DNA

MDPI

Review

Evolution of Acquired Drug Resistance in BRAF-Mutant Melanoma

Josué Ballesteros-Álvarez [1,*] and Ana M. Blázquez-Medela [2]

[1] Buck Institute for Research on Aging, Novato, CA 94945, USA
[2] Department of Basic Medical Sciences, Western University of Health Sciences, Pomona, CA 91766, USA; ablazquezmedela@gmail.com
* Correspondence: jballesteros@buckinstitute.org

Abstract: Melanoma is a highly aggressive type of skin cancer. Metastatic melanoma tumors have historically featured a particularly poor prognosis and have often been considered incurable. Recent advances in targeted therapeutic interventions have radically changed the landscape in metastatic melanoma management, significantly increasing the overall survival of patients. Hyperactive BRAF is the most common mutational event found in metastatic melanoma and its inhibition has proven to be a successful approach in a number of patients. Unfortunately, initial tumor retreat is followed by relapse in most cases, highlighting the elusiveness of finding a widely effective treatment. Melanoma tumors often carry a particularly high number of mutations in what is known as a high level of inter- and intra-patient tumor heterogeneity, driving resistance to treatment. The various mutations that are present in these tumors, in addition to impacting the root cause of the malignancy and the potential for therapeutic interventions, have also been known to arise during tumor clonal evolution leading to the establishment of drug resistance, a major issue in melanoma management.

Keywords: melanoma; drug resistance; mutation; tumor clonal evolution; BRAF; MITF

Citation: Ballesteros-Álvarez, J.; Blázquez-Medela, A.M. Evolution of Acquired Drug Resistance in BRAF-Mutant Melanoma. *DNA* **2024**, *4*, 355–369. https://doi.org/10.3390/dna4040025

Academic Editor: Darren Griffin

Received: 26 July 2024
Revised: 4 October 2024
Accepted: 9 October 2024
Published: 12 October 2024

1. Introduction

Skin cancer is the most common of all cancers. The three main types are basal cell carcinoma, cutaneous squamous cell carcinoma and melanoma. Whereas melanoma accounts for roughly 2% of skin cancer diagnoses, it is the most aggressive form of skin cancer [1]. Although melanoma is not exclusively confined to the Caucasian population, as it can affect people of all ethnicities, it is more commonly diagnosed in fair-skinned individuals with a history of sun exposure. Its incidence has been rising during the past few decades, increasing by circa 3% annually from 1982 to 2011 [2]. From 2006 to 2015, the rate increased among men and women ages 50 and older by 3% per year in the United States [3]. The melanoma incidence rate is projected to continue rising, as long as there are no significant improvements regarding sun-seeking and tanning behavior and use of protective clothing or sunscreen [4].

Metastatic melanoma historically exhibited a particularly poor prognosis with a median survival period of 6–9 months and an overall survival of 10–15% in patients treated with dacarbazine as in 2008 [5,6]. However, great advances have been made in recent years in the management of metastatic melanoma. Approximately 35% of cutaneous melanomas express programmed death ligand-1 (PD-L1) [7]. Treatment with the immune checkpoint inhibitor targeting programmed death-1 (PD-1), nivolumab, increased the overall survival to 73% at 1 year and 51% at 3 years [8]. Ninety-five percent of melanomas are cutaneous melanoma. However, ocular, mucosal, genitourinary, meningeal and gastrointestinal melanomas can occur [1].

In this review, we are presenting insights on what are the most widespread events arising in BRAF-mutant cutaneous melanoma that confer an evolutionary advantage to the

tumors and that need to be identified as key targets for effective long-lasting management of this predominant subtype of cutaneous melanomas.

2. The Melanoma Genome

One of the signature characteristics of the cancer genome is the high number of alterations that it contains—both genetic and irreversible or epigenetic and reversible. A crucial event in tumorigenesis is the establishment of driver mutations. These genetic lesions drive cancer formation and give a selection advantage to a determined cell lineage during cancer development, followed by the subsequent clonal expansion of that lineage [9]. The field of cancer genomics aims to identify the genetic and epigenetic targets behind tumorigenesis, making use of various testing platforms as tools for the discovery of somatic mutations involved in tumorigenesis. These are tumor DNA sequencing platforms such as conventional single-gene Sanger sequencing and pyrosequencing, but also whole-genome and whole-exome sequencing [10]. The identification of somatic mutations and tumor evolution events are crucial for prognostic and therapeutic purposes, an effort that is especially arduous in melanoma.

Melanoma exhibits a broad inter-patient heterogeneity due to several factors including a particularly rich variety of initiating events. Melanoma tumor genomes are notoriously unstable and carry the highest mutational burden among all cancers [11]. Exposure to UV can trigger the formation of photoproducts that distort the DNA helix, generating C > T and CC > TT transitions, namely UV-fingerprint mutations, which contribute to >90% of all non-synonymous single nucleotide variants (nsSNVs) in melanoma [12]. BRAF is a human gene that encodes the protein called B-Raf which belongs to the RAF family of serine/threonine protein kinases. The most frequent mutation found in cutaneous melanoma is the hotspot BRAFV600E somatic mutation, present in approximately 50% of tumors, that results in a hyperactive BRAF-MAPK pathway [13]. NRAS somatic mutations are the second major mutation, found in 28% of cutaneous melanoma tumors. Interestingly, hotspot BRAF and NRAS mutations are mutually exclusive [11,14].

In addition to the exposure to UV radiation in susceptible populations, the most important risk for the development of cutaneous malignant melanoma is the number of melanocytic nevi. Nevi are a benign clonal proliferation of melanocytic cells with a heterogeneous genetic background [15]; 20–30% of melanomas arise from preexisting nevi [16]. Congenital nevi have been reported to feature a high prevalence of BRAF mutations [17,18], while other studies have pointed to NRAS mutations [19,20]. A reason for this discrepancy is the study methodology. Nevus size and whether its origin is congenital or acquired after birth are factors that mark different nevi subpopulations that need to be taken into consideration for the analysis of genetic alterations. In fact, genetic characterization of large/giant congenital nevi shows that they feature NRAS and not BRAF mutations [21]. A majority of nevi are acquired after birth. However, individuals with fair skin and a propensity to sunburn are more prone to developing nevi during their lifetime. In one study, 78% of all acquired nevi studied had a *BRAF* mutation, whereas *NRAS* was mutated in only 6% of cases [22].

In light of the high frequency of BRAF mutations in nevi, it is clear that this mutation is not sufficient to drive melanomagenesis. Nevi carrying mutated BRAF eventually undergo oncogene-induced senescence, involving increased expression of the p16 cell cycle inhibitor of kinase 4A ($p16^{INK4A}$) [23]. $p16^{INK4A}$ and p14 alternate reading frame protein ($p14^{ARF}$) are tumor suppressor proteins encoded by the cyclin-dependent kinase inhibitor 2A (*CDK2A*) gene, which is crucial for controlling cell cycle arrest. $p16^{INK4A}$ has been found to be frequently silenced epigenetically in BRAF-mutated melanomas; however, it was not sufficient to drive melanoma transformation in vitro, suggesting that other genes are involved in bypassing BRAF-induced senescence [24]. Mutations occurring in other tumor suppressors, such as the phosphatase and tensin homolog (PTEN) and tumor protein 53 (TP53), have been found in advanced invasive primary melanomas [25].

Another gene that has been associated with an increased risk of developing melanoma is melanocortin 1 receptor (*MC1R*), which is highly polymorphic in the Caucasian population [26]. Several *MC1R* loss-of-function germline variant alleles have been associated with red hair, fair skin and freckling, which fail to stimulate cAMP production in response to α-MSH signaling to induce melanin production [27,28]. Furthermore, an increased risk of skin melanoma has been found for carriers of some of these variants, such as Val60Leu, Arg151Cys, and Arg160Trp [29].

3. The BRAF–MEK–ERK Highway to Melanoma

The Ras–Raf–MEK–ERK pathway is one of the most studied intracellular signaling cascades. It consists of a series of proteins that communicates an extracellular stimulus from a cell surface receptor to the nucleus of the cell. Small peptides, namely mitogens, bind to receptors in the cell membrane, triggering phosphorylation of the cytoplasmic domain of the receptor. This allows the GDP-bound Ras protein to exchange its GDP for GTP. Subsequently, GTP-bound Ras can modify the conformation of the Raf protein, which leads to activation of Raf. Raf is a family of serine/threonine kinases, found to be mutated in many types of human cancers. Ras-induced activated Raf forms homo- or heterodimers with other proteins like kinase suppressor of Ras (KSR) [30], and then recruits downstream mitogen-activated protein kinase kinase (MAPKK), which is phosphorylated and, in turn, phosphorylates MAPK/ERK. Phosphorylated extracellular signal-regulated kinase (ERK) is active and modulates the activity of transcription factors that are involved in cell cycle regulation or the translation of mRNA to proteins. MAPK activation phosphorylates 40S ribosomal protein S6 kinase (RSK), which in turn phosphorylates ribosomal protein S6 (RPS6) [31]. RPS6 induces the translation of proteins involved in the regulation of glucose homeostasis, cell growth and proliferation [32]. RSK can also directly regulate transcription factors such as c-MYC through phosphorylation [33].

