cancers

MDPI

Review

Histone Modifications, Modifiers and Readers in Melanoma Resistance to Targeted and Immune Therapy

Stuart J Gallagher [1,2,*], Jessamy C Tiffen [1,2] and Peter Hersey [1,2]

Received: 31 July 2015 ; Accepted: 18 September 2015 ; Published: 25 September 2015
Academic Editor: Chyi-Chia Richard Lee

[1] Melanoma Immunology and Oncology Group, Centenary Institute, University of Sydney, Camperdown 2050, Australia; j.tiffen@centenary.org.au (J.C.T.); peter.hersey@sydney.edu.au (P.H.)
[2] Melanoma Institute Australia, Crow's Nest 2065, Sydney, Australia
* Correspondence: s.gallagher@centenary.org.au; Tel.: +61-2-9565-6226; Fax: +61-2-9565-6101

Abstract: The treatment of melanoma has been revolutionized by new therapies targeting MAPK signaling or the immune system. Unfortunately these therapies are hindered by either primary resistance or the development of acquired resistance. Resistance mechanisms involving somatic mutations in genes associated with resistance have been identified in some cases of melanoma, however, the cause of resistance remains largely unexplained in other cases. The importance of epigenetic factors targeting histones and histone modifiers in driving the behavior of melanoma is only starting to be unraveled and provides significant opportunity to combat the problems of therapy resistance. There is also an increasing ability to target these epigenetic changes with new drugs that inhibit these modifications to either prevent or overcome resistance to both MAPK inhibitors and immunotherapy. This review focuses on changes in histones, histone reader proteins and histone positioning, which can mediate resistance to new therapeutics and that can be targeted for future therapies.

Keywords: BRAF; RAF; MEK; immunotherapy; HDAC; histones; methyltransferase; bromodomain; BET; chromatin modifiers; resistance; PRC2; EZH2; PD-L1; PD-1

1. Melanoma Therapy with MAPK and Immunotherapy

Historically, unresectable metastatic melanoma was almost impossible to treat because of its resistance to most chemotherapy and radiotherapy [1]. This has changed with the advent of two new types of therapies for melanoma—mitogen-activated protein kinase (MAPK) inhibitors and immunotherapy. Approximately 40% of cutaneous melanoma patients have tumors with an activating mutation in the BRAF gene (BRAF V600E/K), which activates the MAPK pathway via a phosphorylation cascade that activates MEK and ERK [2]. Phosphorylated ERK has a number of targets including transcription factors ELK and ETS that lead to enhanced cell proliferation and survival signals. Patients with tumors harboring BRAF mutations are now treated with inhibitors of mutant BRAF (vemurafenib or dabrafenib), increasingly in combination with MEK inhibitors such as trametinib or cobimetinib [3,4]. Although initial response to these therapies is high (~80%), almost all patients relapse with tumors due to acquired resistance to these drugs [5–7]. Primary resistance in the 20% of patients that do not show an initial response is also a problem.

Immunotherapy has also provided breakthroughs in melanoma treatment by overcoming immune evasion mechanisms frequently used by melanoma cells. Cutaneous melanoma has the highest mutation rate of all cancers, which in theory should provide plenty of neo-antigens to attract immune surveillance and T-cell mediated killing [8]. However melanoma evades the immune system

by expressing Programmed Cell Death Ligand 1 (PD-L1), which binds to the Program Cell Death 1 (PD-1) receptor on T-cells and inhibits their cytotoxic T-cell activity. PD-1 inhibitors (pembrolizumab and nivolumab) and another immune checkpoint inhibitor against CTLA4 (ipilimumab) provide more durable responses than MAPK inhibitors but are also plagued by primary and acquired resistance [9]. Response rates to immunotherapy are lower than to MAPK inhibitors—10%–20% for ipilimumab, 30%–40% for PD-1 inhibitors and 50%–60% for combination of ipilimumab and anti-PD-1 [10–13]. The superiority of combined PD-1/CTLA4 therapy over single drug treatment was shown in a recent randomized trial in which the progression free survival (PFS) was 11.5 months for the combination compared to 6.9 months for Nivolumab alone or 2.9 months for ipilimumab alone [11–17].

Despite these encouraging successes, acquired and intrinsic resistance to MAPK inhibitors and immunotherapy are the most important challenges facing melanoma therapy today. Concerted efforts of researchers worldwide have found a number of different gene mutations or amplifications that cause resistance to MAPK inhibitors, explaining resistance in about half of patients failing MAPK inhibitors. Resistance to immunotherapy has been associated with low numbers of tumor infiltrating lymphocytes (TILs) [18] but the mechanisms involved remain poorly understood.

2. Melanoma Resistance to MAPK Inhibitors and Immunotherapy

Diverse mechanisms cause resistance against MAPK treatment in melanoma [19,20]. Mechanisms of resistance involve either reactivation of MAPK-pathway signaling or activation of other survival and growth pathways, allowing the melanoma cells to proliferate despite MAPK inhibition. Alternatively, the cell state of the melanoma isn't entirely dependent on MAPK signaling, allowing cell survival and growth even when MAPK signaling is blocked. These mechanisms will be discussed more fully in the subsequent sections, but can be divided into a number of classes.

- Resistance caused by strong anti-apoptotic signaling
- Mutations that reactivate the MAPK pathway
- Mutations that activate alternative survival pathways
- Receptor tyrosine kinase activation/upregulation to provide alternate survival signals
- Presence and selection of slow cycling, drug resistant melanoma cells
- MITF, cAMP and NF-κB related mechanisms

Resistance of melanoma to immunotherapy is less well defined. Primary resistance is the largest problem—even with combined anti-PD-1/anti-CTLA4 treatment, progressive disease is the best response in almost 30% of patients [11]. Both the biology of the immune system and melanoma cells needs to be addressed when looking to modulate immunotherapy—from antigen presentation, expression of immune checkpoints on melanoma and immune cells, and the activity, type and number of immune cells. Epigenetic histone modifiers have been shown to modulate both immunogenicity of melanoma cells and immune function but often in an opposing fashion. This review investigates the contribution of histone changes and histone modifiers in causing resistance and how they can be leveraged to overcome or prevent resistance.

3. Epigenetic Changes—A Brief Overview

Epigenetic regulation refers to (mitotically) heritable changes in gene expression that are not directly the result of changes in DNA sequence. This review focuses on covalent modifications of histones, histone modifiers and histone-binding proteins. DNA methylation and non-coding RNAs are also important epigenetic events and are reviewed elsewhere [21–23].

Histone modifications provide epigenetic control of gene expression and are viewed as being more dynamic than DNA methylation marks, which, with the exception of TET-meditated changes, are considered to be more permanent [24]. Different chemical groups can be added to histones by proteins broadly classified as histone writer proteins [25,26]. The best studied of these modifying

"marks" is acetylation and methylation. These groups can be removed by histone "eraser" proteins. Finally, modifications on histones act as a template, allowing binding by modification-sensitive histone reader proteins. The reader proteins recruit transcription factors or repressors to modulate gene expression [27]. Finally, nucleosome structure and the exact histone variants that make up a nucleosome affect gene transcription. A brief summary of these main epigenetic factors and their context in melanoma biology is given below.

