and eventually apoptosis [109].

The transition of fast-growing melanoma cells towards a slow-cycling phenotype under stress conditions has been described as a hallmark of drug resistance in *BRAF*^{mut} melanoma [111,112]. This process is also attributed to high levels of Wnt5A and subsequent expression of p53, partially accomplished by phosphorylation of MDM2 [113]. Following DNA damage, Wnt5A inhibits p53-mediated apoptotic signaling in melanoma cells through activation of the inhibitor of apoptosis stimulating protein p53 (iASPP) [113]. Webster et al. therefore proposed inhibition of p53 together with MAPK signaling in slow-cycling melanoma cells to prevent BRAFi/MEKi resistance [113]. Such an approach might be worthwhile testing in *NRAS*^{mut} melanoma.

Next, the bromodomain and extra-terminal (BET) protein family members (e.g. BRD2, BRD4) are often overexpressed in melanoma [114-116]. *NRAS*^{mut} melanoma patients with elevated expression of *BRD4* exhibited overall poorer survival in comparison to patients harboring low levels of BRD4, suggesting therapeutic criticality of BETi [114]. Therefore, concomitant *NRAS*^{mut} melanoma administration with MEKi (PD901) and BETi (OTX-015 or JQ-1) perturbed cell cycle progression of *NRAS*^{mut} melanoma cells at different phases and promoted apoptosis *in vitro* and tumor growth suppression *in vivo*. Combined MEKi/BETi therapy resulted in hypophosphorylation of RB, reduced levels of PLK1 and cyclin D1, upregulation of p27 and was accompanied by downregulation of TCF19 transcriptional activity. Notably, the unaltered activity of TCF19 upon targeting MEK/BET was characteristic for non-responding melanoma cells [114].

Simultaneous inhibition of phosphoglycerate dehydrogenase (PHGDH) and MEK in MEKi-resistant *NRAS*^{mut} melanoma cells reduced cell tolerance to oxidative stress, marked by decreased levels of oxidized glutathione, and a subsequent re-sensitization to MEKi (PD901) treatment. *NRAS*^{mut} melanoma cells that harbor higher levels of PHGDH were more prone to respond to combined PHGDHi/MEKi in comparison to cells with lower expression of this enzyme [117]. Concerning melanoma metabolic processes, we have previously shown that MAPKi treatment leads to phosphorylation of pyruvate dehydrogenase (PDH) in both *BRAF*^{mut} and *NRAS*^{mut} cell lines, through ROS production and activation of the PDH upstream repressor pyruvate dehydrogenase kinase (PDK) [118]. Interestingly, the pan-PDKi (AZD7545) also inhibited the growth of *NRAS*^{mut} cells [118].

Lastly, the combination of targeted drugs with oncolytic viruses might become a very rewarding avenue for some melanoma patients. In melanoma, concurrent MEKi and T-VEC therapy promoted T-VEC replication, viral protein production, and MEKi-induced apoptosis [119].

Other combinatorial approaches

Additional therapeutic strategies have been tested in melanoma to evade escape from cell cycle arrest initially induced by CDK4/6i. Protein arginine methyltransferase 5 (PRMT5) inhibition in combination with CDK4/6i profoundly reduced PRMT5-mediated MDM4 activity and subsequently enhanced p53 signaling in *TP53*^{wt} melanoma cells [120]. PRMT5i also increased expression of the p53 transcriptional target genes MDM2 and CDKN1A (encoding p21) in CDK4/6i resistant cells [120]. Considering activation of CDK2-cyclin E1 in CDK4/6i resistant melanoma cells, inactivation of CDK2 by MDM4-p53-induced p21 is a

promising strategy to evade melanoma drug resistance and proliferation. Therefore, concomitant CDK4/6i/PRMT5i treatment proved to be a well-tolerated curative strategy that improved CDK4/6i efficacy in both drug naïve and CDK4/6i resistant melanoma [120]. The amplification of CDK4 has been associated with simultaneous amplification of MDM2, arguing that MDM2 and CDK4 co-inhibition might be an effective anti-tumor approach [121]. Indeed, co-administration of CDK4/6i/MDM2i inhibited melanoma cell growth and remained effective even upon weeklong treatment intermissions, which was also confirmed *in vivo* [122].

Resistance mechanisms

Resisting MEK inhibition

MEKi monotherapy is only beneficial for a small subset of patients [59] as intrinsic and acquired resistance rapidly lead to MEKi insensitivity and disease relapse. Initially, MEKi induce a strong reduction of p-ERK levels in *NRAS*^{mut} melanoma; however, p-ERK signaling is rapidly restored and remains constant during drug renewal over prolonged examination periods [79,99]. Several specific mechanisms of MAPK signaling reactivation, which are driving MEKi resistance in *NRAS*^{mut} melanoma cells, have been elucidated: e.g. ERK5 was shown to be upregulated upon MEKi through platelet-derived growth factor receptor beta (PDGFRβ) signaling and was implicated in acquired drug resistance, reflected by sustained cell survival [82]. Activation of pro-survival signaling involving MITF, increased activity of Bcl-2, as well as activation of the metabolic enzyme PHGDH, have all been reported as critical nodes leading to a survival advantage of *NRAS*^{mut} melanoma under MEKi [108,117]. Recent studies have revealed several markers with prominent expression changes under MEKi in different *NRAS*^{mut} cancer cell lines (e.g. *KEAP1*, *FBXO42*, *CIC*), making them interesting candidates involved in cell fate decisions leading to resistance [99,123,124].

Inconsistent findings regarding AKT activity have been reported, suggesting both unaltered and increased p-AKT levels upon MEKi, which could be explained by the heterogeneity of melanoma cells [98,99]. In *NRAS*^{mut} melanoma cell lines expressing *AKT1*, *PI3K*, or *EGFR*, MEKi treatment did not reduce p-ERK levels, implicating that MEKi resistance is not induced by reactivation of MAPK signaling [99]. Instead, p-S6 levels were increased in response to *AKT*, *PI3K*, or *EGFR* expression upon MEKi, suggesting recovery of its upstream activator mTOR and involvement of mTOR signaling in the modulation of MEKi sensitivity [99]. Similarly, constitutive phosphorylation of S6 has been reported as a contributor to acquired resistance in MEKi-insensitive *BRAF*^{mut} melanoma [45]. Targeting S6 with siRNAs resulted in G0/G1 cell cycle arrest of resistant cells, evidenced by a strong downregulation of RB, cyclin D1, and CDK6. These findings assign an important role to S6, promoting cell cycle progression and proliferation as an underlying property when cells evade MAPK inhibition [45]. Therefore, S6K1-mediated regulation of S6 is switched from MAPK signaling in MEKi-naïve cells to PI3K signaling pathway in resistant cells [45]. In addition, inconclusive data have been published regarding the role of RB in MEKi resistance of *NRAS*^{mut} melanoma. *NRAS*^{mut} melanoma cell lines overexpressing *AKT*, *PI3K*, or *EGFR* constructs showed increased p-RB levels following MEK inhibition, although levels of p-RB varied among cell lines [99].

Resisting CDK4/6i-mediated cell cycle arrest

Responses to combined MEKi/CDK4/6i treatment provoke G1 cell cycle arrest, however, upon prolonged therapy, cancer cells cope with inhibitory stress and re-enter the cell cycle [85,99,125]. Amplification of both *CDK4* and *CDK6* has been implicated in reduced CDK4/6i sensitivity [126,127] with elevated cyclin D1 levels as an early indicator of resistance to CDK4/6i across different cancer cell lines [99,122,125,128]. During early CDK4/6i-adaptation, activated cyclin D1 directly interacts with CDK2, consequently deactivating RB by phosphorylation, which in turn induces transcriptional E2F activity and thus promoting cell cycle re-entry upon CDK4/6i therapy [38,85,128] (Fig. 3).