3.1. BRAF

The Raf protein features a catalytic domain with kinase activity and an N-terminal regulatory domain that contains the Ras-binding activating domain and a cysteine-rich domain responsible for autoinhibition of the catalytic domain [34,35]. The basis of this self-regulatory mechanism is common to the three different members of the Raf family—A-Raf, B-Raf and C-Raf [36,37]. However, these proteins are differentially regulated at the level of post-translational modifications, which has an impact on their autoinhibitory activity [38]. Whereas B-Raf is constitutively phosphorylated at serine 445 [39] and is more readily activated by Ras [40], A-Raf and C-Raf need supplementary phosphorylation of activating residues and dephosphorylation of inhibitory residues in order to display full catalytic activity [41].

As stated before, hyperactivating BRAF mutations are extremely common in metastatic melanomas. Over 90% of these BRAF mutations are at codon 600, and over 90% of these are the single nucleotide mutation BRAFV600E. The second most frequent mutation is BRAFV600K in about 5% of cases [13]. Hyperactive BRAF is able to continue the signaling cascade independently of RAS activation and is functional as a monomer [41]. The homologous mutations of the ARAF or CRAF genes are rare events in human cancer, since these proteins do not share the constitutively phosphorylated residues occurring in B-Raf. The BRAFV600E mutation causes a higher basal kinase activity of this protein and makes it a key player in tumorigenesis [42].

To this date, there is no effective cure for metastatic melanoma, although great advances have been made with the development of immunotherapy [43] and targeted therapies using BRAF-MAPK inhibitors such as vemurafenib, which disrupts the B-Raf/MEK step of the pathway with a higher affinity when the V600E mutation in BRAF is present [44]. However, a broad interpatient heterogeneity and a variety of initiating events in the onset of melanoma lead to drug resistance and therapy failure in the majority of cases. Dacarbazine is a chemotherapeutic agent that methylates guanine, causing the DNA strands

to stick together and thus preventing cell division [6]. Until recently, dacarbazine has been the most widely used treatment for metastatic melanoma with unsatisfactory results [45]. The knowledge of the melanoma signature BRAFV600E mutation propelled the development of targeted therapies using BRAF pathway inhibitors, such as vemurafenib [44] and dabrafenib [46]. BRAF inhibitors provided striking anti-tumor responses and have been a breakthrough in the treatment of metastatic melanoma. However, the response to the treatment is short (average 7 months) and the tumors progress as resistance develops [47–50].

A study addressing the evolution of tumors under BRAF inhibition (BRAFi) therapy identified several subsets of genes involved in leukocyte extravasation—G-protein coupled receptor (GPCR), cAMP and PKA signaling—as the most significantly altered pathways in cells that have undergone BRAFi treatment, compared to the parental untreated cell line [51]. Similarly, overexpressing GPCR pathway-related genes in melanoma cells conferred resistance to BRAFi [52].

Another study found that among MAPK-reactivating mechanisms, *NRAS* mutations were detected in 18% of progressive tumors, *KRAS* mutations in 7%, *BRAF* amplification in 19% and mutant BRAF alternative splice variants in 13% of progressive tumors [53]. A subset of melanoma cells resistant to BRAFi with vemurafenib expressed a 61 kDa variant of the BRAFV600E protein, the p61BRAFV600E, lacking exons 4–8 which include the Ras-binding domain, and render the protein able to dimerize in a Ras-independent manner [54]. A MEK1-activating mutation and *CDKN2A* loss were also detected at a lower proportion. As a whole, among all the samples studied featuring progressive disease, a reactivation of the MAPK pathway as a mechanism of resistance to BRAFi was found at a 70% frequency (Table 1). Moreover, the study identified that PI3K–PTEN–AKT pathway mutations constituted a second core acquired resistance pathway at a 22% frequency that overlapped with the MAPK core pathway. This study also showed that the mutational signature of the progressive tumors has a reduction in C > T transitions as well as an attenuated dipyrimidine motif, indicating non-UV-related DNA damage [53].

Table 1. Incidence of MAPK-reactivating events in patient samples upon BRAF inhibition therapy.

MAPK-Reactivating Event	Ref.	Sample	Incidence (Count)	Incidence (%)	Comments
NRAS mutation	[52]	Progressive tumors after vemurafenib treatment	13/71	18%	G12D/R, G13R, Q61K/R/L
NRAS mutation	[53]	Biopsies from BRAFi-resistant patients	4/19	21%	Mutually exclusive with BRAF splicing variants
KRAS mutation	[52]	Progressive tumors after vemurafenib treatment	5/71	7%	G12C, G12R, Q61H
BRAF amplification	[52]	Progressive tumors after vemurafenib treatment	11/57	19%	2–15 fold
BRAF splice mutants	[52]	Progressive tumors after vemurafenib treatment	6/48	13%	Deletion exons 2–8, 2–10, 4–8
BRAF splice mutants	[53]	Biopsies from BRAFi-resistant patients	6/19	32%	Deletion of exons 4–10 (1), exons 4–8, (1), exons 2–8 (1), or exons 2–10 (3)
MEK1	[52]	Progressive tumors after vemurafenib treatment	2/71	3%	K57N, C121S
CDKN2A loss	[52]	Progressive tumors after vemurafenib treatment	3/44	7%	---

3.2. PI3K-PTEN-AKT

Unexpectedly, a fraction of BRAF-mutated metastatic melanoma patients does not respond to BRAFi. Studying the genetic background of those tumors that display an intrinsic resistance to this treatment would be beneficial to understand what mechanisms of tumor evolution may confer resistance to BRAFi and increased tumor fitness. Turajlic and colleagues analyzed the genomes of five metastatic BRAFV600E (all T > A, p.V600E) melanoma lesions in one patient who presented an intrinsic resistance to vemurafenib. Their results showed pre-vemurafenib ubiquitous GNAQ mutation that sustained ERK activity and PTEN frame-shift deletion that was associated with AKT hyperactivation [55], features that are consistent with a common intrinsic mechanism of resistance. They also observed a mixture of cells with distinct genomic and phenotypic features, suggesting that, while the tumors may have all evolved from a common metastatic progenitor, some sites have acquired additional genomic alterations after disease dissemination, including chromosome 18 loss, which is linked to chromosomal instability [56], and where the secondary effects of treatment with the DNA damaging agents cisplatin and decarbazine cannot be excluded [55].

Shi and colleagues pointed likewise to a rewired signaling landscape after BRAFi, involving the PI3K–PTEN–AKT axis. Analysis of 100 tumor samples from 44 patients that were resistant to vemurafenib or dabrafenib, a MEK inhibitor, found upregulating genetic alterations within the PI3K–PTEN–AKT axis in 22% of those tumors that progressed. These included AKT1/3 mutations and copy number increases [53].

Additionally, it has been shown that PI3K and MEK inhibitors synergized to block the growth of the tumor cells, suggesting that this might be an efficient approach in a subset of patients whose disease has followed this pattern of acquired resistance. IGF-1R/PI3K signaling was found to be upregulated in BRAFi-resistant cells. The absence of mutations and alterations in copy number in IGF-1R suggests that its persistent activity is likely regulated at the post-transcriptional level. The combined use of IGF-1R/PI3K and MEK inhibitors led to cytotoxicity in these melanoma cells resistant to BRAFi [57].

3.3. NRAS

The RAS GTPase NRAS, when active, can stimulate proliferation and survival, but also apoptosis, differentiation, and interactions between cells or between cells and extracellular matrix [58]. Second only to BRAF, NRAS is the most commonly mutated gene in melanoma. In particular, a striking 13–25% of all melanoma tumors carry a specific mutation in codon 61 of NRAS [59,60], which is associated with high tumor aggressiveness [59]. Mutated NRAS remains bound to GTP and is constitutively active [61,62], leading to hyperactivation of the BRAF/MAPK and PI3K/AKT/mTOR pathways. In melanocytes with normal copies of NRAS, the activation of the MAPK pathway is achieved through its effector BRAF. However, in the case of melanocytes with mutated NRAS, this takes place through the action of the CRAF effector, which renders BRAF inhibitors ineffective in this type of melanoma [63,64]. Surprisingly, combined treatment with BRAF/MAPK and PI3K inhibitors has yielded limited success in clinical trials [65]. MEK inhibitors have been suggested as possible treatment. However, they are more effective in patients that have already undergone immunotherapy [66]. Another theoretical possibility would have been the use of CRAF inhibitors; unfortunately, this resulted in a rapid switch to BRAF signaling [67]. The combined use of BRAF and CRAF inhibitors originated resistant cells in which MEK was strongly activated via ARAF [68]. As a consequence of this, several different approaches have been tried combining MEK inhibitors with cell cycle inhibitors or drugs that target the NRAS pathway. At this moment, there are promising in vivo results for the combined use of MEK inhibitors with inhibitors of cyclin CDK4/6 [69]. Further investigation in determining the downstream mechanisms directed by NRAS signaling would prove crucial to a large fraction of melanoma patients with a particularly poor prognosis associated with increased tumor thickness, mitotic rate and lymph node colonization [70].