3.1. Histone Acetylation

Histones can be acetylated at lysine (K) residues by the transfer of acetyl groups from acetyl-CoA to the ε-amino group at the terminal of the lysine side chain [28]. The transfer of the acetyl groups is catalyzed by histone acetyl transferases (HATs) and the groups can be removed by histone deacetylases (HDACs), with the balance of these opposing factors determining the level and state of histone acetylation. The addition of the acetyl group neutralizes the lysine's positive charge, thereby weakening the interaction with the negative charge of DNA, leading to a more open chromatin structure allowing easier access to transcription factors and increased transcription. Lysine residues on the tails of H3 and H4 are the best studied targets of histone acetylation but other more internal residues (such as H3K56) may also be acetylated. Histone acetylation also influences transcription by acting as a binding target for histone reader proteins. Finally, acetyl groups on histones must be removed to allow the deposition of other histone marks, such as the transcriptionally permissive H3K4me3 or repressive H3K27me3, thereby adding another layer of transcriptional control.

Changes in histone acetylation have been noted for many genes during melanomagenesis and may contribute to the downregulation of specific tumor suppressor genes such as p14ARF and p16INK4a [29,30], thus factors modulating histone acetylation have been the subject of much study. HDACs have commanded the most attention due to the increasing number of inhibitors available for these proteins. HDACs are divided into four classes including class I (HDAC 1, 2, 3 and 8), which are predominantly nuclear, class II HDAC (HDAC 4, 5, 6, 7, 9 and 10) that shuttle between the nucleus and cytoplasm, class III that consist of NAD dependent sirtuins, and class IV (HDAC11) [31–33]. HDAC expression is generally elevated in cancer for example HDAC1, 2, 3 and 6 are reported to be upregulated in various cancers [31] but some HDACs, such as HDAC1, 2 and SIRT1, may also be lost or decreased [34,35]. Relatively little is known about HDAC expression in melanoma. HDAC8 expression is associated with improved survival in melanoma, but HDAC1 and HDAC8 also correlate with increase phosphorylated p65—a subunit of the NF-κB complex, which is associated with resistance to MAPK inhibitors [36,37].

Much of the work studying HDAC function has been performed using small molecule inhibitors and the specificity of these inhibitors varies [31]. Additionally, HDACs and HATs have histone-independent targets, which can have significant cellular effects, thus, one must be cautious when comparing the results of studies using different HDAC inhibitors. Histone-independent targets of HDAC proteins include HSP90, p53 and NF-κB subunit p65. Hyperacetylation of chaperone protein HSP90 following HDAC inhibition leads to degradation of signaling molecules c-RAF and Akt—both important mediators of growth and MAPK inhibitor resistance in melanoma. Additionally, HSP90 hyperacetylation causes degradation of the upstream receptor tyrosine kinases ERBB1 and ERBB2 which can promote MAPK inhibitor resistance. Alteration of p53 activity by HDAC inhibitor driven hyperacetylation may alter melanoma resistance to therapy [38–40] and NF-κB activation may alter cytokine production, anti-apoptotic protein transcription and immune response [41–43]. More extensive summaries of histone-independent HDAC targets can be found elsewhere [31,44,45].

3.2. Histone Methylation

Histones are methylated by histone methyltransferases on lysine or arginine (R) amino acids, often leading to repressive chromatin states (e.g., methylation of H3R2, H3R8, H3K9, H3K27).

A number of histone methyltransferases are implicated as having a role in melanoma, especially enhancer of zeste homolog 2 (EZH2)—the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2) complex which represses transcription by adding the H3K27me3 mark [46]. Subsequent histone ubiquitination by the PRC1 complex leads to deep transcriptional repression. EZH2 can also associate with DNA methylases to link histone methylation with DNA methylation [46]. Activating mutations in EZH2 occur in 3% of melanoma, where it functions as a driver of melanoma progression, and its expression may be upregulated in a further cohort of melanoma [47–50]. EZH2 function can be inhibited by a number of small molecules and EZH2 inhibition in melanoma reduces cell growth and metastases [48,50]. Not all histone methylation marks are repressive and H3K4 methylation is a marker of active transcription from a gene.

3.3. Histone Readers

Histone modifications not only strengthen or weaken the interaction between DNA and histones, but also serve to allow the recruitment of epigenetic regulators, which bind specific covalent modifications or patterns of modifications on histones [51]. Reader proteins have a variety of different domains which bind to specific alterations—acetylated lysine residues are bound by bromodomains and plant homology domains (PHD), methylated lysines are bound by HD, chromo, WD40, Tudor, double/tandem Tudor, MBT, Ankyrin Repeats, Zf-CW and PWWP domain proteins. Phosphorylated serine residues of histones are bound by BRCT domain containing MDC1 and by 14-3-3 family (see [27] for full review). Histone reader proteins can alter transcription by recruiting other enzymes, such as the positive transcription elongation factor (P-TEFb) or cause further histone modifications and many histone readers also have catalytic ability [27,51]. The bromodomain and extra terminal (BET) family of histone readers bind acetylated histones and have attracted some attention in melanoma. BET family members BRD2 and BRD4 are over expressed in melanoma and are able to be inhibited by a number of relatively novel drugs such as JQ-1 and I-BET151, leading to reduced melanoma growth, cell death and reduction in NF-κB activity [52–55]. The PhD domain containing ING1 protein has also been reported to be over expressed in melanoma [56].

3.4. Chromatin Structure (SWI/SNF)

While 146 nucleotides wrap around a histone to form a nucleosome, there is a variable number of nucleotides between each nucleosome. These more exposed nucleotides, which may typically number 50–100, are more accessible to transcription factors and therefore nucleosome spacing and location is an important determinant of transcription. The SWI/SNF chromatin remodeling complex can determine nucleosome location. It is comprised of an ATPase subunit (either BRG1 or BRM) and 8–14 BAF members. Inactivating mutations in this complex (ARID2A, ARID1A and SMARCA4) are found in 13% of melanomas [47,57], highlighting their role as tumor suppressors and emphasizing the importance of this complex in normal biology and disease. Ovarian cancer cell lines that harbor an inactivating mutation in ARID1A were recently found to be dependent on PRC2 and thus sensitive to EZH2 inhibition [58]. At least one of the two core ATPases in SWI/SNF (BRG1 or BRM) is required for melanoma growth, and they may regulate different sets of target genes [59]. ATRX, another SWI/SNF chromatin remodeler has also been shown to be reduced with melanoma progression [60].

3.5. Histone Variants

The histone core around which DNA is wrapped typically contains two of each of the canonical histones—H2A, H2B, H3, and H4 [61]. However canonical histones can be replaced by variant histones which have a different sequence and properties. Variants of H2A and H3 are most common and are inserted into specific genomic areas by histone chaperones, resulting in altered chromatin structure, modifications, and gene transcription [61]. Histone variant H2A.Z is increased in melanoma and other cancers [62] and consists of two sub-forms (H2A.Z.1 and H2A.Z.2) that only differ by three amino acids but are transcribed from distinct gene loci. H2A.Z.2 is highly expressed

in melanoma and is bound by and stabilizes histone reader protein BRD2, leading to activation of genes, especially E2F targets that promote cell cycle progression [53]. H2A.Z.2 deficiency increases sensitivity to MEK inhibitor treatment and the involvement of H2A.Z.2 in causing resistance to MAPK inhibitors or immunotherapy in patients remains to be explored [53,63]. Histone 3 variant H3.3 is also associated with E2F target gene expression and overexpression of H3.3 leads to repression of E2F target genes and senescence [64]. Histone variant macroH2A suppresses melanoma progression via suppression of CDK8 expression and expression of macroH2A is generally lost with melanoma progression [62]. Targeting of these variants or the chaperones that deposit them into specific regions of chromatin could alter the sensitivity of melanoma cells to MAPK inhibitors or immunotherapy.