Given that intact RB is necessary for CDK4/6 activity, intrinsic resistance to CDK4/6i is also driven by *RB* loss in many cancer types [129], hence *RB* knockout in *NRAS*^{mut} melanoma cells resulted in resistance to CDK4/6i therapy [99]. The overexpression of *CDKN2*, mediated by CDKN2 family members (p16INK4A (*CDKN2A*), p15INK4B (*CDKN2B*), p18INK4C (*CDKN2C*), and p19INK4D (*CDKN2D*)), might also contribute to intrinsic CDK4/6i resistance, by interacting with the ATP-binding pocket of CDK4/6 [85,130]. However, there is no clear evidence that low levels or knockout of *CDKN2* enhance the binding of CDK4/6 inhibitors to their target and thus increase melanoma sensitivity to CDK4/6 inhibition [130,131].

Another escape mechanism from cell cycle arrest in CDK4/6i treated melanoma cells involves the potential of activated cyclin D1 to bind and sequester CDK inhibitors p21 and p27 [122]. Consistently, initial CDK4/6i treatment showed many p21 and cyclin E1 complexes, which are reduced in CDK4/6i resistant melanoma cells [120]. Inhibition of p21 and p27 can affect the activity of CDK1 and CDK2 to promote cell cycle progression and influence the outcome of CDK4/6i monotherapy [122]. In this context, increased CDK2 levels may result in a hyperactivated CDK2-cyclin E complex that phosphorylates RB, replacing CDK4/6 function in cell cycle progression [120]. Besides, *CCNE1* overexpression is a well-described mechanism of CDK4/6i cancer resistance [128,132]. Similar to MEKi resistance, acquired or intrinsic alterations leading to CDK4/6i resistance also involve the PI3K pathway, both *in vivo* and *in vitro* [85,99,122,128,133]. These and other potential mechanisms leading to cell cycle progression and acquired resistance to CDK4/6i have been reviewed elsewhere [85,129].

Resisting combined MEK plus CDK4/6 inhibition

Despite promising pharmacological results, the development of resistance to MEKi/CDK4/6i co-treatment in *NRAS*^{mut} melanoma remains a serious clinical issue. In a first study, following the clinical trial NCT01781572, that aimed to characterize resistance mechanisms to MEKi/CDK4/6i co-administration, whole-exome sequencing (WES) was performed on serial longitudinal biopsies (pre-, on-, and post-treatment) [134]. Post-treatment biopsy collection was conducted during follow-up anti-PD-1 treatment from both responding and non-responding lesions [134]. WES revealed a small fraction of pre-existing *PIK3CA*^{E545K} mutations at an early stage of MEK/CDK4/6 co-inhibition, suggesting a resistant subpopulation with intrinsic potential to expand under drug pressure. Functional validation of this finding confirmed that *NRAS*^{mut} melanoma cells expressing *PIK3CA*^{E545K} have reduced sensitivities to single MEKi or combined MEKi (MEK162) plus CDK4/6i (LEE011) [134].

Further, increased levels of p-S6K1 and its target ribosomal protein S6 were identified in these *NRAS*^{mut} and *PIK3CA*^{E545K} mutant melanoma cells [134]. Likewise, S6 activity has been implicated as a predictor of resistance after analyzing *BRAF*^{mut} melanoma samples derived from clinical trials (NCT01543698 and NCT02159066), which evaluated triple BRAFi/MEKi/CDK4/6i treatment. Despite a limited number of patients, there was an evident induction of p-S6 in post- versus pre-treatment patient samples with shorter therapy response. Notably, the best responses were observed in patients with low p-S6 protein levels [102]. The importance of upregulated mTOR-S6 signaling for acquired resistance to MEKi/CDK4/6i has also been confirmed in both *BRAF*^{mut} and *NRAS*^{mut} melanoma xenografts [102].

In addition, E2F transcription factor levels were elevated in melanomas resistant to combined MEKi/CDK4/6i treatment, resulting in tumor progression during drug holidays [102]. Therapeutic arms consisting of continuous CDK4/6i and intermittent MEKi, or continuous MEKi and intermittent CDK4/6i, retained stable and low E2F activity for a prolonged period but rapid E2F increase was recorded after the third

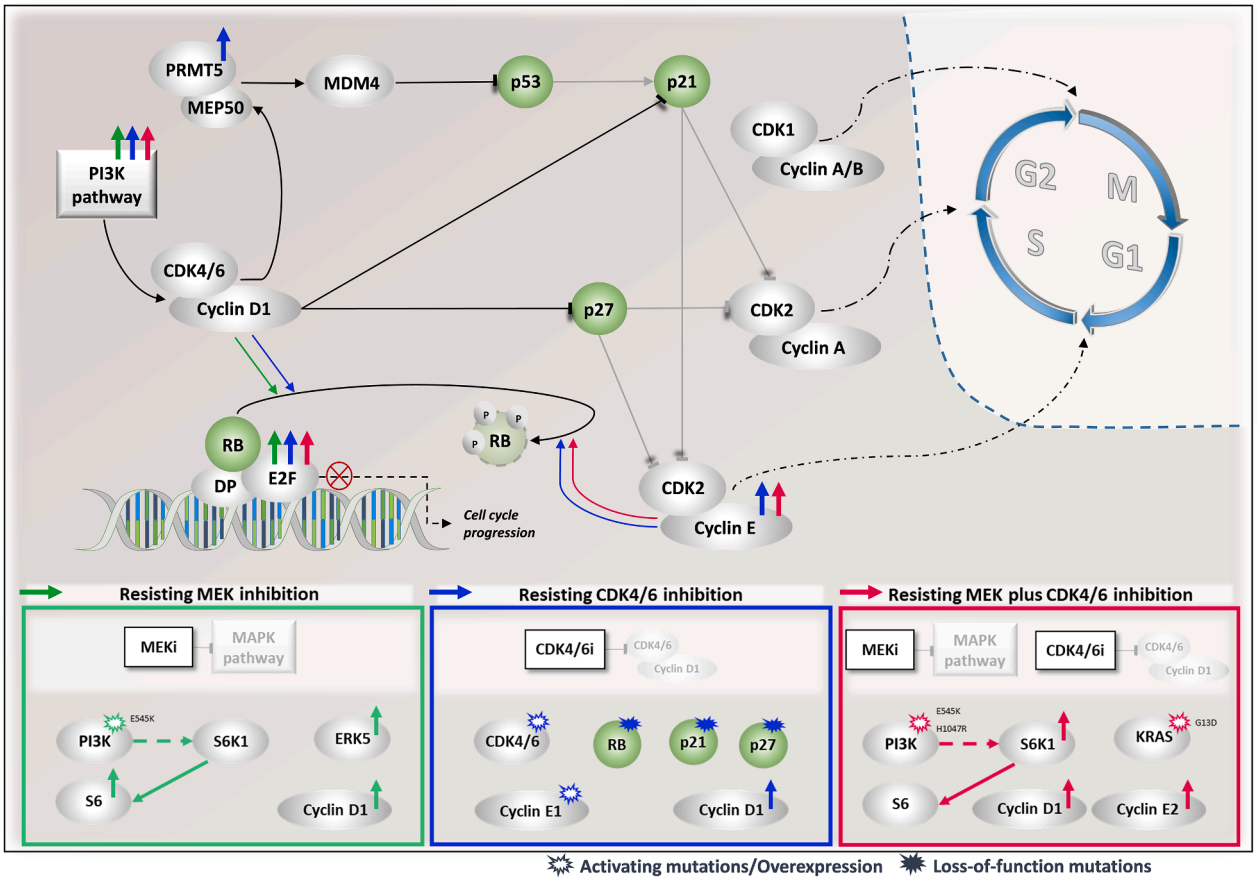

Fig. 3. Key molecular players in resistance to MEKi-, CDK4/6i-, or combined MEKi + CDK4/6i. Melanoma cells resistant to single MEKi or combined MEKi/CDK4/6i treatment often display alterations in the PI3K pathway. CDK2/cyclin E complexes have been described as one of the main promoters of cell cycle re-entrance under single CDK4/6i or dual MEKi/CDK4/6i therapy. PRMT5-mediated suppression of MDM4 protein levels by CDK4/6i in sensitive melanoma cells is disturbed in CDK4/6i-resistant melanoma settings. Color code: Light gray, affected pathway signaling under single/dual therapy; Dashed line, indirect effect.