3.4. mTOR

Another player that has been linked to the BRAF-dependent landscape of cutaneous melanoma tumors is the mammalian target of rapamycin complexes (mTOR). The mTOR protein is a serine/threonine kinase that forms an integral part of two distinct complexes, the mTORC1/2, that can sense a rich variety of stimuli and signaling inputs and regulate the synthesis of mRNA, cellular growth, proliferation and autophagy, among several other cellular processes [71–73]. ERK1/2 downstream of the hyperactive BRAF/MAPK pathway, a hallmark in melanoma tumors, can phosphorylate RSK which, in turn, activates mTORC1 in melanoma cells and its downstream targets, promoting increased protein translation, growth and proliferation [74]. A role of mTORC1/2 in bypassing oncogene-induced senescence has been described, suggesting that combined inhibition of the BRAF/MAPK pathway and mTORC1/2 could be effective under certain conditions [75]. On a similar note, another study showed that BRAFV600E mutation and CDKN2A loss were not sufficient to drive melanomagenesis in a mouse model, as this leads to repression of the PI3K–AKT axis, oncogene-induced senescence (OIS) and growth arrest. In contrast, the melanocytes that underwent transformation exhibited increased mTORC1 and AKT-mTORC2 signaling, suggesting that mTORC1/2 activation is a mechanism used by tumor cells to bypass BRAF-induced senescence and trigger progression [76]. Dysregulation of mTORC1 has an impact on the signaling mechanisms affecting tumor progression, which defines the complex as an oncogene that may be relevant in several types of cancer. Nonsynonymous mutations have been suggested in 2–3% of all cancers and 4–7% of all cutaneous melanomas [11,77], whereas increased activity of the Akt/mTOR axis has been observed in about 70% of melanoma metastases [78], suggesting the importance of this pathway in advanced tumor progression. Of note, rapamycin-mediated inhibition of mTOR failed to induce cell death of BRAF/MEK-inhibition-resistant cells. However, when rapamycin was administered in combination with a PI3K inhibitor, the mTOR-inhibition-dependent upregulation of AKT was prevented, leading to cell death [79], suggesting that inhibiting the PI3K–AKT pathway could be a viable approach to induce cell death in mTOR-elevated tumors.

3.5. MITF: Directing the Melanoma Orchestra

The microphthalmia-associated transcription factor (MITF) was first discovered due to coat color mutations in mice [80]. MITF belongs to the basic helix-loop-helix leucine zipper (bHLHZip) family of transcription factors. Mice that are deprived of MITF cannot produce melanocytes [81]. Mutations at the mouse *Mitf* locus result in pigmentation defects of the coat, small eyes and deafness. Moreover, mast cell defects have also been recognized for certain *Mitf* alleles [82]. Therefore, it is regarded as the master regulator of the melanocytic lineage as it is required for the development, growth and survival of melanocytes, where it regulates the expression of various differentiation and cell cycle progression genes [83,84]. MITF is an evolutionarily conserved transcription factor subject to differential splicing, thus being expressed as multiple isoforms that differ in their first exon and promoter usage [85]. In most isoforms, the initial variable exon is spliced onto the exon 1B1b and then continues with exons 2–9 that include all the functionally important motifs necessary for protein dimerization and transactivation ability [86]. The shortest isoform, termed MITF-M, which is the predominant isoform in melanocytes, contains a short exon 1 M directly spliced onto exons 2–9 [85].

The other isoforms of MITF have been described in a variety of cell types, including osteoclasts, natural killer cells, macrophages, mast cells, B cells, and cardiac muscle cells. MITF-MC is expressed in bone marrow-derived mast cells [82]. MITF-D is mostly expressed in the human retinal pigment epithelium (RPE) [87], while MITF-A, MITF-B, MITF-E and MITF-H are more ubiquitous [84]. In the RPE, A, D, H and M isoforms of MITF have been detected at comparable levels [88], in contrast with other studies that reported that MITF-M is not expressed in RPE cell lines [89]. Whether these different isoforms have tissue-restricted functions is not well known. MITF-MC has been shown to selectively activate the promoter of the gene encoding the mouse mast cell protease 6 (*Mmcp6*) and to fail to activate the pigmentation-related gene tyrosinase (*TYR*), known to be a target of MITF in

melanocytes, as shown by gene transactivation assays [90]. MITF-H has been shown to have a greater transactivation potential than MITF-M in cardiac cells, suggesting that the activity of MITF in the heart is isoform-specific [91]. Moreover, two isoforms of MITF called (+) and (−) exist, differing in exon 6a. The (+) isoform of MITF encodes six additional amino acids between Leu185 and Thr186, upstream of the DNA-binding basic domain [92,93]. Both the MITF-M and MITF-H isoforms have been found to be expressed as (−) and (+) versions, although it is possible that this could be the case for all the MITF isoforms [94]. Interestingly, the (+) isoform that includes the extra exon 6a exhibits a strong inhibitory effect on DNA synthesis [92]. Furthermore, isoform quantification across melanoma samples revealed that differential expression is dependent on MEK1–ERK2 activation and that MITF (−) expression is enriched in a subset of metastatic melanomas [95]. Interestingly, a PAX3-mediated upregulation of MITF was identified as a driver of an early non-mutational mechanism of drug resistance in response to long-term BRAF and MEK inhibition [96].

MITF has also been termed as a lineage "addiction" oncogene with a role in acquired drug resistance (Table 2). Focal amplification of MITF has been described in 10% of primary melanomas and over 15% of metastatic melanomas, in addition to having an association with decreased 5-year survival [97]. A patient with a tumor relapse-associated focal amplification of MITF has been reported [98]. MITF can cooperate with hyperactive mutant BRAFV600E in transforming human melanocytes in vitro; however, elevating the expression of MITF has been shown to display anti-proliferative effects [99]. These two seemingly contrasting observations respond to two different mechanisms. On one hand, $MITF^{high}$ melanomas display greater resistance to BRAF/MEK-inhibition-targeted therapy by overcoming the cytotoxic effects of the inhibitors [100], by means of increasing the expression of anti-apoptotic and pro-survival genes when compared to control cells [98]. On the other hand, hyperactive BRAF lowers the expression of MITF to basal levels that might be required for the survival of melanoma cells [99]. It could be hypothesized that a genomic amplification of MITF counteracts the BRAF-induced reduction of MITF which is required for sustaining melanoma survival, without impairing proliferation and clonal expansion. These findings indicate that MITF presents a finely tuned control in melanoma cells and that its role in melanoma is complex and needs further investigation. A model for MITF function as a rheostat has been proposed, wherein different levels of expression of MITF dictate phenotype outcome in melanoma [101]. This model says that the level of MITF activity determines cellular function. Long-term depletion of MITF drives senescence in melanoma cells [102], impairing DNA replication, mitosis and genomic stability [96]. Low expression has been associated with an invasive phenotype, whereas intermediate levels promote proliferation, and high expression of MITF activates a differentiation-associated cell cycle arrest via an increase in cyclin-dependent kinase inhibitors, leading to a non-proliferating phenotype with elevated differentiation [103,104] (Figure 1).

Figure 1. Diagram representing how the balance between BRAF and MITF different levels of expression (as depicted by arrows) affects the melanoma tumor phenotype.

Additionally, the E318K germline mutation of MITF has been associated with increased risk of melanoma. The mutant protein can delay BRAFV600E-induced senescence in human melanocytes, concomitant with decreased expression of $p16^{INK4A}$. This role of MITFE318K in bypassing BRAF-induced senescence may be the underlying mechanism that favors melanomagenesis in familial or sporadic melanoma [105]. Interestingly, this variant has been found to abrogate SUMOylation of K316 [106,107]. SUMOylation of MITF is known to render it less active transcriptionally. The receptor activator of NF-kappa B ligand (RANKL)/p38 pathway phosphorylates MITF at Ser307 in osteoclasts, which allows a SUMO protease to deSUMOylate Lys316 of MITF [108]. This enables MITF to recruit cofactors FUS (fused in sarcoma protein) and Brahma-related gene 1 (BRG1) to form a complex that stimulates transcription in these cells [108,109].

Table 2. MITF-driven mechanisms of drug resistance in melanoma tumors.