4. Overcoming Resistance to MAPK Inhibitors by Epigenetic Modulators

4.1. Reducing Intrinsic Anti-Apoptotic Signaling in Melanoma

Melanoma cells are intrinsically resistant to apoptosis which confers resistance to a variety of cytotoxic insults—from DNA damaging drugs, radiation and a variety of chemotherapeutic agents [65]. There are many pathways to cell death [66], but a central pathway is the caspase driven apoptosis pathway, which is mediated in-large part by the Bcl-2 family of proteins which control depolarization of the mitochondria [67,68]. Mitochondria are depolarized by Bax and Bak which are controlled by the balance of other pro or anti-apoptotic members of the Bcl-2 family [69]. Once depolarized, the contents of the mitochondria are released which activates caspases or caspase-independent molecules which complete the induction of cell death [65,70]. In the balancing act of pro- *vs.* anti-apoptotic proteins, melanoma cells have the dial turned to survival [65,71–73]. High levels of MAPK and/or PI3K signaling typical in melanoma increase levels of anti-apoptotic Bcl-2, Mcl-1, Bcl-XL, survivin and XIAP [65,74–79] while suppressing expression of the potent apoptotic inducer Bim and sequestering pro-apoptotic Bmf to the cytoskeleton [75,78,80–83].

MAPK inhibitors cause apoptosis by adjusting this balance of proteins, most notably by inducing BIM, Bmf and reducing Mcl-1 [82,84,85]. This shift towards an apoptotic state is partly offset by a decrease in NOXA [86] but is often enough to trigger apoptosis. Melanoma cells which have high enough levels anti-apoptotic proteins can resist death in response to MAPK inhibitors and instead the primary response is cell cycle arrest. Epigenetic modifiers—especially HDAC inhibitors, can play a key role in switching the balance to an apoptotic state (Figure 1).

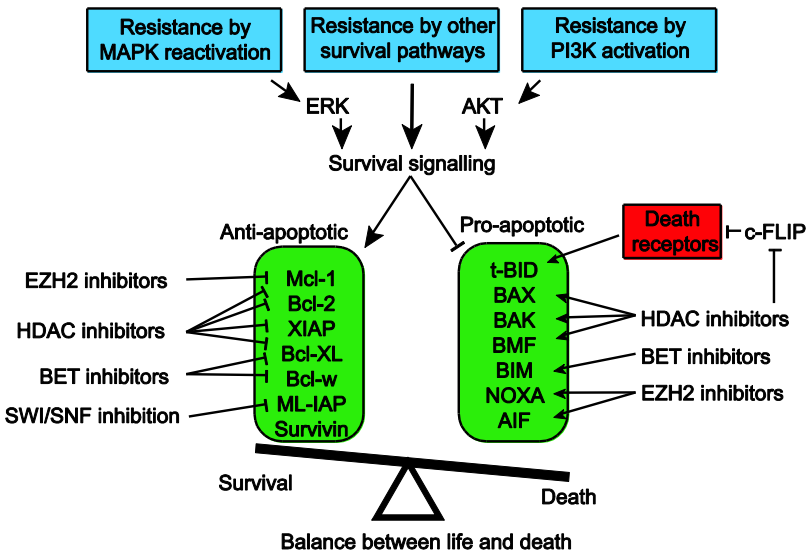

Figure 1. Mechanisms conferring resistance to MAPK inhibitors restore survival signaling by decreasing pro-apoptotic proteins and increasing anti-apoptotic signals. A variety of epigenetic modulators can push the balance back to signaling that favors cell death.

4.1.1. Histone Acetylation, HDAC Inhibitors and Apoptotic Proteins in Melanoma

Inhibitors of HDACs create a pro-apoptotic environment that increases the cell death in response to MAPK inhibitors and can also cause cell death alone [87–89]. HDAC inhibitors increase or decrease the expression of hundreds to thousands of genes [90,91] and many of the gene changes shift the balance towards apoptosis. In melanoma, HDAC inhibitors inhibit expression of anti-apoptotic proteins survivin, Bcl-XL, Bcl-2, Mcl-1, XIAP, mostly via decreased transcription [87,92–96]. HDAC inhibitors differ in their ability to suppress individual anti-apoptotic proteins—for example suberanilohydroxamic acid (SAHA/Vorinostat) suppressed Bcl-XL levels in melanoma cells while valproic acid (VPA) did not [94]. These differences are likely due to differences in the HDACs targeted by different inhibitors—SAHA has a broader inhibitory effect on HDAC than VPA [31].

Pro-apoptotic proteins are also induced by HDAC inhibitors, for example suberohydroxamic acid (SBHA) induced BIM, BAX and BAK in melanoma cells [87]. Bax can be upregulated by the HDAC inhibitor sodium butyrate, although this was p53 dependent and may be a result of p53 acetylation, rather than direct changes in histone acetylation [97]. Cell cycle inhibitor and p53 target p21 (CDKN1A) is frequently reported to be upregulated by HDAC inhibitor treatment in a p53 independent fashion, which is the result of direct action of increased histone acetylation, chromatin remodeling and RNApol II recruitment to the CDKN1A promoter [98].

The extrinsic pathway of apoptosis, which activates caspases independent of mitochondria, can also be targeted by HDAC inhibitors which reduce levels of c-FLIP—an inhibitor of TNF-related apoptosis inducing ligand (TRAIL)—the class I HDAC inhibitor MS-275 rendered melanoma more sensitive to TRAIL killing [92,99].

4.1.2. Histone Methylation

Inhibition of the histone methyltransferase and PRC2 member EZH2 leads to melanoma cell death by induction of AIF (apoptosis inducing factor) release from mitochondria [48]. Caspase independent apoptosis was also associated with a decrease in Mc1-1 and an increase Noxa, although the events preceding mitochondrial depolarization remain unclear. As persistently high Mcl-1 levels and decreased NOXA impede MAPK inhibitor-induced cell death, the combination of EZH2 and MAPK inhibition is rational [70].

4.1.3. Histone Readers

The Bromodomain and Extra Terminal (BET) family of histone reader proteins also push melanoma cells towards apoptosis. BET proteins bind acetylated lysines on histone H3 and H4 and recruit RNA polymerase II to drive transcription and two BET proteins—BRD2 and BRD4 are increased during melanoma progression [52,53]. Inhibition of BET proteins leads to an increase in BIM, which may cooperate with BIM induction following MAPK inhibition and early data suggest that combining BET inhibitors and MAPK inhibition may be advantageous in melanoma [52,55,100]. Combining HDAC with BET further enhances apoptosis in melanoma and could be an alternate strategy [95].

4.1.4. SWI/SNF

SWI/SNF can promote melanoma survival by remodeling the IAP promoter leading to enhanced MITF-driven BIRC7 (Livin/ML-IAP) expression [101].

4.2. Mutations that Re-Activate the MAPK Signaling Pathway

Many of the described mechanisms causing BRAF inhibitor resistance are mutations that reactivate the MAPK signaling pathway—for review see [19,20,102,103]. These may be mutations activating upstream components (NRAS Q61K/R or inactivation of NF-1), activating mutations in

downstream targets such (MEK mutation or COT upregulation) and BRAF amplifications or BRAF truncation and splice mutants that are resistant to inhibition [104]. Epigenetic modifiers may serve to overcome some of these resistance mechanisms as well (Figure 2).