MEKi-free phase, confirming the association of E2F activity with drug resistance [102]. Reduced survivin and p-RB levels accompanied combinatorial MEKi/CDK4/6i treatment and have therefore been suggested as potential indicators of treatment success [131]. Along these lines, Tikvah and colleagues recently showed that increased levels of p-RB, p-S6 and other cell cycle markers such as cyclin E2 and cyclin D1 were associated with resistance to MEKi/CDK4/6i in *NRAS*^{mut} melanoma [99]. Lastly, activated *KRAS*^{G13D} was among the top candidates to overcome MEKi/CDK4/6i treatment followed by *RB* loss-of-function and genes involved in MAPK and PI3K pathways [99] (Fig. 3).

Circumventing resistance to dual therapy

Melanoma cells resisting either CDK4/6i monotherapy or dual MEKi/CDK4/6i share common molecular characteristics, including frequent reactivation of CDK4/6-RB signaling and sensitivity to PI3K pathway inhibitors, making these pathways interesting pharmacological targets to prevent MEKi/CDK4/6i drug-insensitivity [102,122,131,135]. Given that inhibition of mTORC1/2 leads to decreased cyclin D1 levels, RB deactivation and elevated E2F activity in pre-clinical settings, the simultaneous CDK4/6 and PI3K pathway inhibition might be able to maintain durable repression of cancer growth [135]. In estrogen (ER)-positive breast cancer cells, combined targeting of mTORC1/2 (AZD2014) and CDK4/6 (palbociclib) suppressed E2F activity and retained a quiescent-like state by postponing transition into a resistant state [135]. Likewise, upregulated mTOR activity in both *BRAF*^{mut} and *NRAS*^{mut} melanoma cell lines resistant to MEKi/CDK4/6i co-treatment could be targeted using mTORC1/2i to delay melanoma progression. Elevated phosphorylation of S6 during MEKi/CDK4/6i co-treatment of melanoma cell lines as well as in biopsies from melanoma patients treated with MEKi/CDK4/6i, support the inclusion of mTORC1/2i in a triple treatment regimen [102]. Indeed, triple-drug approaches targeting MEK (PD'901), CDK4/6 (palbociclib), and mTORC1/2 (AZD2014) disturbed colony formation and S6 phosphorylation in MEKi/CDK4/6i-resistant melanoma, resulting in a significant increase in cell death, but treatment success might be hampered by elevated toxicities [102].

Perspectives

To this date, there is an unmet clinical need for effective therapeutic strategies for *NRAS*^{mut} melanoma patients. Considerable efforts have been invested in the inhibition of the MAPK pathway alone or in combination with other drugs. Disrupting cell cycle-progression with specific drugs in combination with MEKi appears to be particularly effective in several tumors, including *NRAS*^{mut} melanoma. Still, the emergence of intrinsic and acquired resistance in this aggressive melanoma subtype calls for innovative approaches. Novel classes of inhibitors such as the proteolysis-targeting chimera (PROTAC) with enhanced efficacy in *BRAF*^{mut} melanoma are encouraging in this respect [136]. An entirely different but exciting approach involves mRNA vaccines that stimulate a targeted immune response against personalized tumor antigens, which, when combined with checkpoint inhibition could be transforming the way we treat melanoma in the not-so-far future [137].

A focus should be placed on determining the most beneficial treatment schedules that involve the application of novel mono-, dual-, or even triple-targeted therapies in treatment-naïve patients or upon

disease relapse following checkpoint inhibition. Longer median PFS upon MEKi treatment was observed in a patient subgroup previously exposed to immunotherapy in comparison with those patients that received only MEKi, prioritizing inhibition of the MAPK pathway in a post-immunotherapy setting [61]. Neoadjuvant therapy of local metastatic lesions has recently been put forward as an encouraging approach to improve surgical excision [138]. As indicated previously, triple-drug therapy that combines dual MEKi/CDK4/6i with some of the herein mentioned targets holds promise in the treatment of *NRAS*^{mut} melanoma patients. Still, there is a need to remedy toxicities and side effects and to optimize dosing schedules. The prediction of patient subgroups that will respond to combined therapies based on their genetic profiles may facilitate *NRAS*^{mut} melanoma drug-administration. Discovering specific markers that correlate with drug sensitivity or emerging resistance will greatly aid therapeutic interventions for all melanoma patients, *BRAF*^{mut}, *NRAS*^{mut}, *NF1*^{mut}, or triple WT.

The inevitable intrinsic or acquired drug resistance and sometimes intolerable toxicities and side effects call for additional treatment avenues. Checkpoint inhibition, alone or in combination with targeted compounds only shows durable responses in a fraction of patients. Innovative concepts such as oncolytic viruses, mRNA vaccines, and/or novel classes of pharmacological compounds will have to be considered for the majority of advanced stage melanoma patients in order to have a long and well-tolerated PFS.

Funding

This work was supported by a grant from the Fondation Cancer, Luxembourg (SecMelPro grant). TR is supported by the CriTiCS DTU of the Fonds National de la Recherche (FNR), Luxembourg.

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Can. J Clin 2018;68:394–424. https://doi.org/10.3322/caac.21492.

[2] Schadendorf D, van Akkooi ACJ, Berking C, Griewank KG, Gutzmer R, Hauschild A, et al. Melanoma. Lancet (London, England) 2018;392:971–84. https://doi.org/10.1016/S0140-6736(18)31559-9.

[3] Abdel-Malek ZA, Kadekaro AL, Swope VB. Stepping up melanocytes to the challenge of UV exposure. Pigment Cell Melanoma Res 2010;23:171–86. https://doi.org/10.1111/j.1755-148X.2010.00679.x.

[4] White RM, Zon LI. Melanocytes in development, regeneration, and cancer. Cell Stem Cell 2008;3:242–52. https://doi.org/10.1016/j.stem.2008.08.005.

[5] Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat J-P, et al. A landscape of driver mutations in melanoma. Cell 2012;150:251–63. https://doi.org/10.1016/j.cell.2012.06.024.

[6] Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415–21. https://doi.org/10.1038/nature12477.

[7] Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017;9:34. https://doi.org/10.1186/s13073-017-0424-2.

[8] Lotz M, Budden T, Furney SJ, Virós A. Molecular subtype, biological sex and age shape melanoma tumour evolution. Br J Dermatol 2021;184:328–37. https://doi.org/10.1111/bjd.19128.

[9] Ticha I, Hojny J, Michalkova R, Kodet O, Krkavcova E, Hajkova N, et al. A comprehensive evaluation of pathogenic mutations in primary cutaneous melanomas, including the identification of novel loss-of-function variants. Sci Rep 2019;9:17050. https://doi.org/10.1038/s41598-019-53636-x.

[10] Casula M, Paliogiannis P, Ayala F, De Giorgi V, Stanganelli I, Mandalà M, et al. Germline and somatic mutations in patients with multiple primary melanomas: a next generation sequencing study. BMC Can 2019;19:772. https://doi.org/10.1186/s12885-019-5984-7.