MITF-Driven Resistance Mechanism	Ref.	Effect	Comments
PAX3-mediated upregulation of MITF	[96]	Drug resistance in response to BRAF/MEK inhibition	-
MITF focal amplification	[97–100]	Associated with tumor relapse and resistance to BRAF/MEK inhibition	Counteraction of BRAF-induced reduction of MITF
E318K mutation of MITF	[105–107]	Bypass of BRAF-induced senescence	E318K MITF variant increases its transcriptional activity
MITF-mediated upregulation of BCL2A1	[110]	Reduced apoptosis of melanoma cells	Combined treatment with BRAF and BCL2A1 inhibitors increase apoptosis and reduce tumor volume
BRN2-mediated downregulation of MITF	[111]	Promotes migration and cell survival	Intratumor heterogeneity with proliferative or invasive cell subpopulations

The BCL2A1 locus, a member of the BCL2 family of anti-apoptotic proteins, has been found to be amplified in ~30% of melanomas. Interestingly, BCL2A1 expression is directly regulated by MITF in the melanocytic lineage. BRAFi therapy in melanoma was found to be less effective in the $BCL2A1^{high}$ context, whereas combined treatment with a BCL2 inhibitor yielded increased apoptosis and reduced tumor volume [110].

BRN2 (also known as POU3F2 or N-Oct-3) is a transcription factor that plays a significant role in melanoma. BRN2 represses MITF expression, marking two distinct subpopulations of melanoma cells that highlight melanoma tumor heterogeneity [111]. As the master regulator of the melanocytic lineage and an oncogene important for melanoma survival, motility, oxidative stress and DNA repair [104], modulating MITF is likely to be crucial in controlling tumor proliferation and a relevant target of future research in melanomagenesis. In contrast, melanoma cells expressing BRN2 are more migratory and feature a more invasive phenotype [111]. BRN2 has also been linked to melanoma cell survival as elevated BRN2 expression correlated with increased cell survival and resistance to cell death signals [112]. As a consequence, the role of BRN2 in promoting invasive aggressive phenotypes of melanoma indicates that understanding the downstream effectors of BRN2 and their mechanism of action could lead to identifying targets of interest and the development of novel therapeutics.

4. Final Remarks

BRAF is the most commonly mutated gene found in metastatic melanomas and, thus, BRAFi has been established as the first targeted therapeutic approach against this highly lethal form of cancer. Unfortunately, most of the patients that undergo BRAF inhibition treatment relapse and although novel combination therapies that include immunotherapy are radically changing the landscape, as of today, no current therapy has robustly proven to extend survival beyond the 5-year mark in more than 50% of these patients. This is, in part, due to a variety of acquired drug-resistance mechanisms that arise and confer an evolutionary advantage to the tumors that harbor them (Figure 2). It is an important

consequence and feature of the clonal nature of cancers—the geographical and temporal variation in tumor composition [113]. Spatial heterogeneity has been demonstrated in several tumor types and BRAF-mutant melanomas are no exception, often harboring an especially complicated genetic background triggered by UV-related DNA damage and able to elaborate multiple resistance mechanisms simultaneously. Therefore, when considering targeted therapy in melanoma treatment, it is crucial to fully understand the genetic landscape comprising all the intra- and inter-tumor heterogeneity. This is further entangled with "non-genetic" mechanisms, such as BRAF alternative splicing [114], that contribute to BRAF-targeting therapy resistance but have not been discussed in this review. As a remark on this complexity, it has been shown in a melanoma patient that progressing melanoma tumors followed a branched evolution and that this clonal diversification is associated with an increased mutational burden, but the extent of genetic diversification of the progressive tumors was, interestingly, not co-linear with the timing of clinical emergence [53]. It is a challenging effort to identify the BRAF resistance-associated genetic lesions, taking into account the extreme genetic heterogeneity present in melanoma patients, but one that would prove crucial for the management of this predominant subtype of cutaneous melanomas.

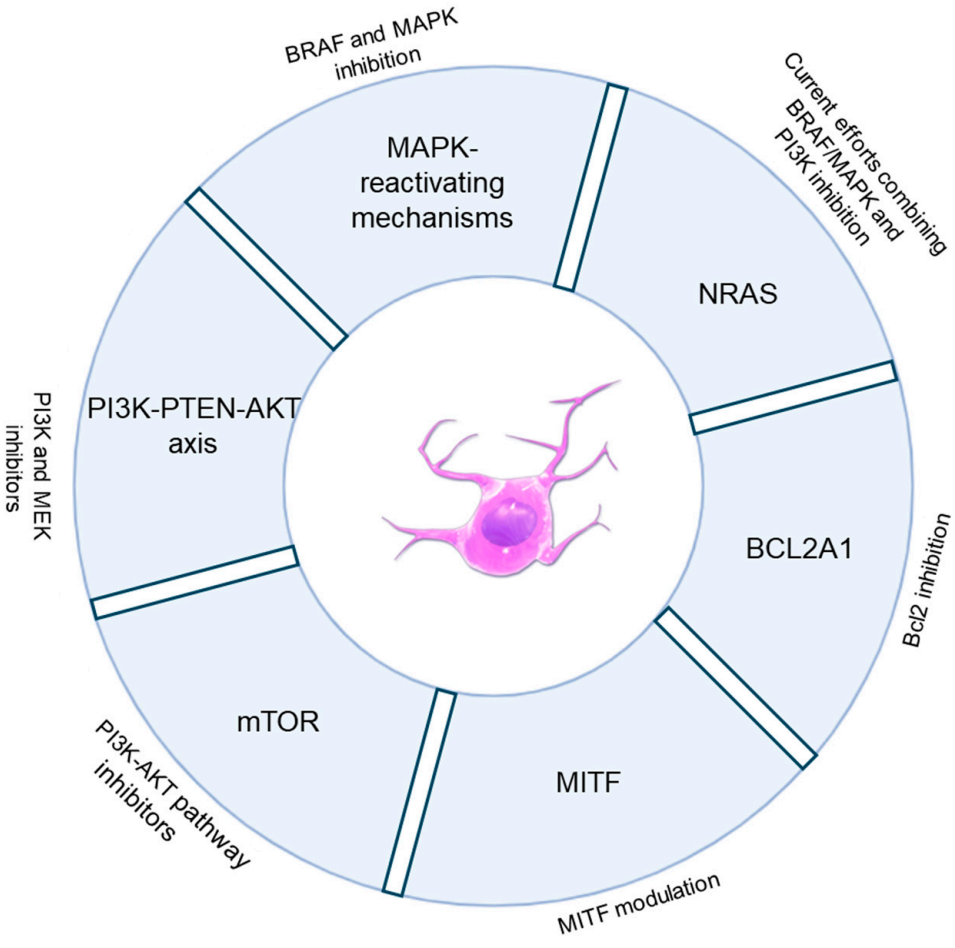

Figure 2. *Cont.*

Resistance Mechanisms	Incidence	Bypass	References
MAPK-reactivating mechanisms	70%	BRAF inhibition MAPK inhibition	Shi, H. et al., 2014
PI3K–PTEN–AKT axis	22%	PI3K and MEK inhibitors	Shi, H. et al., 2014
NRAS	13–25%	Combined treatment with BRAF/MAPK and PI3K inhibitors has yielded limited success Research underway targeting NRAS signaling	Randic et al., 2021 Jakob et al., 2012
mTOR	2–3% of all cancers and 4–7% of all cutaneous melanomas Increased activity up to 70% of melanoma metastases	Research suggests that inhibiting PI3K–AKT pathway could be a viable approach	The Cancer Genome Atlas Network 2015 Kong et al., 2016 Chamcheu et al., 2019
MITF	10% of primary melanomas, 15% of metastatic melanomas	MITF modulation	Garraway et al., 2005
BCL2A1	30% of melanomas	Bcl2 inhibition	Haq et al., 2011

Figure 2. Summary of acquired drug-resistance mechanisms that arise in melanoma tumors conferring an evolutionary advantage [11,53,59,60,77,78,97,110].