Not all mutations that cause resistance are created equal. For example a number of mutations in MEK I or II have been reported [19], and while some clearly provide strong BRAF-independent activation, others seem to be only weakly activating. Likewise, BRAF amplifications may not be completely protective, but may provide just enough survival signaling to allow a tumor to escape therapy. This "just enough" signaling model is supported by work showing that weak upstream MAPK pathway activation is sufficient to cause resistance to BRAF inhibitors [105]. In these situations, combination with HDAC inhibitors may be advantageous, as the HDAC inhibitor would increase death signaling while also assist to block cell cycle through p21 induction, as described in the previous section.

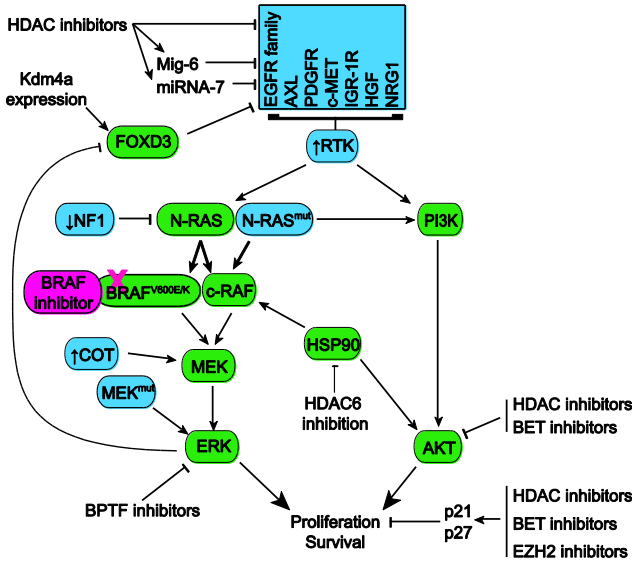

Figure 2. Melanoma cells develop resistance to MAPK inhibitors by mutating (mut) or altering expression of a number of proteins which reactivate MAPK signaling and/or activate alternative survival pathways such as the PI3K pathway. Some alterations known to drive resistance are shown in blue. Targeting epigenetic regulators may be able to overcome these resistance mechanisms by targeting the resistance mechanism directly or downstream signaling.

Upregulation of CRAF or reactivation of MAPK signaling via CRAF may be attenuated by the HDAC inhibitor LAQ826. LAQ826 inhibits HDAC6 activity to increase acetylation of HSP90, leading to a reduction in CRAF levels [106]. Another HSP90 client protein, AKT is also reduced by this treatment, which would be beneficial in reducing survival signals mediated by the PI3K pathway.

Blocking ERK signaling may be possible via targeting of the chromatin remodeler BPTF. BPTF has recently been reported to confer resistance to MAPK inhibitors and suppressing BPTF expression lead to a decrease in transcription of ERK, reduced total- and phospho-ERK protein levels and resulted in an increase in sensitivity to BRAF inhibitors [107].

4.3. Mutations that Activate Alternative Survival Pathways

The PI3K pathway is frequently activated in MAPK-inhibitor resistant melanomas to provide survival and growth signals [108]. HDAC inhibitors can blunt the signaling of the AKT pathway in a number of ways. Reduction of the AKT-inhibitor phosphatidylinositol 4,5-bisphosphate-5-phosphatase by histone hypoacetylation is mediated by HDAC2 and HDAC3 and can be reversed by HDAC inhibitor treatment [109]. HDAC inhibitors also prevent HDAC deacetylation of HSP90, leading to reduced HSP90 chaperone activity and a decrease in HSP90 target

AKT3 [106]. Combined inhibition of HDAC and BET reader proteins also lead to a potent reduction in AKT phosphorylation that was related to a decrease in activation of YAP [95]. As discussed in the next section, HDAC inhibitors can also reduce RTK signaling, which reduces activation of AKT and other pathways. Dual PI3K/HDAC inhibitors have been designed and may offer benefits of tighter PI3K control and concomitant HDAC inhibition [110,111].

4.4. Receptor Tyrosine Kinases Driving Resistance

Upregulation or activation of receptor tyrosine kinases (RTKs) is one resistance strategy used by melanoma cells. RTK signaling activates either the PI3K signaling pathway, to provide alternative growth signals to overcome MAPK inhibition, or reactivates the MAPK pathway by signaling though CRAF—a pathway that can be blocked to some extent by MEK inhibition, explaining in part the advantages seen with concurrent BRAF and MEK inhibition. Upregulation of various RTKs or their ligands have been reported in MAPK-inhibitor resistant melanoma, including PDGFRβ, IGF-R1, HGF, FGFR2, NRG1 and the EGFR family (EGFR, ERBB2, ERBB3, ERBB4) [112–116]. While melanoma cell populations that express high levels of RTK may be selected and expand following MAPK inhibitor treatment, FOXD3 is increased by BRAF inhibitor treatment and transcriptionally induces ERBB3 expression, providing an early adaptive resistance response in melanoma [117–119]. FOXD3 expression is epigenetically controlled during development by histone and DNA methylation and inhibition of the activating H3K9me3 mark by overexpression of histone lysine demethylase Kdm4a can reduce its expression [120].

Multiple RTKs can be co-expressed in resistant melanoma cells, with expression clusters involving EGFR, ERBB3, AXL and PDGFR being identified [37,121,122] leading to multiple resistance drivers that are difficult to target with single drugs. The RTK driven resistance is often difficult to overcome with RTK inhibitors, which vary in specificity and effect between different RTKs [121].

HDAC inhibitors could play a role in treating these melanomas with RTK driven resistance. HDAC inhibitors have been reported to downregulate a variety of RTK including EGFR, ERBB2, ERBB3, c-MET, IGF-1R [123–128], or induce EGFR inhibitor MIG-6 [129]. The effects of HDAC inhibitors on RTK suppression are dependent on the HDAC inhibitor used—trichostatin A (TSA) reduced EGFR expression by up-regulating miRNA-7 but SAHA did not have this affect [130]. Expression of any of HDAC1, 2 or 3 was sufficient to increase EGFR expression in colorectal cells, and in a similar fashion specific reduction of any of these individual HDAC proteins by shRNA could decrease EGFR expression, although not as potently as pan-HDAC inhibition by TSA or SAHA [124]. The inhibition of EGFR was caused by reduced histone acetylation and SP1 recruitment to the EGRF promoter [124].

4.5. Slow-Cycling, Drug Resistant Melanoma

Epigenetic modifiers may also benefit MAPK inhibitor treatment by killing off slowly cycling melanoma cells which are resistant to BRAF/MEK inhibition. Drug resistant populations of cells have been identified as having high levels of JARID1A, a histone demethylase that associates with HDAC and is associated with elevated IGF-1R expression and resistance to RAF inhibition. Although JARID1A is a histone demethylase, its association with HDAC means that it is affected by HDAC inhibitor treatment, and HDAC treatment increased death in response to the AZ628 RAF inhibitor as well as the emergence of resistant cells. The existence of these slow growing, drug-resistant side populations, which have also been reported to have expression of JARID1B has been reported by others [131–134] and might be effectively targeted by HDAC inhibitors, which also kill non-proliferating cells [135].

4.6. MITF Related Resistance Mechanisms

MITF is the master melanocytic regulator, a transcription factor that drives the expression of a suite of genes necessary for the formation of melanocytes and melanogenesis. Expression of MITF

in melanoma is variable and is driven by a number of factors including LEF/β-catenin and SOX10 in conjunction with CREB (cyclic AMP response element binding protein), a cyclic AMP responsive cofactor [136–138].