[11] Robles-Espinoza CD, Roberts ND, Chen S, Leacy FP, Alexandrov LB, Pornputtapong N, et al. Germline MC1R status influences somatic mutation burden in melanoma. Nat Commun 2016;7:12064. https://doi.org/10.1038/ncomms12064.

[12] Goldstein AM, Chan M, Harland M, Hayward NK, Demenais F, Bishop DT, et al. Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. J Med Genet 2007;44:99–106. https://doi.org/10.1136/jmg.2006.043802.

[13] Genomic Classification of Cutaneous Melanoma. Cell 2015;161:1681–96. https://doi.org/10.1016/j.cell.2015.05.044.

[14] Shain AH, Bastian BC. From melanocytes to melanomas. Nat Rev Can 2016;16:345–58. https://doi.org/10.1038/nrc.2016.37.

[15] Hayward NK, Wilmott JS, Waddell N, Johansson PA, Field MA, Nones K, et al. Whole-genome landscapes of major melanoma subtypes. Nature 2017;545:175–80. https://doi.org/10.1038/nature22071.

[16] Hobbs GA, Der CJ, Rossman KL. RAS isoforms and mutations in cancer at a glance. J Cell Sci 2016;129:1287–92. https://doi.org/10.1242/jcs.182873.

[17] Prior IA, Lewis PD, Mattos C. A comprehensive survey of Ras mutations in cancer. Cancer Res 2012;72:2457–67. https://doi.org/10.1158/0008-5472.CAN-11-2612.

[18] Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvas I, Dhomen N, et al. Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell 2010;140:209–21. https://doi.org/10.1016/j.cell.2009.12.040.

[19] Heppt MV, Siepmann T, Engel J, Schubert-Fritschle G, Eckel R, Mirlach L, et al. Prognostic significance of BRAF and NRAS mutations in melanoma: a German study from routine care. BMC Can 2017;17:536. https://doi.org/10.1186/s12885-017-3529-5.

[20] Lokhandwala PM, Tseng L-H, Rodriguez E, Zheng G, Pallavajjalla A, Gocke CD, et al. Clinical mutational profiling and categorization of BRAF mutations in melanomas using next generation sequencing. BMC Can 2019;19:665. https://doi.org/10.1186/s12885-019-5864-1.

[21] Matthews NH, Li W-Q, Qureshi AA, Weinstock MA, Cho E. Epidemiology of Melanoma. In: Ward WH, Farma JM, editors., Brisbane (AU): 2017. https://doi.org/10.15586/codon.cutaneousmelanoma.2017.ch1.

[22] Mrazek AA, Chao C. Surviving cutaneous melanoma: a clinical review of follow-up practices, surveillance, and management of recurrence. Surg Clin North Am 2014;94:989–1002, vii–viii. https://doi.org/10.1016/j.suc.2014.07.003.

[23] Jakob JA, Bassett RLJ, Ng CS, Curry JL, Joseph RW, Alvarado GC, et al. NRAS mutation status is an independent prognostic factor in metastatic melanoma. Can 2012;118:4014–23. https://doi.org/10.1002/cncr.26724.

[24] Thumar J, Shahbazian D, Aziz SA, Jilaveanu LB, Kluger HM. MEK targeting in N-RAS mutated metastatic melanoma. Mol Can 2014;13:45. https://doi.org/10.1186/1476-4598-13-45.

[25] Robert C, Grob JJ, Stroyakovskiy D, Karaszewska B, Hauschild A, Levchenko E, et al. Five-year outcomes with dabrafenib plus trametinib in metastatic melanoma. N Engl J Med 2019;381:626–36. https://doi.org/10.1056/NEJMoa1904059.

[26] Larkin J, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Lao CD, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med 2019;381:1535–46. https://doi.org/10.1056/NEJMoa1910836.

[27] Simanshu DK, Nissley DV, McCormick F. RAS proteins and their regulators in human disease. Cell 2017;170:17–33. https://doi.org/10.1016/j.cell.2017.06.009.

[28] Fedorenko IV, Gibney GT, Smalley KSM. NRAS mutant melanoma: biological behavior and future strategies for therapeutic management. Oncogene 2013;32:3009–18. https://doi.org/10.1038/onc.2012.453.

[29] Buday L, Downward J. Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. Cell 1993;73:611–20. https://doi.org/10.1016/0092-8674(93)90146-h.

[30] Lim JKM, Leprivier G. The impact of oncogenic RAS on redox balance and implications for cancer development. Cell Death Dis 2019;10:955. https://doi.org/10.1038/s41419-019-2192-y.

[31] Ryan MB, Corcoran RB. Therapeutic strategies to target RAS-mutant cancers. Nat Rev Clin Oncol 2018;15:709–20. https://doi.org/10.1038/s41571-018-0105-0.

[32] Li S, Balmain A, Counter CM. A model for RAS mutation patterns in cancers: finding the sweet spot. Nat Rev Cancer 2018;18:767–77. https://doi.org/10.1038/s41568-018-0076-6.

[33] Yang L, Zheng L, Chng WJ, Ding JL. Comprehensive analysis of ERK1/2 substrates for potential combination immunotherapies. Trends Pharmacol Sci 2019;40:897–910. https://doi.org/10.1016/j.tips.2019.09.005.

[34] Benary M, Bohn S, Lüthen M, Nolis IK, Blüthgen N, Loewer A. Disentangling pro-mitotic signaling during cell cycle progression using time-resolved single-cell imaging. Cell Rep 2020;31:107514. https://doi.org/10.1016/j.celrep.2020.03.078.

[35] Harbour JW, Dean DC. The Rb/E2F pathway: expanding roles and emerging paradigms. Genes Dev 2000;14:2393–409. https://doi.org/10.1101/gad.813200.

[36] Kato J, Matsushime H, Hiebert SW, Ewen ME, Sherr CJ. Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. Genes Dev 1993;7:331–42. https://doi.org/10.1101/gad.7.3.331.

[37] Classon M, Harlow E. The retinoblastoma tumour suppressor in development and cancer. Nat Rev Cancer 2002;2:910–7. https://doi.org/10.1038/nrc950.

[38] Ingham M, Schwartz GK. Cell-cycle therapeutics come of age. J Clin Oncol Off J Am Soc Clin Oncol 2017;35:2949–59. https://doi.org/10.1200/JCO.2016.69.0032.

[39] Goel S, DeCristo MJ, McAllister SS, Zhao JJ. CDK4/6 inhibition in cancer: beyond cell cycle arrest. Trends Cell Biol 2018;28:911–25. https://doi.org/10.1016/j.tcb.2018.07.002.

[40] Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: mission possible? Nat Rev Drug Discov 2014;13:828–51. https://doi.org/10.1038/nrd4389.

[41] Burd CE, Liu W, Huynh MV, Waqas MA, Gillahan JE, Clark KS, et al. Mutation-specific RAS oncogenicity explains NRAS codon 61 selection in melanoma. Cancer Discov 2014;4:1418–29. https://doi.org/10.1158/2159-8290.CD-14-0729.