Author Contributions: J.B.-Á.: Conceptualization, investigation, writing, reviewing, and editing. A.M.B.-M.: Conceptualization, investigation, writing, reviewing, and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: The authors would like to acknowledge that major sections of this manuscript are inspired in Ballesteros-Alvarez's doctoral dissertation [115] and thank Eiríkur Steingrímsson at the University of Iceland for critical guidance and mentorship.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Saginala, K.; Barsouk, A.; Aluru, J.S.; Rawla, P.; Barsouk, A. Epidemiology of Melanoma. *Med. Sci.* **2021**, *9*, 63. [CrossRef] [PubMed]
2. Whiteman, D.C.; Green, A.C.; Olsen, C.M. The Growing Burden of Invasive Melanoma: Projections of Incidence Rates and Numbers of New Cases in Six Susceptible Populations through 2031. *J. Investig. Dermatol.* **2016**, *136*, 1161–1171. [CrossRef] [PubMed]
3. American Cancer Society. *Facts & Figures 2019*; American Cancer Society: Atlanta, GA, USA, 2019.
4. Leiter, U.; Eigentler, T.; Garbe, C. Epidemiology of skin cancer. *Adv. Exp. Med. Biol.* **2014**, *810*, 120–140. [PubMed]
5. Thirlwell, C.; Nathan, P. Melanoma—Part 2: Management. *BMJ* **2008**, *337*, a2488. [CrossRef]
6. Lapkina, E.Z.; Esimbekova, A.R.; Beleniuk, V.D.; Savchenko, A.A.; Ruksha, T.G. The Distribution of B16 Melanoma Cells in Cell-Cycle Phases under the Influence of Dacarbazine. *Cell Tissue Biol.* **2023**, *17*, 161–168. [CrossRef]
7. Kaunitz, G.J.; Cottrell, T.R.; Lilo, M.; Muthappan, V.; Esandrio, J.; Berry, S.; Taube, J.M. Melanoma subtypes demonstrate distinct PD-L1 expression profiles. *Lab. Investig.* **2017**, *97*, 1063–1071. [CrossRef]
8. Ascierto, P.A.; Long, G.V.; Robert, C.; Brady, B.; Dutriaux, C.; Di Giacomo, A.M.; Mortier, L.; Hassel, J.C.; Rutkowski, P.; McNeil, C.; et al. Survival Outcomes in Patients with Previously Untreated *BRAF* Wild-Type Advanced Melanoma Treated with Nivolumab Therapy. *JAMA Oncol.* **2019**, *5*, 187. [CrossRef]
9. Martincorena, I. Somatic mutation and clonal expansions in human tissues. *Genome Med.* **2019**, *11*, 35. [CrossRef]
10. Pei, X.M.; Yeung, M.H.Y.; Wong, A.N.N.; Tsang, H.F.; Yu, A.C.S.; Yim, A.K.Y.; Wong, S.C.C. Targeted Sequencing Approach and Its Clinical Applications for the Molecular Diagnosis of Human Diseases. *Cells* **2023**, *12*, 493. [CrossRef]
11. The Cancer Genome Atlas Network. Genomic Classification of Cutaneous Melanoma. *Cell* **2015**, *161*, 1681–1696. [CrossRef]
12. Hodis, E.; Watson, I.R.; Kryukov, G.V.; Arold, S.T.; Imielinski, M.; Theurillat, J.-P.; Nickerson, E.; Auclair, D.; Li, L.; Place, C.; et al. A landscape of driver mutations in melanoma. *Cell* **2012**, *150*, 251–263. [CrossRef] [PubMed]

13. Colombino, M.; Paliogiannis, P.; Cossu, A.; De Re, V.; Miolo, G.; Botti, G.; Scognamiglio, G.; Ascierto, P.A.; Santeufemia, D.A.; Fraggetta, F.; et al. BRAF Mutations and Dysregulation of the MAP Kinase Pathway Associated to Sinonasal Mucosal Melanomas. *J. Clin. Med.* **2019**, *8*, 1577. [CrossRef] [PubMed]
14. Dumaz, N.; Jouenne, F.; Delyon, J.; Mourah, S.; Bensussan, A.; Lebbé, C. Atypical BRAF and NRAS Mutations in Mucosal Melanoma. *Cancers* **2019**, *11*, 1133. [CrossRef] [PubMed]
15. Magana-Garcia, M.; Ackerman, A.B. What are nevus cells? *Am. J. Dermatopathol.* **1990**, *12*, 93–102. [CrossRef]
16. Shreberk-Hassidim, R.; Ostrowski, S.M.; Fisher, D.E. The Complex Interplay between Nevi and Melanoma: Risk Factors and Precursors. *Int. J. Mol. Sci.* **2023**, *24*, 3541. [CrossRef]
17. Pollock, P.M.; Harper, U.L.; Hansen, K.S.; Yudt, L.M.; Stark, M.; Robbins, C.M.; Moses, T.Y.; Hostetter, G.; Wagner, U.; Kakareka, J.; et al. High frequency of BRAF mutations in nevi. *Nat. Genet.* **2003**, *33*, 19–20. [CrossRef]
18. Yazdi, A.S.; Palmedo, G.; Flaig, M.J.; Puchta, U.; Reckwerth, A.; Rütten, A.; Mentzel, T.; Hügel, H.; Hantschke, M.; Schmid-Wendtner, M.-H.; et al. Mutations of the BRAF gene in benign and malignant melanocytic lesions. *J. Investig. Dermatol.* **2003**, *121*, 1160–1162. [CrossRef]
19. Francis, J.H.; Grossniklaus, H.E.; Habib, L.A.; Marr, B.; Abramson, D.H.; Busam, K.J. *BRAF*, *NRAS*, and *GNAQ* Mutations in Conjunctival Melanocytic Nevi. *Investig. Opthalmology Vis. Sci.* **2018**, *59*, 117. [CrossRef]
20. Bauer, J.; Curtin, J.A.; Pinkel, D.; Bastian, B.C. Congenital melanocytic nevi frequently harbor NRAS mutations but no BRAF mutations. *J. Investig. Dermatol.* **2007**, *127*, 179–182. [CrossRef]
21. da Silva, V.M.; Martinez-Barrios, E.; Tell-Marti, G.; Dabad, M.; Carrera, C.; Aguilera, P.; Brualla, D.; Esteve-Codina, A.; Vicente, A.; Puig, S.; et al. Genetic Abnormalities in Large to Giant Congenital Nevi: Beyond NRAS Mutations. *J. Investig. Dermatol.* **2019**, *139*, 900–908. [CrossRef]
22. Stark, M.S.; Tan, J.-M.; Tom, L.; Jagirdar, K.; Lambie, D.; Schaider, H.; Soyer, H.P.; Sturm, R.A. Whole-Exome Sequencing of Acquired Nevi Identifies Mechanisms for Development and Maintenance of Benign Neoplasms. *J. Investig. Dermatol.* **2018**, *138*, 1636–1644. [CrossRef] [PubMed]
23. Mackiewicz-Wysocka, M.; Czerwińska, P.; Filas, V.; Bogajewska, E.; Kubicka, A.; Przybyła, A.; Dondajewska, E.; Kolenda, T.; Marszałek, A.; Mackiewicz, A. Oncogenic BRAF mutations and p16 expression in melanocytic nevi and melanoma in the Polish population. *Adv. Dermatol. Allergol.* **2017**, *5*, 490–498. [CrossRef] [PubMed]
24. Dhomen, N.; Reis-Filho, J.S.; Dias, S.d.R.; Hayward, R.; Savage, K.; Delmas, V.; Larue, L.; Pritchard, C.; Marais, R. Oncogenic Braf induces melanocyte senescence and melanoma in mice. *Cancer Cell* **2009**, *15*, 294–303. [CrossRef] [PubMed]
25. Loureiro, J.B.; Raimundo, L.; Calheiros, J.; Carvalho, C.; Barcherini, V.; Lima, N.R.; Gomes, C.; Almeida, M.I.; Alves, M.G.; Costa, J.L.; et al. Targeting p53 for Melanoma Treatment: Counteracting Tumour Proliferation, Dissemination and Therapeutic Resistance. *Cancers* **2021**, *13*, 1648. [CrossRef] [PubMed]
26. Valverde, P.; Healy, E.; Jackson, I.; Rees, J.L.; Thody, A.J. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat. Genet.* **1995**, *11*, 328–330. [CrossRef]
27. Frändberg, P.-A.; Doufexis, M.; Kapas, S.; Chhajlani, V. Human pigmentation phenotype: A point mutation generates nonfunctional MSH receptor. *Biochem. Biophys. Res. Commun.* **1998**, *245*, 490–492. [CrossRef]
28. Schiöth, H.B.; Phillips, S.R.; Rudzishd, R.; Birch-Machin, M.A.; Wikberg, J.E.; Rees, J.L. Loss of function mutations of the human melanocortin 1 receptor are common and are associated with red hair. *Biochem. Biophys. Res. Commun.* **1999**, *260*, 488–491. [CrossRef]
29. Palmer, J.S.; Duffy, D.L.; Box, N.F.; Aitken, J.F.; O'Gorman, L.E.; Green, A.C.; Hayward, N.K.; Martin, N.G.; Sturm, R.A. Melanocortin-1 receptor polymorphisms and risk of melanoma: Is the association explained solely by pigmentation phenotype? *Am. J. Hum. Genet.* **2000**, *66*, 176–186. [CrossRef]
30. Rohrer, L.; Spohr, C.; Beha, C.; Griffin, R.; Braun, S.; Halbach, S.; Brummer, T. Analysis of RAS and drug induced homo- and heterodimerization of RAF and KSR1 proteins in living cells using split Nanoluc luciferase. *Cell Commun. Signal.* **2023**, *21*, 136. [CrossRef]
31. Cronin, R.; Brooke, G.N.; Prischi, F. The role of the p90 ribosomal S6 kinase family in prostate cancer progression and therapy resistance. *Oncogene* **2021**, *40*, 3775–3785. [CrossRef]
32. Yi, Y.W.; You, K.S.; Park, J.-S.; Lee, S.-G.; Seong, Y.-S. Ribosomal Protein S6: A Potential Therapeutic Target against Cancer? *Int. J. Mol. Sci.* **2021**, *23*, 48. [CrossRef] [PubMed]
33. Zhu, J.; Blenis, J.; Yuan, J. Activation of PI3K/Akt and MAPK pathways regulates Myc-mediated transcription by phosphorylating and promoting the degradation of Mad1. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6584–6589. [CrossRef] [PubMed]
34. Cutler, R.E.; Stephens, R.M.; Saracino, M.R.; Morrison, D.K. Autoregulation of the Raf-1 serine/threonine kinase. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9214–9219. Available online: https://www.ncbi.nlm.nih.gov/pubmed/9689060 (accessed on 25 July 2024). [CrossRef] [PubMed]
35. Zhang, M.; Jang, H.; Li, Z.; Sacks, D.B.; Nussinov, R. B-Raf autoinhibition in the presence and absence of 14-3-3. *Structure* **2021**, *29*, 768–777.e2. [CrossRef]
36. Maurer, G.; Tarkowski, B.; Baccarini, M. Raf kinases in cancer-roles and therapeutic opportunities. *Oncogene* **2011**, *30*, 3477–3488. [CrossRef] [PubMed]
37. Tran, N.H.; Frost, J.A. Phosphorylation of Raf-1 by p21-activated kinase 1 and Src regulates Raf-1 autoinhibition. *J. Biol. Chem.* **2003**, *278*, 11221–11226. [CrossRef]