4.6.1. The MITF/NF-κB/AXL Axis

Melanomas with low levels of MITF are resistant to MAPK inhibitors and tend to exhibit high levels of NF-κB and AXL, while melanoma with high MITF levels exhibit low NF-κB activity and are more sensitive to MAPK inhibition [37,122,139]. AXL is not the main mediator of resistance in the MITF-low; NF-κB high cells, despite being able to confer resistance when overexpressed in MAPK-sensitive cell lines [137]. These MITF-low/NF-κB high cells may actually be regulated by high AP-1/TEAD transcription factor activity, which can confer resistance to MAPK inhibition [140].

Despite MITF high/NF-κB low melanoma showing sensitivity to MAPK inhibitors, the exact role of MITF in causing sensitivity to MAPK inhibitors is not clear as ectopic expression of MITF can also cause MAPK resistance [108,137] and NF-κB activity may be the main driver of resistance in melanoma naturally displaying the MITF low/NF-κB high phenotype [37]. If this is the case, treatment with Bromodomain and Extra Terminal inhibitors such as I-BET151 and JQ-1 potently suppress NF-κB signaling in melanoma, providing a rationale the use of these inhibitors in combination with MAPK inhibitors [54].

MITF activates the expression of anti-apoptotic genes BCL2A1, BCL2 and BIRC7 (MC-IAP/Livin) which may contribute to resistance that high MITF levels confer [65,136]. As MITF is normally suppressed by BRAF activity, a rebound in MITF levels following MAPK inhibition could be an early adaptive resistance.

4.6.2. HDAC Inhibitors and MITF

MITF may be modulated by a number of epigenetic factors. HDAC inhibitors can suppress MITF expression [141] and HDAC inhibitors have been shown to blunt the effectiveness of ectopic MITF driven resistance to MAPK inhibitors [137]. However the activity of MITF may be increased by HDAC inhibitors as the suppressor of MITF transcription HINT1 associates with HDAC1 and mSIN3a to repress transcription of MITF targets [142]. MITF activity may also be modulated by heterochromatin protein 1, a protein associated with heterochromatin formation, although the direction of MITF regulation was not consistent between all cell lines [143].

4.6.3. SWI/SNF and MITF

The SWI/SNF complex could be targeted to modulate MITF activity. The BRG1 containing SWI/SNF complex is required by MITF to activate melanocyte specific genes as MITF associates with SWI/SNF which increases chromatin accessibility at MITF target genes [59,144]. The SWI/SNF complex is also required to activate transcription of MITF itself, demonstrating the importance of the SWI/SNF complex in MITF processes [145]. BRG1 may be increased during melanoma progression [101,146], potentially causing a shift of MITF target genes towards anti-apoptotic signaling via BIRC7/ML-IAP, although conflicting reports exist about BRG1 expression in melanoma progression [147]. Another member of the SWI/SNF complex, SNF5 is also lost in melanoma progression and correlates to poor survival [148].

SWI/SNF complexes contain either BRG1 or BRM, which confer slightly different target specificity [59]. BRAF inhibits BRM expression and enhances BRG1 expression and following BRAF inhibition BRM is increased and BRG1 is reduced. BRAF inhibition leads to an increase in histone H4 acetylation at the BRM promoter (as did HDAC inhibitor treatment). HDAC3 and HDAC9 have been shown to regulate BRM expression—thus, linking HDAC with SWI/SNF [149]. It is likely that increases in BRM and an altered ratio of BRM:BRG1 expression following MAPK inhibition alters the transcriptional program of MITF leading to early alterations in drug sensitivity.

5. Overcoming Resistance to Immunotherapy

Melanoma cells have all the hallmarks of cells that should be eradicated by the immune system. They have a high mutation rate (the highest of all cancers [8]) and express a range of novel antigens, such as the cancer-testis-antigen family, which should provide plenty of antigens to attract the immune system. Tumor infiltrating lymphocytes are also frequently observed in melanoma tissue, indicating the presence of immune cells—and yet the immune system fails to kill the melanoma cells. This immune evasion is due to two main processes—inactivation of immune attack and resistance to apoptosis elicited by immune attack.

Immunotherapy against PD-1 and CTLA-4 targets two mechanisms that allow melanoma to evade immune attack—PD-L1 expression on melanoma cells to inactivate cytotoxic T-cells and CTLA-4 expression on lymphocytes which inhibits T-cell activation. The relatively high levels of primary resistance seen in approximately 30% of patients, as well as development of secondary resistance to these immune therapies in approximately 30%–40% of patients [150] is a major clinical problem and the causes of resistance are still being elucidated. Some resistance mechanisms are known and are driven by the biology of the melanoma, including downregulation of MHC molecules, loss of antigen expression, negative feedback causing PD-L1 upregulation in response to INF-γ production by T-cells and reduction in chemokine expression including CCL3, CSCL1, CXCL2 and CCL4 [151]. While these resistance mechanisms may be targeted by epigenetic modifiers (Figure 3), immune cells will also be affected by any drugs that are systemically administered and the effects may not always be advantageous.

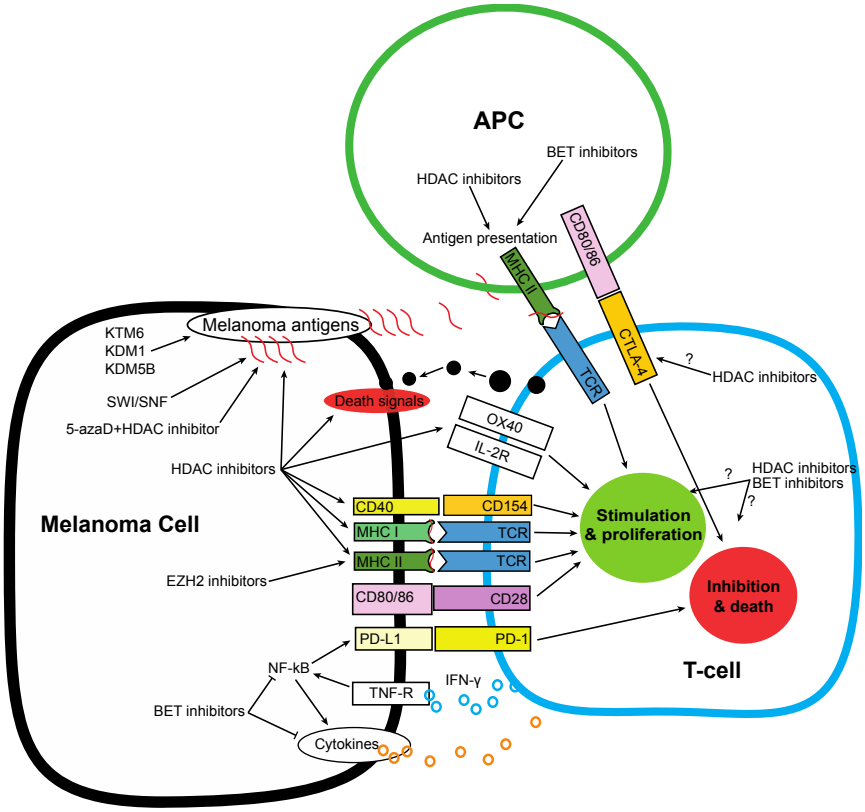

Figure 3. Melanoma cells evade immune attack using a number of mechanisms. Inhibitors of CTLA-4 and PD-1/PD-L1 have shown success in the clinic but some melanoma tumors are resistant. Epigenetic modulators can stimulate immune killing of melanoma by modulating antigen presentation, receptor expression and cytokine production in both melanoma and immune cells. However the effect of epigenetic modulators on immune cell viability and function is not fully understood and the effects differ for different immune cells. This makes the net result of many epigenetic modulators on anti-tumor immune function difficult to predict.