[42] Dumaz N, Hayward R, Martin J, Ogilvie L, Hedley D, Curtin JA, et al. In melanoma, RAS mutations are accompanied by switching signaling from BRAF to CRAF and disrupted cyclic AMP signaling. Can Res 2006;66:9483–91. https://doi.org/10.1158/0008-5472.CAN-05-4227.
[43] Marquette A, André J, Bagot M, Bensussan A, Dumaz N. ERK and PDE4 cooperate to induce RAF isoform switching in melanoma. Nat Struct Mol Biol 2011;18:584–91. https://doi.org/10.1038/nsmb.2022.
[44] Posch C, Moslehi H, Feeney L, Green GA, Ebaee A, Feichtenschlager V, et al. Combined targeting of MEK and PI3K/mTOR effector pathways is necessary to effectively inhibit NRAS mutant melanoma in vitro and in vivo. Proc Natl Acad Sci USA 2013;110:4015–20. https://doi.org/10.1073/pnas.1216013110.
[45] Gao M-Z, Wang H-B, Chen X-L, Cao W-T, Fu L, Li Y, et al. Aberrant modulation of ribosomal protein S6 phosphorylation confers acquired resistance to MAPK pathway inhibitors in BRAF-mutant melanoma. Acta Pharmacol Sin 2019;40:268–78. https://doi.org/10.1038/s41401-018-0020-z.
[46] Sheppard KE, McArthur GA. The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma. Clin Can Res Off J Am Assoc Can Res 2013;19:5320–8. https://doi.org/10.1158/1078-0432.CCR-13-0259.
[47] Kwong LN, Costello JC, Liu H, Jiang S, Helms TL, Langsdorf AE, et al. Oncogenic NRAS signaling differentially regulates survival and proliferation in melanoma. Nat Med 2012;18:1503–10. https://doi.org/10.1038/nm.2941.
[48] Ackermann J, Frutschi M, Kaloulis K, McKee T, Trumpp A, Beermann F. Metastasizing melanoma formation caused by expression of activated N-RasQ61K on an INK4a-deficient background. Cancer Res 2005;65:4005–11. https://doi.org/10.1158/0008-5472.CAN-04-2970.
[49] Stott FJ, Bates S, James MC, McConnell BB, Starborg M, Brookes S, et al. The alternative product from the human CDKN2A locus, p14(ARF), participates in a regulatory feedback loop with p53 and MDM2. EMBO J 1998;17:5001–14. https://doi.org/10.1093/emboj/17.17.5001.
[50] Shattuck-Brandt RL, Chen S-C, Murray E, Johnson CA, Crandall H, O'Neal JF, et al. Metastatic melanoma patient-derived xenografts respond to MDM2 inhibition as a single agent or in combination with BRAF/MEK inhibition. Clin Can Res Off J Am Assoc Can Res 2020;26:3803–18. https://doi.org/10.1158/1078-0432.CCR-19-1895.
[51] Li A, Ma Y, Jin M, Mason S, Mort RL, Blyth K, et al. Activated mutant NRas(Q61K) drives aberrant melanocyte signaling, survival, and invasiveness via a Rac1-dependent mechanism. J Invest Dermatol 2012;132:2610–21. https://doi.org/10.1038/jid.2012.186.
[52] Siroy AE, Boland GM, Milton DR, Roszik J, Frankian S, Malke J, et al. Beyond BRAF(V600): clinical mutation panel testing by next-generation sequencing in advanced melanoma. J Invest Dermatol 2015;135:508–15. https://doi.org/10.1038/jid.2014.366.
[53] Escobar-Hoyos LF, Penson A, Kannan R, Cho H, Pan C-H, Singh RK, et al. Altered RNA splicing by mutant p53 activates oncogenic RAS signaling in pancreatic cancer. Can Cell 2020;38:198–211.e8. https://doi.org/10.1016/j.ccell.2020.05.010.
[54] Michielin O, van Akkooi ACJ, Ascierto PA, Dummer R, Keilholz U. Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up†. Ann Oncol Off J Eur Soc Med Oncol 2019;30:1884–901. https://doi.org/10.1093/annonc/mdz411.
[55] Thomas NE, Edmiston SN, Alexander A, Groben PA, Parrish E, Kricker A, et al. Association between NRAS and BRAF mutational status and melanoma-specific survival among patients with higher-risk primary melanoma. JAMA Oncol 2015;1:359–68. https://doi.org/10.1001/jamaoncol.2015.0493.
[56] Johnson DB, Lovly CM, Flavin M, Panageas KS, Ayers GD, Zhao Z, et al. Impact of NRAS mutations for patients with advanced melanoma treated with immune therapies. Cancer Immunol Res 2015;3:288–95. https://doi.org/10.1158/2326-6066.CIR-14-0207.
[57] Guida M, Bartolomeo N, Quaglino P, Madonna G, Pigozzo J, Di Giacomo AM, et al. No Impact of NRAS Mutation on Features of Primary and Metastatic Melanoma or on Outcomes of Checkpoint Inhibitor Immunotherapy: An Italian Melanoma Intergroup (IMI) Study. Cancers (Basel) 2021;13. https://doi.org/10.3390/cancers13030475.
[58] Lee J, Lee SJ, Kim K, Kim ST, Jang K-T, Lee J. Comprehensive molecular and clinical characterization of Asian melanoma patients treated with anti-PD-1 antibody. BMC Cancer 2019;19:805. https://doi.org/10.1186/s12885-019-6030-5.
[59] Kirchberger MC, Ugurel S, Mangana J, Heppt MV, Eigentler TK, Berking C, et al. MEK inhibition may increase survival of NRAS-mutated melanoma patients treated with checkpoint blockade: Results of a retrospective multicentre analysis of 364 patients. Eur J Cancer 2018;98:10–6. https://doi.org/10.1016/j.ejca.2018.04.010.
[60] Rose AAN, Armstrong SM, Hogg D, Butler MO, Saibil SD, Arteaga DP, et al. Biologic subtypes of melanoma predict survival benefit of combination anti-PD1+anti-CTLA4 immune checkpoint inhibitors versus anti-PD1 monotherapy. J Immunother Cancer 2021;9. https://doi.org/10.1136/jitc-2020-001642.
[61] Dummer R, Schadendorf D, Ascierto PA, Arance A, Dutriaux C, Di Giacomo AM, et al. Binimetinib versus dacarbazine in patients with advanced NRAS-mutant melanoma (NEMO): a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol 2017;18:435–45. https://doi.org/10.1016/S1470-2045(17)30180-8.
[62] Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RHI, Michielin O, et al. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. Cell 2017;170:1109–1119.e10. https://doi.org/10.1016/j.cell.2017.08.027.
[63] Whyte DB, Kirschmeier P, Hockenberry TN, Nunez-Oliva I, James L, Catino JJ, et al. K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. J Biol Chem 1997;272:14459–64. https://doi.org/10.1074/jbc.272.22.14459.
[64] Janes MR, Zhang J, Li L-S, Hansen R, Peters U, Guo X, et al. Targeting KRAS mutant cancers with a covalent G12C-specific inhibitor. Cell 2018;172:578–589.e17. https://doi.org/10.1016/j.cell.2018.01.006.
[65] Dummer R, Hauschild A, Lindenblatt N, Pentheroudakis G, Keilholz U. Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol Off J Eur Soc Med Oncol 2015;26(Suppl 5):v126–32. https://doi.org/10.1093/annonc/mdv297.
[66] Maio M, Lewis K, Demidov L, Mandalà M, Bondarenko I, Ascierto PA, et al. Adjuvant vemurafenib in resected, BRAF(V600) mutation-positive melanoma (BRIM8): a randomised, double-blind, placebo-controlled, multicentre, phase 3 trial. Lancet Oncol 2018;19:510–20. https://doi.org/10.1016/S1470-2045(18)30106-2.
[67] Long GV, Flaherty KT, Stroyakovskiy D, Gogas H, Levchenko E, de Braud F, et al. Dabrafenib plus trametinib versus dabrafenib monotherapy in patients with metastatic BRAF V600E/K-mutant melanoma: long-term survival and safety analysis of a phase 3 study. Ann Oncol Off J Eur Soc Med Oncol 2017;28:1631–9. https://doi.org/10.1093/annonc/mdx176.
[68] Koelblinger P, Thuerigen O, Dummer R. Development of encorafenib for BRAF-mutated advanced melanoma. Curr Opin Oncol 2018;30:125–33. https://doi.org/10.1097/CCO.0000000000000426.
[69] Oberholzer PA, Kee D, Dziunycz P, Sucker A, Kamsukom N, Jones R, et al. RAS mutations are associated with the development of cutaneous squamous cell tumors in patients treated with RAF inhibitors. J Clin Oncol Off J Am Soc Clin Oncol 2012;30:316–21. https://doi.org/10.1200/JCO.2011.36.7680.
[70] Vakana E, Pratt S, Blosser W, Dowless M, Simpson N, Yuan X-J, et al. LY3009120, a panRAF inhibitor, has significant anti-tumor activity in BRAF and KRAS mutant preclinical models of colorectal cancer. Oncotarget 2017;8:9251–66. https://doi.org/10.18632/oncotarget.14002.
[71] Ascierto PA, Schadendorf D, Berking C, Agarwala SS, van Herpen CM, Queirolo P, et al. MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study. Lancet Oncol 2013;14:249–56. https://doi.org/10.1016/S1470-2045(13)70024-X.
[72] Lito P, Saborowski A, Yue J, Solomon M, Joseph E, Gadal S, et al. Disruption of CRAF-mediated MEK activation is required for effective MEK inhibition in KRAS mutant tumors. Cancer Cell 2014;25:697–710. https://doi.org/10.1016/j.ccr.2014.03.011.
[73] Dorard C, Estrada C, Barbotin C, Larcher M, Garancher A, Leloup J, et al. RAF proteins exert both specific and compensatory functions during tumour progression of NRAS-driven melanoma. Nat Commun 2017;8:15262. https://doi.org/10.1038/ncomms15262.
[74] Monaco K-A, Delach S, Yuan J, Mishina Y, Fordjour P, Labrot E, et al. LXH254, a potent and selective ARAF-sparing inhibitor of BRAF and CRAF for the treatment of MAPK-driven tumors. Clin Cancer Res an Off J Am Assoc Cancer Res 2021;27:2061–73. https://doi.org/10.1158/1078-0432.CCR-20-2563.
[75] Kim TW, Lee J, Shin SJ, Kim J-S, Kim YJ, Han HS, et al. Belvarafenib, a novel pan-RAF inhibitor, in solid tumor patients harboring BRAF, KRAS, or NRAS mutations: Phase I study. 2019.
[76] Hong YS, Lee J, Han HS, Lee SJ, Kim J-S, Ryoo B-Y, et al. First-in-human study of HM95573, a novel oral RAF inhibitor, in patients with solid tumors. 2016.
[77] Atefi M, Titz B, Avramis E, Ng C, Wong DJL, Lassen A, et al. Combination of pan-RAF and MEK inhibitors in NRAS mutant melanoma. Mol Cancer 2015;14:27. https://doi.org/10.1186/s12943-015-0293-5.
[78] Sullivan RJ, Infante JR, Janku F, Wong DJL, Sosman JA, Keedy V, et al. First-in-class ERK1/2 inhibitor ulixertinib (BVD-523) in patients with MAPK mutant advanced solid tumors: results of a phase I dose-escalation and expansion study. Cancer Discov 2018;8:184–95. https://doi.org/10.1158/2159-8290.CD-17-1119.
[79] Rebecca VW, Alicea GM, Paraiso KHT, Lawrence H, Gibney GT, Smalley KSM. Vertical inhibition of the MAPK pathway enhances therapeutic responses in NRAS-mutant melanoma. Pigment Cell Melanoma Res 2014;27:1154–8. https://doi.org/10.1111/pcmr.12303.
[80] Merchant M, Moffat J, Schaefer G, Chan J, Wang X, Orr C, et al. Combined MEK and ERK inhibition overcomes therapy-mediated pathway reactivation in RAS mutant tumors. PLoS ONE 2017;12:e0185862. https://doi.org/10.1371/journal.pone.0185862.
[81] Germann UA, Furey BF, Markland W, Hoover RR, Aronov AM, Roix JJ, et al. Targeting the MAPK signaling pathway in cancer: promising preclinical activity with the novel selective ERK1/2 inhibitor BVD-523 (Ulixertinib). Mol Can Ther 2017;16:2351–63. https://doi.org/10.1158/1535-7163.MCT-17-0456.
[82] Adam C, Fusi L, Weiss N, Goller SG, Meder K, Frings VG, et al. Efficient suppression of NRAS-driven melanoma by co-inhibition of ERK1/2 and ERK5 MAPK pathways. J Invest Dermatol 2020. https://doi.org/10.1016/j.jid.2020.03.972.
[83] Tolcher AW, Peng W, Calvo E. Rational approaches for combination therapy strategies targeting the MAP kinase pathway in solid tumors. Mol Cancer Ther 2018;17:3–16. https://doi.org/10.1158/1535-7163.MCT-17-0349.
[84] Kiessling MK, Curioni-Fontecedro A, Samaras P, Lang S, Scharl M, Aguzzi A, et al. Targeting the mTOR complex by everolimus in NRAS mutant neuroblastoma. PLoS ONE 2016;11:e0147682. https://doi.org/10.1371/journal.pone.0147682.
[85] Álvarez-Fernández M, Malumbres M. Mechanisms of Sensitivity and Resistance to CDK4/6 inhibition. Can Cell 2020;37:514–29. https://doi.org/10.1016/j.ccell.2020.03.010.
[86] Klein ME, Kovatcheva M, Davis LE, Tap WD, Koff A. CDK4/6 inhibitors: the mechanism of action may not be as simple as once thought. Can Cell 2018;34:9–20. https://doi.org/10.1016/j.ccell.2018.03.023.