38. Tran, N.H.; Wu, X.; Frost, J.A. B-Raf and Raf-1 are regulated by distinct autoregulatory mechanisms. *J. Biol. Chem.* **2005**, *280*, 16244–16253. [CrossRef]
39. Marais, R.; Light, Y.; Paterson, H.F.; Mason, C.S.; Marshall, C.J. Differential regulation of Raf-1, A-Raf, and B-Raf by oncogenic ras and tyrosine kinases. *J. Biol. Chem.* **1997**, *272*, 4378–4383. Available online: https://www.ncbi.nlm.nih.gov/pubmed/9020159 (accessed on 25 July 2024). [CrossRef]
40. Wellbrock, C.; Karasarides, M.; Marais, R. The RAF proteins take centre stage. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 875–885. [CrossRef]
41. Takahashi, M.; Li, Y.; Dillon, T.J.; Kariya, Y.; Stork, P.J.S. Phosphorylation of the C-Raf N Region Promotes Raf Dimerization. *Mol. Cell. Biol.* **2017**, *37*, e00132-17. [CrossRef]
42. TShabaneh, T.B.; Molodtsov, A.K.; Steinberg, S.M.; Zhang, P.; Torres, G.M.; Mohamed, G.A.; Boni, A.; Curiel, T.J.; Angeles, C.V.; Turk, M.J. Oncogenic BRAFV600E Governs Regulatory T-cell Recruitment during Melanoma Tumorigenesis. *Cancer Res.* **2018**, *78*, 5038–5049. [CrossRef] [PubMed]
43. AKnight, A.; Karapetyan, L.; Kirkwood, J.M. Immunotherapy in Melanoma: Recent Advances and Future Directions. *Cancers* **2023**, *15*, 1106. [CrossRef]
44. Porcelli, L.; Porcelli, L.; Di Fonte, R.; Di Fonte, R.; Pierri, C.L.; Pierri, C.L.; Fucci, L.; Fucci, L.; Saponaro, C.; Saponaro, C.; et al. BRAFV600E;K601Q metastatic melanoma patient-derived organoids and docking analysis to predict the response to targeted therapy. *Pharmacol. Res.* **2022**, *182*, 106323. [CrossRef]
45. Gogas, H.J.; Kirkwood, J.M.; Sondak, V.K. Chemotherapy for metastatic melanoma: Time for a change? *Cancer* **2007**, *109*, 455–464. [CrossRef] [PubMed]
46. Robert, C.; Grob, J.J.; Stroyakovskiy, D.; Karaszewska, B.; Hauschild, A.; Levchenko, E.; Chiarion Sileni, V.; Schachter, J.; Garbe, C.; Bondarenko, I.; et al. Five-Year Outcomes with Dabrafenib plus Trametinib in Metastatic Melanoma. *N. Engl. J. Med.* **2019**, *381*, 626–636. [CrossRef]
47. Chapman, P.B.; Hauschild, A.; Robert, C.; Haanen, J.B.; Ascierto, P.; Larkin, J.; Dummer, R.; Garbe, C.; Testori, A.; Maio, M.; et al. Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. *N. Engl. J. Med.* **2011**, *364*, 2507–2516. [CrossRef] [PubMed]
48. Flaherty, K.T.; Puzanov, I.; Kim, K.B.; Ribas, A.; McArthur, G.A.; Sosman, J.A.; O'Dwyer, P.J.; Lee, R.J.; Grippo, J.F.; Nolop, K.; et al. Inhibition of Mutated, Activated BRAF in Metastatic Melanoma. *N. Engl. J. Med.* **2010**, *363*, 809–819. [CrossRef]
49. Proietti, I.; Skroza, N.; Bernardini, N.; Tolino, E.; Balduzzi, V.; Marchesiello, A.; Michelini, S.; Volpe, S.; Mambrin, A.; Mangino, G.; et al. Mechanisms of Acquired BRAF Inhibitor Resistance in Melanoma: A Systematic Review. *Cancers* **2020**, *12*, 2801. [CrossRef]
50. Sosman, J.A.; Kim, K.B.; Schuchter, L.; Gonzalez, R.; Pavlick, A.C.; Weber, J.S.; McArthur, G.A.; Hutson, T.E.; Moschos, S.J.; Flaherty, K.T.; et al. Survival in BRAF V600-Mutant Advanced Melanoma Treated with Vemurafenib. *N. Engl. J. Med.* **2012**, *366*, 707–714. [CrossRef]
51. Kansler, E.R.; Verma, A.; Langdon, E.M.; Simon-Vermot, T.; Yin, A.; Lee, W.; Attiyeh, M.; Elemento, O.; White, R.M. Melanoma genome evolution across species. *BMC Genom.* **2017**, *18*, 136. [CrossRef]
52. Johannessen, C.M.; Johnson, L.A.; Piccioni, F.; Townes, A.; Frederick, D.T.; Donahue, M.K.; Narayan, R.; Flaherty, K.T.; Wargo, J.A.; Root, D.E.; et al. A melanocyte lineage program confers resistance to MAP kinase pathway inhibition. *Nature* **2013**, *504*, 138–142. [CrossRef] [PubMed]
53. Shi, H.; Hugo, W.; Kong, X.; Hong, A.; Koya, R.C.; Moriceau, G.; Chodon, T.; Guo, R.; Johnson, D.B.; Dahlman, K.B.; et al. Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. *Cancer Discov.* **2014**, *4*, 80–93. [CrossRef] [PubMed]
54. Alabi, S.; Jaime-Figueroa, S.; Yao, Z.; Gao, Y.; Hines, J.; Samarasinghe, K.T.G.; Vogt, L.; Rosen, N.; Crews, C.M. Mutant-selective degradation by BRAF-targeting PROTACs. *Nat. Commun.* **2021**, *12*, 920. [CrossRef] [PubMed]
55. Turajlic, S.; Furney, S.J.; Stamp, G.; Rana, S.; Ricken, G.; Oduko, Y.; Saturno, G.; Springer, C.; Hayes, A.; Gore, M.; et al. Whole-genome sequencing reveals complex mechanisms of intrinsic resistance to BRAF inhibition. *Ann. Oncol.* **2014**, *25*, 959–967. [CrossRef] [PubMed]
56. Andrade, J.R.; Gallagher, A.D.; Maharaj, J.; McClelland, S.E. Disentangling the roles of aneuploidy, chromosomal instability and tumour heterogeneity in developing resistance to cancer therapies. *Chromosome Res.* **2023**, *31*, 28. [CrossRef]
57. Villanueva, J.; Vultur, A.; Lee, J.T.; Somasundaram, R.; Fukunaga-Kalabis, M.; Cipolla, A.K.; Wubbenhorst, B.; Xu, X.; Gimotty, P.A.; Kee, D.; et al. Acquired Resistance to BRAF Inhibitors Mediated by a RAF Kinase Switch in Melanoma Can Be Overcome by Cotargeting MEK and IGF-1R/PI3K. *Cancer Cell* **2010**, *18*, 683–695. [CrossRef]
58. Muñoz-Maldonado, C.; Zimmer, Y.; Medová, M. A Comparative Analysis of Individual RAS Mutations in Cancer Biology. *Front. Oncol.* **2019**, *9*, 1088. [CrossRef]
59. Randic, T.; Kozar, I.; Margue, C.; Utikal, J.; Kreis, S. NRAS mutant melanoma: Towards better therapies. *Cancer Treat. Rev.* **2021**, *99*, 102238. [CrossRef]
60. Jakob, J.A.; Bassett, R.L.; Ng, C.S.; Curry, J.L.; Joseph, R.W.; Alvarado, G.C.; Rohlfs, M.L.; Richard, J.; Gershenwald, J.E.; Kim, K.B.; et al. NRAS mutation status is an independent prognostic factor in metastatic melanoma. *Cancer* **2012**, *118*, 4014–4023. [CrossRef]
61. Takács, T.; Kudlik, G.; Kurilla, A.; Szeder, B.; Buday, L.; Vas, V. The effects of mutant Ras proteins on the cell signalome. *Cancer Metastasis Rev.* **2020**, *39*, 1051–1065. [CrossRef]
62. Simanshu, D.K.; Nissley, D.V.; McCormick, F. RAS Proteins and Their Regulators in Human Disease. *Cell* **2017**, *170*, 17–33. [CrossRef] [PubMed]