5.1. Anti-Apoptotic Signaling Mediates Resistance to Immunotherapy

The general resistance of melanoma cells to apoptosis not only enables them to resist MAPK inhibitors, but also contributes to resistance to immunotherapy. Cells selected to be resistant to BRAF inhibition were also cross resistant to CTL and NK cell-death [152]. HDAC inhibitor restored sensitivity by shifting the balance of apoptotic protein towards apoptosis. This shows that histone modifiers can be used in the same way in immunotherapy as they might be for MAPK inhibitor therapy—to shift the balance towards a more pro-apoptotic state in the melanoma. A more direct extension of this is the increase in TRAIL sensitivity that HDAC inhibitors induce. TRAIL is used by T lymphocytes and NK cells to kill melanoma cells and TRAIL-resistance is another immune evasion mechanism [153]. Pan-HDAC inhibitors sensitize melanoma cells to TRAIL-induced death by down-regulating the inhibitory c-FLIP, increasing expression of TRAIL receptors such as DR4 and DR5 and shifting in expression of apoptotic proteins towards a state more permissive to apoptosis [92,94,99,154,155].

5.2. HDAC Inhibitors and Immune Function

While histone deacetylase inhibitors may drive melanoma toward an apoptotic state, they have a number of immune inhibitory effects [156]. HDAC inhibitors have been viewed as immunosuppressive, causing side effects such as lymphopenia, leukopenia, neutropenia, and thrombocytopenia, and are cytotoxic to PBMC's at IC50 concentrations lower than melanoma cells, suggesting that they would negatively impact immunotherapy [157–161]. However, this view is no longer certain as HDAC inhibitors have a number of potentially beneficial effects and an increasing number of studies show HDAC inhibitors can improve immune related killing of melanoma.

HDAC inhibitors were shown to increase tumor immunogenicity as shown by studies on panobinostat [162]. Panobinostat enhanced the proliferation, retention and polyfunctional status of tumor specific T-cells in the B16 mouse model and induced higher IL-2R (CD25) and co-stimulatory OX-40 receptors in T-cells [163]. In these studies panobinostat improved the effectiveness of gp100 specific T-cell immunotherapy and maintained systemic pro-inflammatory levels. HDAC inhibitors may specifically enhance T-cell survival and function and can prevent activation-induced death of tumor infiltrating lymphocytes, promoting anti-tumor immunity [164]. These effects were predominantly mediated by CD4+ T-cells and enhanced memory T-cell populations. Activation of T-cells could also be targeted by HDAC inhibitors as the CTLA4 promoter is differentially acetylated in CD4+ *vs.* CD8+ T cells and may be able to be targeted to alter activation of these T-cell populations [165]. Cytokine production is also altered by HDAC inhibitors: IL-24, which activates monocytes and Th2 cells, is lost during melanoma progression but is re-expressed following HDAC inhibitor treatment [166].

HDAC inhibitors have also been used to enhance vaccine strategies. Depsipeptide promoted immune killing of B16/F10 melanoma cells [167] and only mice vaccinated with TSA treated B16 melanoma cells were effectively vaccinated from subsequent B16 tumor challenge [168,169]. In these studies, HDAC inhibitor treatment enhanced the expression of MHC class II, CD40 and B7-1/2 on B16 cells and vaccination with HDAC inhibitor-treated melanoma cells elicited tumor specific immunity in both prevention and treatment models. Cytotoxic and IFN-γ-producing cells were identified in splenocytes and CD4+, CD8+ T cells and NK cells were all involved in the induction of immunity. Apoptotic cells derived from HDAC inhibitor treatments, but not H_2O_2, significantly enhanced the effectiveness of the vaccine.

5.3. MHC Expression

Melanoma cells decrease MHC class I and II expression to evade immune response. The decrease of MHC class I expression in melanoma can be due to a transcriptional repression resulting from epigenetic modifications [170]. Histone deacetylases upregulate MHC class I expression in murine

and human melanoma cells [170–174]. While class I HDAC are reported to be involved in MHC class I expression [174], the class IIb HDAC6 seems also involved as specific inhibition of HDAC6 via genetic means or with small molecule inhibitors Nexturastat A or Tubastatin A increased MHC class I expression in melanoma [175].

MHC class II, which present exogenous proteins following endocytosis can be downregulated in melanoma. HDAC treatment of B16 melanoma cells and human melanoma upregulated MHC class II expression and allowed them to become antigen presenting cells [169,174,176].

EZH2 causes H3K27me3 marks on the MHC2TA gene, leading to downregulation of MHC class II genes that may dampen the anti-tumor immune response [177,178]. Supporting this hypothesis, EZH2 has been implicated in the activation and maintenance of regulatory T-cells that suppress the immune system [179,180]. Histone methylation has also been reported to be an important determinant of cancer-testis antigen (NY-ESO-1, MAGEA1, MAGE-A3) expression in lung cancer, and repression of histone methyltransferase KMT6 and demethylases KDM1 and KDM5B induced antigen expression [181]. These antigens are commonly expressed in melanoma and may be similarly affected by histone methylation.

5.4. Expression and Presentation of Melanoma Antigens

A number of epigenetic modifiers have been shown to increase antigen exposure on melanoma. Antigen expression can be lost by melanoma, contributing to its immune evasion [182,183]. Depsipeptide, which preferentially inhibits class I HDAC, augmented NY-ESO-1 expression following 5-AzadC treatment (but not by itself) in melanoma and increased subsequent killing by CTL [184]. Likewise, pan-HDAC inhibitor TSA increased 5-aza-2′-deoxycytidine-induced expression of MAGE-A1 -A2, -A3 and -A12 genes. Examination of melanoma showed that MAGE-A is silenced by DNA hypermethylation and histone deacetylation [185]. Specific inhibition of class IIb HDAC6 can also increase tumor associated antigen production, such as gp100, Mart1 and Tryp1/2 in both human and murine melanoma cells [175]. Inhibition of HDAC11, the sole member of the HDAC IV family, can also increase antigen presentation by APCs [186]. Relatively little is known about HDAC11 but its expression is limited to relatively few tissues, making it an interesting target for immunotherapy. As well as increased antigen presentation, HDAC inhibitors may allow increased immune infiltration to the tumors as they increase ICAM-1 expression in tumor endothelial cells, increasing leucocyte interaction and infiltration [187].

5.5. SWI/SNF and Immunotherapy

SWI/SNF is also a potential target for immunomodulation. As SWI/SNF modulates melanocyte/melanoma specific genes, enhanced SWI/SNF may allow enhanced expression of melanoma specific antigens [144]. Melanomas undergoing the switch to a mesenchymal state are more able to escape T-cell immunity [188].