[87] Chaikovsky AC, Sage J. Beyond the cell cycle: enhancing the immune surveillance of tumors via CDK4/6 inhibition. Mol Can. Res 2018;16:1454–7. https://doi.org/10.1158/1541-7786.MCR-18-0201.

[88] Miller DM, Flaherty KT. Cyclin-dependent kinases as therapeutic targets in melanoma. Pigment Cell Melanoma Res 2014;27:351–65. https://doi.org/10.1111/pcmr.12211.

[89] Margue C, Philippidou D, Kozar I, Cesi G, Felten P, Kulms D, et al. Kinase inhibitor library screening identifies synergistic drug combinations effective in sensitive and resistant melanoma cells. J Exp Clin Cancer Res 2019;38:56. https://doi.org/10.1186/s13046-019-1038-x.

[90] Zhou J, Zhang S, Chen X, Zheng X, Yao Y, Lu G, et al. Palbociclib, a selective CDK4/6 inhibitor, enhances the effect of selumetinib in RAS-driven non-small cell lung cancer. Can Lett 2017;408:130–7. https://doi.org/10.1016/j.canlet.2017.08.031.

[91] Yadav V, Burke TF, Huber L, Van Horn RD, Zhang Y, Buchanan SG, et al. The CDK4/6 inhibitor LY2835219 overcomes vemurafenib resistance resulting from MAPK reactivation and cyclin D1 upregulation. Mol Can Ther 2014;13:2253–63. https://doi.org/10.1158/1535-7163.MCT-14-0257.

[92] Tao Z, Le Blanc JM, Wang C, Zhan T, Zhuang H, Wang P, et al. Coadministration of trametinib and palbociclib radiosensitizes KRAS-mutant non-small cell lung cancers in vitro and in vivo. Clin Can Res an Off J Am Assoc Cancer Res 2016;22:122–33. https://doi.org/10.1158/1078-0432.CCR-15-0589.

[93] de Leeuw R, McNair C, Schiewer MJ, Neupane NP, Brand LJ, Augello MA, et al. MAPK reliance via acquired CDK4/6 inhibitor resistance in cancer. Clin Cancer Res an Off J Am Assoc Cancer Res 2018;24:4201–14. https://doi.org/10.1158/1078-0432.CCR-18-0410.