63. Vakana, E.; Pratt, S.; Blosser, W.; Dowless, M.; Simpson, N.; Yuan, X.-J.; Jaken, S.; Manro, J.; Stephens, J.; Zhang, Y.; et al. LY3009120, a panRAF inhibitor, has significant anti-tumor activity in BRAF and KRAS mutant preclinical models of colorectal cancer. *Oncotarget* **2017**, *8*, 9251–9266. [CrossRef] [PubMed]
64. AAl Mahi, A.; Ablain, J. RAS pathway regulation in melanoma. *Dis. Model. Mech.* **2022**, *15*, dmm049229. [CrossRef] [PubMed]
65. Florent, L.; Saby, C.; Slimano, F.; Morjani, H. BRAF V600-Mutated Metastatic Melanoma and Targeted Therapy Resistance: An Update of the Current Knowledge. *Cancers* **2023**, *15*, 2607. [CrossRef]
66. Dummer, R.; Schadendorf, D.; Ascierto, P.A.; Arance, A.; Dutriaux, C.; Di Giacomo, A.M.; Rutkowski, P.; Del Vecchio, M.; Gutzmer, R.; Mandala, M.; 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*, 435–445. [CrossRef]
67. Dorard, C.; Estrada, C.; Barbotin, C.; Larcher, M.; Garancher, A.; Leloup, J.; Beermann, F.; Baccarini, M.; Pouponnot, C.; Larue, L.; et al. RAF proteins exert both specific and compensatory functions during tumour progression of NRAS-driven melanoma. *Nat. Commun.* **2017**, *8*, 15262. [CrossRef]
68. Monaco, K.-A.; Delach, S.; Yuan, J.; Mishina, Y.; Fordjour, P.; Labrot, E.; McKay, D.; Guo, R.; Higgins, S.; Wang, H.Q.; et al. LXH254, a Potent and Selective ARAF-Sparing Inhibitor of BRAF and CRAF for the Treatment of MAPK-Driven Tumors. *Clin. Cancer Res.* **2021**, *27*, 2061–2073. [CrossRef]
69. Teh, J.L.; Cheng, P.F.; Purwin, T.J.; Nikbakht, N.; Patel, P.; Chervoneva, I.; Ertel, A.; Fortina, P.M.; Kleiber, I.; HooKim, K.; et al. In Vivo E2F Reporting Reveals Efficacious Schedules of MEK1/2–CDK4/6 Targeting and mTOR–S6 Resistance Mechanisms. *Cancer Discov.* **2018**, *8*, 568–581. [CrossRef]
70. Devitt, B.; Liu, W.; Salemi, R.; Wolfe, R.; Kelly, J.; Tzen, C.; Dobrovic, A.; McArthur, G. Clinical outcome and pathological features associated with NRAS mutation in cutaneous melanoma. *Pigment. Cell Melanoma Res.* **2011**, *24*, 666–672. [CrossRef]
71. Ballesteros-Álvarez, J.; Andersen, J.K. mTORC2: The other mTOR in autophagy regulation. *Aging Cell* **2021**, *20*, e13431. [CrossRef]
72. DGuertin, D.A.; Sabatini, D.M. Defining the Role of mTOR in Cancer. *Cancer Cell* **2007**, *12*, 9–22. [CrossRef] [PubMed]
73. Zou, Z.; Tao, T.; Li, H.; Zhu, X. mTOR signaling pathway and mTOR inhibitors in cancer: Progress and challenges. *Cell Biosci.* **2020**, *10*, 31. [CrossRef] [PubMed]
74. Romeo, Y.; Moreau, J.; Zindy, P.-J.; Saba-El-Leil, M.; Lavoie, G.; Dandachi, F.; Baptissart, M.; Borden, K.L.B.; Meloche, S.; Roux, P.P. RSK regulates activated BRAF signalling to mTORC1 and promotes melanoma growth. *Oncogene* **2013**, *32*, 2917–2926. [CrossRef]
75. Wang, B.; Zhang, W.; Zhang, G.; Kwong, L.; Lu, H.; Tan, J.; Sadek, N.; Xiao, M.; Zhang, J.; Labrie, M.; et al. Targeting mTOR signaling overcomes acquired resistance to combined BRAF and MEK inhibition in BRAF-mutant melanoma. *Oncogene* **2021**, *40*, 5590–5599. [CrossRef] [PubMed]
76. Damsky, W.; Micevic, G.; Meeth, K.; Muthusamy, V.; Curley, D.P.; Santhanakrishnan, M.; Erdelyi, I.; Platt, J.T.; Huang, L.; Theodosakis, N.; et al. mTORC1 activation blocks BrafV600E-induced growth arrest but is insufficient for melanoma formation. *Cancer Cell* **2015**, *27*, 41–56. [CrossRef]
77. Kong, Y.; Si, L.; Li, Y.; Wu, X.; Xu, X.; Dai, J.; Tang, H.; Ma, M.; Chi, Z.; Sheng, X.; et al. Analysis of *mTOR* Gene Aberrations in Melanoma Patients and Evaluation of Their Sensitivity to PI3K–AKT–mTOR Pathway Inhibitors. *Clin. Cancer Res.* **2016**, *22*, 1018–1027. [CrossRef]
78. Chamcheu, J.C.; Roy, T.; Uddin, M.B.; Banang-Mbeumi, S.; Chamcheu, R.-C.N.; Walker, A.L.; Liu, Y.-Y.; Huang, S. Role and Therapeutic Targeting of the PI3K/Akt/mTOR Signaling Pathway in Skin Cancer: A Review of Current Status and Future Trends on Natural and Synthetic Agents Therapy. *Cells* **2019**, *8*, 803. [CrossRef]
79. Deng, W.; Gopal, Y.N.V.; Scott, A.; Chen, G.; Woodman, S.E.; Davies, M.A. Role and therapeutic potential of PI3K-mTOR signaling in de novo resistance to BRAF inhibition. *Pigment. Cell Melanoma Res.* **2012**, *25*, 248–258. [CrossRef] [PubMed]
80. Hertwig, P. Neue Mutationen und Kopplungsgruppen bei der Hausmaus. *Z. Für Indukt. Abstamm.-Und Vererbungslehre* **1942**, *80*, 220–246.
81. Steingrímsson, E.; Copeland, N.G.; Jenkins, N.A. Melanocytes and the Microphthalmia transcription factor network. *Annu. Rev. Genet.* **2004**, *38*, 365–411. [CrossRef]
82. Oppezzo, A.; Rosselli, F. The underestimated role of the microphthalmia-associated transcription factor (MiTF) in normal and pathological haematopoiesis. *Cell Biosci.* **2021**, *11*, 18. [CrossRef] [PubMed]
83. Hodgkinson, C.A.; Moore, K.J.; Nakayama, A.; Steingrímsson, E.; Copeland, N.G.; Jenkins, N.A.; Arnheiter, H. Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. *Cell* **1993**, *74*, 395–404. Available online: https://www.ncbi.nlm.nih.gov/pubmed/8343963 (accessed on 25 July 2024). [CrossRef] [PubMed]
84. Flesher, J.L.; Paterson-Coleman, E.K.; Vasudeva, P.; Ruiz-Vega, R.; Marshall, M.; Pearlman, E.; MacGregor, G.R.; Neumann, J.; Ganesan, A.K. Delineating the role of *MITF* isoforms in pigmentation and tissue homeostasis. *Pigment. Cell Melanoma Res.* **2020**, *33*, 279–292. [CrossRef]
85. Kawakami, A.; Fisher, D.E. The master role of microphthalmia-associated transcription factor in melanocyte and melanoma biology. *Lab. Investig.* **2017**, *97*, 649–656. [CrossRef]
86. Hershey, C.L.; Fisher, D.E. Genomic analysis of the Microphthalmia locus and identification of the MITF-J/Mitf-J isoform. *Gene* **2005**, *347*, 73–82. [CrossRef] [PubMed]