5.6. Histone Reader Proteins and Immunotherapy

Histone reader proteins may also have a role to play in immunotherapy, although like HDAC inhibitors, they also may negative impact on the immune system. BET inhibitor I-BET151 is a potent inhibitor of NF-κB activity and cytokine production in melanoma [54] and prevents the induction of PD-L1 expression on melanoma cells treated with IFN-γ [189]. The reduction in both PD-L1 and anti-inflammatory cytokine production could increase immune attack on melanoma. BET inhibitors may also have beneficial effects on immune function. BET inhibitor JQ1 increased inflammatory response of antigen presenting cells (APCs) by reducing expression PD-L1 and anti-inflammatory cytokines [190]. This lead to APC activation, increased priming of naïve CD4+ T-cells and restored responsiveness of tolerized CD4+ cells [190]. The positive effects of BET inhibition may still be offset by their adverse effects on immune function however. BET inhibitors suppress inflammation [191], macrophage cytokine production [192] and reduce dendritic cell (DC) maturation and function by

inhibiting STAT5, thereby reducing DC-stimulation of CD4+ and CD8+ T-cell proliferation and function, leading to reduced T-cell response [193]. Early clinical use of BET inhibitor OTX015 show it is tolerated at clinically relevant doses, but can cause reversible neutropenia and thrombocytopenia, highlighting potentially negative effects on the immune system [194].

6. Future Directions

It is clear from the above review that treatment with targeted and immunotherapies involve a number of epigenetic changes that may be exploited to increase the effectiveness of these treatments in melanoma. In the case of targeted therapies the use of HDAC inhibitors alone or with BET protein inhibitors appears to be a promising approach to overcome a range of resistance mechanisms. Similarly preclinical studies on immunotherapy provide exciting opportunities to use inhibitors of HDACs, EZH2 and BET proteins to overcome resistance to immunotherapy induced by immune checkpoint inhibitors and to reverse resistance in ongoing treatments. Studies to build on the promising advances reviewed above are now needed.

Acknowledgments: This work was supported by program grant 633004 from the Australian National Health and Medical Research Council (NHMRC).

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Hersey, P.; Bastholt, L.; Chiarion-Sileni, V.; Cinat, G.; Dummer, R.; Eggermont, A.M.; Espinosa, E.; Hauschild, A.; Quirt, I.; Robert, C.; *et al*. Small molecules and targeted therapies in distant metastatic disease. *Ann. Oncol.* **2009**, *20*, vi35–vi40. [CrossRef] [PubMed]
2. Dhomen, N.; Marais, R. Braf signaling and targeted therapies in melanoma. *Hematol. Oncol. Clin. North Am.* **2009**, *23*, 529–545. [CrossRef] [PubMed]
3. Larkin, J.; Ascierto, P.A.; Dreno, B.; Atkinson, V.; Liszkay, G.; Maio, M.; Mandala, M.; Demidov, L.; Stroyakovskiy, D.; Thomas, L.; *et al*. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N. Engl. J. Med.* **2014**, *371*, 1867–1876. [CrossRef] [PubMed]
4. Robert, C.; Karaszewska, B.; Schachter, J.; Rutkowski, P.; Mackiewicz, A.; Stroiakovski, D.; Lichinitser, M.; Dummer, R.; Grange, F.; Mortier, L.; *et al*. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N. Engl. J. Med.* **2015**, *372*, 30–39. [CrossRef] [PubMed]
5. Flaherty, K.T.; Infante, J.R.; Daud, A.; Gonzalez, R.; Kefford, R.F.; Sosman, J.; Hamid, O.; Schuchter, L.; Cebon, J.; Ibrahim, N.; *et al*. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N. Engl. J. Med.* **2012**, *29*, 29. [CrossRef] [PubMed]
6. 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] [PubMed]
7. Flaherty, K.T.; Robert, C.; Hersey, P.; Nathan, P.; Garbe, C.; Milhem, M.; Demidov, L.V.; Hassel, J.C.; Rutkowski, P.; Mohr, P.; *et al*. Improved survival with MEK inhibition in BRAF-mutated melanoma. *N. Engl. J. Med.* **2012**, *367*, 107–114. [CrossRef] [PubMed]
8. Lawrence, M.S.; Stojanov, P.; Polak, P.; Kryukov, G.V.; Cibulskis, K.; Sivachenko, A.; Carter, S.L.; Stewart, C.; Mermel, C.H.; Roberts, S.A.; *et al*. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* **2013**, *499*, 214–218. [CrossRef] [PubMed]
9. Hersey, P.; Gallagher, S. A focus on PD-l1 in human melanoma. *Clin. Cancer Res.* **2013**, *19*, 514–516. [CrossRef] [PubMed]
10. Postow, M.A.; Chesney, J.; Pavlick, A.C.; Robert, C.; Grossmann, K.; McDermott, D.; Linette, G.P.; Meyer, N.; Giguere, J.K.; Agarwala, S.S.; *et al*. Nivolumab and ipilimumab *versus* ipilimumab in untreated melanoma. *N. Engl. J. Med.* **2015**, *372*, 2006–2017. [CrossRef] [PubMed]

11. Larkin, J.; Chiarion-Sileni, V.; Gonzalez, R.; Grob, J.J.; Cowey, C.L.; Lao, C.D.; Schadendorf, D.; Dummer, R.; Smylie, M.; Rutkowski, P.; *et al*. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N. Engl. J. Med.* **2015**, *373*, 23–34. [CrossRef] [PubMed]
12. Ribas, A.; Puzanov, I.; Dummer, R.; Schadendorf, D.; Hamid, O.; Robert, C.; Hodi, F.S.; Schachter, J.; Pavlick, A.C.; Lewis, K.D.; *et al*. Pembrolizumab *versus* investigator-choice chemotherapy for ipilimumab-refractory melanoma (keynote-002): A randomised, controlled, Phase 2 trial. *Lancet Oncol.* **2015**, *16*, 908–918. [CrossRef]
13. Robert, C.; Schachter, J.; Long, G.V.; Arance, A.; Grob, J.J.; Mortier, L.; Daud, A.; Carlino, M.S.; McNeil, C.; Lotem, M.; *et al*. Pembrolizumab *versus* ipilimumab in advanced melanoma. *N. Engl. J. Med.* **2015**, *372*, 2521–2532. [CrossRef] [PubMed]
14. Hodi, F.S.; O'Day, S.J.; McDermott, D.F.; Weber, R.W.; Sosman, J.A.; Haanen, J.B.; Gonzalez, R.; Robert, C.; Schadendorf, D.; Hassel, J.C.; *et al*. Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **2010**, *363*, 711–723. [CrossRef] [PubMed]
15. Hersey, P.; Gallagher, S.; Mijatov, B. Overcoming resistance of melanoma to immunotherapy with monoclonal antibodies against checkpoints inhibitors. In *Resistance to Immunotherapeutic Antibodies in Cancer*; Bonavida, B., Ed.; Springer: New York, NY, USA, 2013; Volume 2, pp. 143–155.
16. Wolchok, J.D.; Kluger, H.; Callahan, M.K.; Postow, M.A.; Rizvi, N.A.; Lesokhin, A.M.; Segal, N.H.; Ariyan, C.E.; Gordon, R.A.; Reed, K.; *et al*. Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J. Med.* **2013**, *2*, 2. [CrossRef] [PubMed]
17. Hersey, P.; Gowrishankar, K. Pembrolizumab joins the anti-PD-1 armamentarium in the treatment of melanoma. *Future Oncol.* **2015**, *11*, 133–140. [CrossRef]
18. Tumeh, P.C.; Harview, C.L.; Yearley, J.H.; Shintaku, I.P.; Taylor, E.J.; Robert, L.; Chmielowski, B.; Spasic, M.; Henry, G.; Ciobanu, V.; *et al*. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **2014**, *515*, 568–571. [CrossRef] [PubMed]
19. Spagnolo, F.; Ghiorzo, P.; Queirolo, P. Overcoming resistance to BRAF inhibition in BRAF-mutated metastatic melanoma. *Oncotarget* **2014**, *5*, 10206–10221. [CrossRef] [PubMed]
20. Little, A.S.; Smith, P.D.; Cook, S.J. Mechanisms of acquired resistance to erk1/2 pathway inhibitors. *Oncogene* **2013**, *32*, 1207–1215. [CrossRef] [PubMed]
21. Jones, P.A. Functions of DNA methylation: Islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.* **2012**, *13*, 484–492. [CrossRef] [PubMed]
22. Fratta, E.; Sigalotti, L.; Covre, A.; Parisi, G.; Coral, S.; Maio, M. Epigenetics of melanoma: Implications for immune-based therapies. *Immunotherapy* **2013**, *5*, 1103–1116. [CrossRef] [PubMed]
23. Lee, J.J.; Murphy, G.F.; Lian, C.G. Melanoma epigenetics: Novel mechanisms, markers, and medicines. *Lab. Investig.* **2014**, *94*, 822–838. [CrossRef] [PubMed]
24. Lian, C.G.; Xu, Y.; Ceol, C.; Wu, F.; Larson, A.; Dresser, K.; Xu, W.; Tan, L.; Hu, Y.; Zhan, Q.; *et al*. Loss of 5-hydroxymethylcytosine is an epigenetic hallmark of melanoma. *Cell* **2012**, *150*, 1135–1146. [CrossRef] [PubMed]
25. Ram, O.; Goren, A.; Amit, I.; Shoresh, N.; Yosef, N.; Ernst, J.; Kellis, M.; Gymrek, M.; Issner, R.; Coyne, M.; *et al*. Combinatorial patterning of chromatin regulators uncovered by genome-wide location analysis in human cells. *Cell* **2011**, *147*, 1628–1639. [CrossRef] [PubMed]
26. Strahl, B.D.; Allis, C.D. The language of covalent histone modifications. *Nature* **2000**, *403*, 41–45. [CrossRef] [PubMed]
27. Yun, M.; Wu, J.; Workman, J.L.; Li, B. Readers of histone modifications. *Cell Res.* **2011**, *21*, 564–578. [CrossRef] [PubMed]
28. Bannister, A.J.; Kouzarides, T. Regulation of chromatin by histone modifications. *Cell Res.* **2011**, *21*, 381–395. [CrossRef] [PubMed]
29. Venza, M.; Visalli, M.; Biondo, C.; Lentini, M.; Catalano, T.; Teti, D.; Venza, I. Epigenetic regulation of P14ARF and P16INK4A expression in cutaneous and uveal melanoma. *Biochim. Biophys. Acta* **2015**, *1849*, 247–256. [CrossRef] [PubMed]
30. Valentini, A.; Gravina, P.; Federici, G.; Bernardini, S. Valproic acid induces apoptosis, P16INK4A upregulation and sensitization to chemotherapy in human melanoma cells. *Cancer Biol. Ther.* **2007**, *6*, 185–191. [CrossRef] [PubMed]