[94] van Herpen CML, Agarwala SS, Hauschild A, Berking C, Beck JT, Schadendorf D, et al. Biomarker results from a phase II study of MEK1/2 inhibitor binimetinib (MEK162) in patients with advanced NRAS- or BRAF-mutated melanoma. Oncotarget 2019;10:1850–9. https://doi.org/10.18632/oncotarget.26753.

[95] Hart LS, Rader J, Raman P, Batra V, Russell MR, Tsang M, et al. Preclinical therapeutic synergy of MEK1/2 and CDK4/6 inhibition in neuroblastoma. Clin Can Res Off J Am Assoc Can Res 2017;23:1785–96. https://doi.org/10.1158/1078-0432.CCR-16-1131.

[96] Tripathy D, Bardia A, Sellers WR. Ribociclib (LEE011): mechanism of action and clinical impact of this selective cyclin-dependent kinase 4/6 inhibitor in various solid tumors. Clin Can Res Off J Am Assoc Can Res 2017;23:3251–62. https://doi.org/10.1158/1078-0432.CCR-16-3157.

[97] Barroso-Sousa R, Shapiro GI, Tolaney SM. Clinical development of the CDK4/6 inhibitors ribociclib and abemaciclib in breast cancer. Breast Care (Basel) 2016;11:167–73. https://doi.org/10.1159/000447284.

[98] Posch C, Sanlorenzo M, Ma J, Kim ST, Zekhtser M, Ortiz-Urda S. MEK/CDK4,6 co-targeting is effective in a subset of NRAS, BRAF and "wild type" melanomas. Oncotarget 2018;9:34990–5. https://doi.org/10.18632/oncotarget.26204.

[99] Hayes TK, Luo F, Cohen O, Goodale AB, Lee Y, Pantel S, et al. A functional landscape of resistance to MEK1/2 and CDK4/6 inhibition in NRAS-mutant melanoma. Cancer Res 2019;79:2352–66. https://doi.org/10.1158/0008-5472.CAN-18-2711.

[100] Schuler MH, Ascierto PA, De Vos FYFL, Postow MA, Van Herpen CM-, Carlino MS, et al. Phase 1b/2 trial of ribociclib+ binimetinib in metastatic NRAS-mutant melanoma. Safety 2017. efficacy, and recommended phase 2 dose (RP2D).

[101] Schwartz GK, LoRusso PM, Dickson MA, Randolph SS, Shaik MN, Wilner KD, et al. Phase I study of PD 0332991, a cyclin-dependent kinase inhibitor, administered in 3-week cycles (Schedule 2/1). Br J Cancer 2011;104:1862–8. https://doi.org/10.1038/bjc.2011.177.

[102] Teh JLF, Cheng PF, Purwin TJ, Nikbakht N, Patel P, Chervoneva I, et al. In vivo E2F reporting reveals efficacious schedules of MEK1/2-CDK4/6 targeting and mTOR-S6 resistance mechanisms. Cancer Discov 2018;8:568–81. https://doi.org/10.1158/2159-8290.CD-17-0699.

[103] Appleton KM, Palsuledesai CC, Misek SA, Blake M, Zagorski J, Gallo KA, et al. Inhibition of the Myocardin-Related Transcription Factor Pathway Increases Efficacy of Trametinib in NRAS-Mutant Melanoma Cell Lines. Cancers (Basel) 2021;13. https://doi.org/10.3390/cancers13092012.

[104] Vogel CJ, Smit MA, Maddalo G, Possik PA, Sparidans RW, van der Burg SH, et al. Cooperative induction of apoptosis in NRAS mutant melanoma by inhibition of MEK and ROCK. Pigment Cell Melanoma Res 2015;28:307–17. https://doi.org/10.1111/pcmr.12364.

[105] Kinsey CG, Camolotto SA, Boespflug AM, Guillen KP, Foth M, Truong A, et al. Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers. Nat Med 2019;25:620–7. https://doi.org/10.1038/s41591-019-0367-9.

[106] Lakhter AJ, Sahu RP, Sun Y, Kaufmann WK, Androphy EJ, Travers JB, et al. Chloroquine promotes apoptosis in melanoma cells by inhibiting BH3 domain-mediated PUMA degradation. J Invest Dermatol 2013;133:2247–54. https://doi.org/10.1038/jid.2013.56.

[107] Maertens O, Kuzmickas R, Manchester HE, Emerson CE, Gavin AG, Guild CJ, et al. MAPK pathway suppression unmasks latent DNA repair defects and confers a chemical synthetic vulnerability in BRAF-, NRAS-, and NF1-mutant melanomas. Cancer Discov 2019;9:526–45. https://doi.org/10.1158/2159-8290.CD-18-0879.

[108] Najem A, Krayem M, Salès F, Hussein N, Badran B, Robert C, et al. P53 and MITF/Bcl-2 identified as key pathways in the acquired resistance of NRAS-mutant melanoma to MEK inhibition. Eur J Cancer 2017;83:154–65. https://doi.org/10.1016/j.ejca.2017.06.033.

[109] Posch C, Cholewa BD, Vujic I, Sanlorenzo M, Ma J, Kim ST, et al. Combined inhibition of MEK and Plk1 Has synergistic antitumor activity in NRAS mutant melanoma. J Invest Dermatol 2015;135:2475–83. https://doi.org/10.1038/jid.2015.198.

[110] Lane HA, Nigg EA. Antibody microinjection reveals an essential role for human polo-like kinase 1 (Plk1) in the functional maturation of mitotic centrosomes. J Cell Biol 1996;135:1701–13. https://doi.org/10.1083/jcb.135.6.1701.

[111] Rambow F, Marine J-C, Goding CR. Melanoma plasticity and phenotypic diversity: therapeutic barriers and opportunities. Genes Dev 2019;33:1295–318. https://doi.org/10.1101/gad.329771.119.

[112] Kozar I, Margue C, Rothengatter S, Haan C, Kreis S. Many ways to resistance: How melanoma cells evade targeted therapies. Biochim Biophys Acta Rev Cancer 2019;1871:313–22. https://doi.org/10.1016/j.bbcan.2019.02.002.

[113] Webster MR, Fane ME, Alicea GM, Basu S, Kossenkov AV, Marino GE, et al. Paradoxical role for wild-type p53 in driving therapy resistance in melanoma. Mol Cell 2020;77:633–644.e5. https://doi.org/10.1016/j.molcel.2019.11.009.

[114] Echevarría-Vargas IM, Reyes-Uribe PI, Guterres AN, Yin X, Kossenkov A V, Liu Q, et al. Co-targeting BET and MEK as salvage therapy for MAPK and checkpoint inhibitor-resistant melanoma. EMBO Mol Med 2018;10. https://doi.org/10.15252/emmm.201708446.

[115] Tiago M, Capparelli C, Erkes DA, Purwin TJ, Heilman SA, Berger AC, et al. Targeting BRD/BET proteins inhibits adaptive kinome upregulation and enhances the effects of BRAF/MEK inhibitors in melanoma. Br J Can 2020;122:789–800. https://doi.org/10.1038/s41416-019-0724-y.

[116] Segura MF, Fontanals-Cirera B, Gaziel-Sovran A, Guijarro MV, Hanniford D, Zhang G, et al. BRD4 sustains melanoma proliferation and represents a new target for epigenetic therapy. Can Res 2013;73:6264–76. https://doi.org/10.1158/0008-5472.CAN-13-0122-T.