87. Takeda, K.; Yasumoto, K.-I.; Kawaguchi, N.; Udono, T.; Watanabe, K.-I.; Saito, H.; Takahashi, K.; Noda, M.; Shibahara, S. Mitf-D, a newly identified isoform, expressed in the retinal pigment epithelium and monocyte-lineage cells affected by Mitf mutations. *Biochim. Biophys. Acta* **2002**, *1574*, 15–23. [CrossRef]
88. Maruotti, J.; Thein, T.; Zack, D.J.; Esumi, N. MITF-M, a 'melanocyte-specific' isoform, is expressed in the adult retinal pigment epithelium. *Pigment. Cell Melanoma Res.* **2012**, *25*, 641–644. [CrossRef] [PubMed]
89. Shibahara, S.; Takeda, K.; Yasumoto, K.-I.; Udono, T.; Watanabe, K.-I.; Saito, H.; Takahashi, K. Microphthalmia-associated transcription factor (MITF): Multiplicity in structure, function, and regulation. *J. Investig. Dermatol. Symp. Proc.* **2001**, *6*, 99–104. [CrossRef]
90. Takemoto, C.M.; Yoon, Y.-J.; Fisher, D.E. The identification and functional characterization of a novel mast cell isoform of the microphthalmia-associated transcription factor. *J. Biol. Chem.* **2002**, *277*, 30244–30252. [CrossRef]
91. Tshori, S.; Sonnenblick, A.; Yannay-Cohen, N.; Kay, G.; Nechushtan, H.; Razin, E. Microphthalmia transcription factor isoforms in mast cells and the heart. *Mol. Cell. Biol.* **2007**, *27*, 3911–3919. [CrossRef]
92. Bismuth, K.; Maric, D.; Arnheiter, H. MITF and cell proliferation: The role of alternative splice forms. *Pigment. Cell Res.* **2005**, *18*, 349–359. [CrossRef] [PubMed]
93. Goding, C.R.; Arnheiter, H. MITF—The first 25 years. *Genes Dev.* **2019**, *33*, 983–1007. [CrossRef] [PubMed]
94. Hallsson, J.H.; Favor, J.; Hodgkinson, C.; Glaser, T.; Lamoreux, M.L.; Magnúsdóttir, R.; Gunnarsson, G.J.; Sweet, H.O.; Copeland, N.G.; Jenkins, N.A.; et al. Genomic, transcriptional and mutational analysis of the mouse microphthalmia locus. *Genetics* **2000**, *155*, 291–300. [CrossRef] [PubMed]
95. Primot, A.; Mogha, A.; Corre, S.; Roberts, K.; Debbache, J.; Adamski, H.; Dreno, B.; Khammari, A.; Lesimple, T.; Mereau, A.; et al. ERK-regulated differential expression of the Mitf 6a/b splicing isoforms in melanoma. *Pigment. Cell Melanoma Res.* **2010**, *23*, 93–102. [CrossRef]
96. Smith, M.P.; Wellbrock, C. Molecular Pathways: Maintaining MAPK Inhibitor Sensitivity by Targeting Nonmutational Tolerance. *Clin. Cancer Res.* **2016**, *22*, 5966–5970. [CrossRef]
97. Garraway, L.A.; Widlund, H.R.; Rubin, M.A.; Getz, G.; Berger, A.J.; Ramaswamy, S.; Beroukhim, R.; Milner, D.A.; Granter, S.R.; Du, J.; et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature* **2005**, *436*, 117–122. [CrossRef]
98. Van Allen, E.M.; Wagle, N.; Sucker, A.; Treacy, D.J.; Johannessen, C.M.; Goetz, E.M.; Place, C.S.; Taylor-Weiner, A.; Whittaker, S.; Kryukov, G.V.; et al. The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov.* **2014**, *4*, 94–109. [CrossRef]
99. Wellbrock, C.; Marais, R. Elevated expression of MITF counteracts B-RAF-stimulated melanocyte and melanoma cell proliferation. *J. Cell Biol.* **2005**, *170*, 703–708. [CrossRef]
100. Arozarena, I.; Wellbrock, C. Overcoming resistance to BRAF inhibitors. *Ann. Transl. Med.* **2017**, *5*, 387. [CrossRef]
101. Seberg, H.E.; Van Otterloo, E.; Cornell, R.A. Beyond MITF: Multiple transcription factors directly regulate the cellular phenotype in melanocytes and melanoma. *Pigment. Cell Melanoma Res.* **2017**, *30*, 454–466. [CrossRef]
102. Giuliano, S.; Cheli, Y.; Ohanna, M.; Bonet, C.; Beuret, L.; Bille, K.; Loubat, A.; Hofman, V.; Hofman, P.; Ponzio, G.; et al. Microphthalmia-Associated Transcription Factor Controls the DNA Damage Response and a Lineage-Specific Senescence Program in Melanomas. *Cancer Res.* **2010**, *70*, 3813–3822. [CrossRef] [PubMed]
103. Ballotti, R.; Cheli, Y.; Bertolotto, C. The complex relationship between MITF and the immune system: A Melanoma ImmunoTherapy (response) Factor? *Mol. Cancer* **2020**, *19*, 170. [CrossRef] [PubMed]
104. Vlčková, K.; Vachtenheim, J.; Réda, J.; Horák, P.; Ondrušová, L. Inducibly decreased MITF levels do not affect proliferation and phenotype switching but reduce differentiation of melanoma cells. *J. Cell. Mol. Med.* **2018**, *22*, 2240–2251. [CrossRef] [PubMed]
105. Ballotti, R.; Bertolotto, C. Deregulated MITF sumoylation: A route to melanoma. *Mol. Cell. Oncol.* **2017**, *4*, e1331154. [CrossRef]
106. Italian Melanoma Intergroup Italian Melanoma Intergroup (I.M.I.); Ciccarese, G.; Dalmasso, B.; Bruno, W.; Queirolo, P.; Pastorino, L.; Andreotti, V.; Spagnolo, F.; Tanda, E.; Ponti, G.; et al. Clinical, pathological and dermoscopic phenotype of MITF p.E318K carrier cutaneous melanoma patients. *J. Transl. Med.* **2020**, *18*, 78. [CrossRef]
107. Guhan, S.M.; Artomov, M.; McCormick, S.; Njauw, C.N.; Stratigos, A.J.; Shannon, K.; Tsao, H. Cancer risks associated with the germline MITF(E318K) variant. *Sci. Rep.* **2020**, *10*, 17051. [CrossRef]
108. Bronisz, A.; Carey, H.A.; Godlewski, J.; Sif, S.; Ostrowski, M.C.; Sharma, S.M. The multifunctional protein fused in sarcoma (FUS) is a coactivator of microphthalmia-associated transcription factor (MITF). *J. Biol. Chem.* **2014**, *289*, 326–334. [CrossRef]
109. Mansky, K.C.; Sankar, U.; Han, J.; Ostrowski, M.C. Microphthalmia transcription factor is a target of the p38 MAPK pathway in response to receptor activator of NF-kappa B ligand signaling. *J. Biol. Chem.* **2002**, *277*, 11077–11083. [CrossRef]
110. Haq, R.; Fisher, D.E. Biology and Clinical Relevance of the Micropthalmia Family of Transcription Factors in Human Cancer. *J. Clin. Oncol.* **2011**, *29*, 3474–3482. [CrossRef]
111. Fane, M.E.; Chhabra, Y.; Smith, A.G.; Sturm, R.A. BRN 2, a POU erful driver of melanoma phenotype switching and metastasis. *Pigment. Cell Melanoma Res.* **2019**, *32*, 9–24. [CrossRef]
112. Herbert, K.; Binet, R.; Lambert, J.-P.; Louphrasitthiphol, P.; Kalkavan, H.; Sesma-Sanz, L.; Robles-Espinoza, C.D.; Sarkar, S.; Suer, E.; Andrews, S.; et al. BRN2 suppresses apoptosis, reprograms DNA damage repair, and is associated with a high somatic mutation burden in melanoma. *Genes Dev.* **2019**, *33*, 310–332. [CrossRef] [PubMed]

113. Gopal, P.; Sarihan, E.I.; Chie, E.K.; Kuzmishin, G.; Doken, S.; Pennell, N.A.; Raymond, D.P.; Murthy, S.C.; Ahmad, U.; Raja, S.; et al. Clonal selection confers distinct evolutionary trajectories in BRAF-driven cancers. *Nat. Commun.* **2019**, *10*, 5143. [CrossRef] [PubMed]
114. Bokharaie, H.; Kolch, W.; Krstic, A. Analysis of Alternative mRNA Splicing in Vemurafenib-Resistant Melanoma Cells. *Biomolecules* **2022**, *12*, 993. [CrossRef] [PubMed]
115. Ballesteros-Álvarez, J. A Network of bHLHZip Transcription Factors in Melanoma: Interactions of MITF, TFEB and TFE3. Ph.D. Thesis, University of Iceland, School of Health Sciences, Faculty of Medicine, Reykjavik, Iceland, 2019.