[117] Nguyen MQ, Teh JLF, Purwin TJ, Chervoneva I, Davies MA, Nathanson KL, et al. Targeting PHGDH upregulation reduces glutathione levels and re-sensitizes resistant NRAS mutant melanoma to MEK inhibition. J Invest Dermatol 2020. https://doi.org/10.1016/j.jid.2020.02.047.

[118] Cesi G, Walbrecq G, Zimmer A, Kreis S, Haan C. ROS production induced by BRAF inhibitor treatment rewires metabolic processes affecting cell growth of melanoma cells. Mol Can 2017;16:102. https://doi.org/10.1186/s12943-017-0667-y.

[119] Bommareddy PK, Aspromonte S, Zloza A, Rabkin SD, Kaufman HL. MEK inhibition enhances oncolytic virus immunotherapy through increased tumor cell killing and T cell activation. Sci Transl Med 2018;10. https://doi.org/10.1126/scitranslmed.aau0417.

[120] AbuHammad S, Cullinane C, Martin C, Bacolas Z, Ward T, Chen H, et al. Regulation of PRMT5-MDM4 axis is critical in the response to CDK4/6 inhibitors in melanoma. Proc Natl Acad Sci U S A 2019;116:17990–8000. https://doi.org/10.1073/pnas.1901323116.

[121] Muthusamy V, Hobbs C, Nogueira C, Cordon-Cardo C, McKee PH, Chin L, et al. Amplification of CDK4 and MDM2 in malignant melanoma. Genes Chromosomes Cancer 2006;45:447–54. https://doi.org/10.1002/gcc.20310.

[122] Vilgelm AE, Saleh N, Shattuck-Brandt R, Riemenschneider K, Slesur L, Chen S-C, et al. MDM2 antagonists overcome intrinsic resistance to CDK4/6 inhibition by inducing p21. Sci Transl Med 2019;11. https://doi.org/10.1126/scitranslmed.aav7171.

[123] Krall EB, Wang B, Munoz DM, Ilic N, Raghavan S, Niederst MJ, et al. KEAP1 loss modulates sensitivity to kinase targeted therapy in lung cancer. Elife 2017;6. https://doi.org/10.7554/eLife.18970.

[124] Nagler A, Vredevoogd DW, Alon M, Cheng PF, Trabish S, Kalaora S, et al. A genome-wide CRISPR screen identifies FBXO42 involvement in resistance toward MEK inhibition in NRAS-mutant melanoma. Pigment Cell Melanoma Res 2020;33:334–44. https://doi.org/10.1111/pcmr.12825.

[125] Garrido-Castro AC, Goel S. CDK4/6 inhibition in breast cancer: mechanisms of response and treatment failure. Curr Breast Cancer Rep 2017;9:26–33. https://doi.org/10.1007/s12609-017-0232-0.

[126] Yang C, Li Z, Bhatt T, Dickler M, Giri D, Scaltriti M, et al. Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. Oncogene 2017;36:2255–64. https://doi.org/10.1038/onc.2016.379.

[127] Olanich ME, Sun W, Hewitt SM, Abdullaev Z, Pack SD, Barr FG. CDK4 amplification reduces sensitivity to CDK4/6 inhibition in fusion-positive rhabdomyosarcoma. Clin Cancer Res an Off J Am Assoc Cancer Res 2015;21:4947–59. https://doi.org/10.1158/1078-0432.CCR-14-2955.

[128] Herrera-Abreu MT, Palafox M, Asghar U, Rivas MA, Cutts RJ, Garcia-Murillas I, et al. Early adaptation and acquired resistance to CDK4/6 inhibition in estrogen receptor-positive breast cancer. Cancer Res 2016;76:2301–13. https://doi.org/10.1158/0008-5472.CAN-15-0728.

[129] Knudsen ES, Witkiewicz AK. The strange case of CDK4/6 inhibitors: mechanisms, resistance, and combination strategies. Trends in Cancer 2017;3:39–55. https://doi.org/10.1016/j.trecan.2016.11.006.

[130] Green JL, Okerberg ES, Sejd J, Palafox M, Monserrat L, Alemayehu S, et al. Direct CDKN2 modulation of CDK4 alters target engagement of CDK4 inhibitor drugs. Mol Can Ther 2019;18:771–9. https://doi.org/10.1158/1535-7163.MCT-18-0755.

[131] Teh JLF, Purwin TJ, Greenawalt EJ, Chervoneva I, Goldberg A, Davies MA, et al. An in vivo reporter to quantitatively and temporally analyze the effects of CDK4/6 inhibitor-based therapies in melanoma. Can Res 2016;76:5455–66. https://doi.org/10.1158/0008-5472.CAN-15-3384.

[132] Min A, Kim JE, Kim Y-J, Lim JM, Kim S, Kim JW, et al. Cyclin E overexpression confers resistance to the CDK4/6 specific inhibitor palbociclib in gastric cancer cells. Can Lett 2018;430:123–32. https://doi.org/10.1016/j.canlet.2018.04.037.

[133] Jansen VM, Bhola NE, Bauer JA, Formisano L, Lee K-M, Hutchinson KE, et al. Kinome-wide RNA interference screen reveals a role for PDK1 in acquired resistance to CDK4/6 inhibition in ER-positive breast cancer. Can Res 2017;77:2488–99. https://doi.org/10.1158/0008-5472.CAN-16-2653.

[134] Romano G, Chen P-L, Song P, McQuade JL, Liang RJ, Liu M, et al. A preexisting rare PIK3CA(E545K) subpopulation confers clinical resistance to MEK plus CDK4/6 inhibition in NRAS melanoma and is dependent on S6K1 signaling. Can Discov 2018;8:556–67. https://doi.org/10.1158/2159-8290.CD-17-0745.

[135] Michaloglou C, Crafter C, Siersbaek R, Delpuech O, Curwen JO, Carnevalli LS, et al. Combined inhibition of mTOR and CDK4/6 is required for optimal blockade of E2F function and long-term growth inhibition in estrogen receptor-positive breast cancer. Mol Cancer Ther 2018;17:908–20. https://doi.org/10.1158/1535-7163.MCT-17-0537.

[136] Posternak G, Tang X, Maisonneuve P, Jin T, Lavoie H, Daou S, et al. Functional characterization of a PROTAC directed against BRAF mutant V600E. Nat Chem Biol 2020;16:1170–8. https://doi.org/10.1038/s41589-020-0609-7.

[137] Sahin U, Oehm P, Derhovanessian E, Jabulowsky RA, Vormehr M, Gold M, et al. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. Nature 2020;585:107–12. https://doi.org/10.1038/s41586-020-2537-9.

[138] Amaria RN, Menzies AM, Burton EM, Scolyer RA, Tetzlaff MT, Antdbacka R, et al. Neoadjuvant systemic therapy in melanoma: recommendations of the International Neoadjuvant Melanoma Consortium. Lancet Oncol 2019;20:e378–89. https://doi.org/10.1016/S1470-2045(19)30332-8.

[139] Lebbé C, Dutriaux C, Lesimple T, et al. Pimasertib versus dacarbazine in patients with unresectable NRAS-mutated cutaneous melanoma: Phase II, randomized, controlled trial with crossover. Cancers (Basel). 2020;12(7):1727. https://doi.org/10.3390/cancers120717.

[140] Lin S, Zhao X, Zhou Z, et al. FCN-159: A novel, potent and selective oral inhibitor of MEK1/2 for the treatment of solid tumors. Published online 2020.

[141] Wang X, Si L, Mao L, et al. A first-in-human phase I/II study of HL-085, a MEK Inhibitor, in Chinese patients with NRASm advanced melanoma. Published online 2020.

[142] Sullivan RJ, Amaria RN, Lawrence DP, et al. Abstract PR06: Phase 1b dose-escalation study of trametinib (MEKi) plus palbociclib (CDK4/6i) in patients with advanced solid tumors. Published online 2015.