31. Bolden, J.E.; Peart, M.J.; Johnstone, R.W. Anticancer activities of histone deacetylase inhibitors. *Nat. Rev. Drug Discov.* **2006**, *5*, 769–784. [CrossRef] [PubMed]
32. Dokmanovic, M.; Clarke, C.; Marks, P.A. Histone deacetylase inhibitors: Overview and perspectives. *Mol. Cancer Res.* **2007**, *5*, 981–989. [CrossRef]
33. Glozak, M.A.; Seto, E. Histone deacetylases and cancer. *Oncogene* **2007**, *26*, 5420–5432. [CrossRef] [PubMed]
34. Ropero, S.; Fraga, M.F.; Ballestar, E.; Hamelin, R.; Yamamoto, H.; Boix-Chornet, M.; Caballero, R.; Alaminos, M.; Setien, F.; Paz, M.F.; *et al*. A truncating mutation of HDAC2 in human cancers confers resistance to histone deacetylase inhibition. *Nat. Genet.* **2006**, *38*, 566–569. [CrossRef] [PubMed]
35. Ozdag, H.; Teschendorff, A.E.; Ahmed, A.A.; Hyland, S.J.; Blenkiron, C.; Bobrow, L.; Veerakumarasivam, A.; Burtt, G.; Subkhankulova, T.; Arends, M.J.; *et al*. Differential expression of selected histone modifier genes in human solid cancers. *BMC Genom.* **2006**, *7*, 90. [CrossRef] [PubMed]
36. Wilmott, J.S.; Colebatch, A.J.; Kakavand, H.; Shang, P.; Carlino, M.S.; Thompson, J.F.; Long, G.V.; Scolyer, R.A.; Hersey, P. Expression of the class 1 histone deacetylases HDAC8 and 3 are associated with improved survival of patients with metastatic melanoma. *Mod. Pathol.* **2015**, *28*, 884–894. [CrossRef] [PubMed]
37. Konieczkowski, D.J.; Johannessen, C.M.; Abudayyeh, O.; Kim, J.W.; Cooper, Z.A.; Piris, A.; Frederick, D.T.; Barzily-Rokni, M.; Straussman, R.; Haq, R.; *et al*. A melanoma cell state distinction influences sensitivity to mapk pathway inhibitors. *Cancer Discov.* **2014**, *4*, 816–827. [CrossRef] [PubMed]
38. Yu, X.; Guo, Z.S.; Marcu, M.G.; Neckers, L.; Nguyen, D.M.; Chen, G.A.; Schrump, D.S. Modulation of P53, ERBB1, ERBB2, and RAF-1 expression in lung cancer cells by depsipeptide FR901228. *J. Natl. Cancer Inst.* **2002**, *94*, 504–513. [CrossRef] [PubMed]
39. Juan, L.J.; Shia, W.J.; Chen, M.H.; Yang, W.M.; Seto, E.; Lin, Y.S.; Wu, C.W. Histone deacetylases specifically down-regulate p53-dependent gene activation. *J. Biol. Chem.* **2000**, *275*, 20436–20443. [CrossRef] [PubMed]
40. Blagosklonny, M.V.; Trostel, S.; Kayastha, G.; Demidenko, Z.N.; Vassilev, L.T.; Romanova, L.Y.; Bates, S.; Fojo, T. Depletion of mutant P53 and cytotoxicity of histone deacetylase inhibitors. *Cancer Res.* **2005**, *65*, 7386–7392. [CrossRef] [PubMed]
41. Ashburner, B.P.; Westerheide, S.D.; Baldwin, A.S., Jr. The p65 (RELA) subunit of NF-κB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. *Mol. Cell. Biol.* **2001**, *21*, 7065–7077. [CrossRef] [PubMed]
42. Chen, L.; Fischle, W.; Verdin, E.; Greene, W.C. Duration of nuclear NF-κB action regulated by reversible acetylation. *Science* **2001**, *293*, 1653–1657. [CrossRef] [PubMed]
43. Chen, C.; Edelstein, L.C.; Gelinas, C. The rel/NF-κB family directly activates expression of the apoptosis inhibitor BCL-X(L). *Mol. Cell. Biol.* **2000**, *20*, 2687–2695. [CrossRef] [PubMed]
44. Glozak, M.A.; Sengupta, N.; Zhang, X.; Seto, E. Acetylation and deacetylation of non-histone proteins. *Gene* **2005**, *363*, 15–23. [CrossRef] [PubMed]
45. Sadoul, K.; Boyault, C.; Pabion, M.; Khochbin, S. Regulation of protein turnover by acetyltransferases and deacetylases. *Biochimie* **2008**, *90*, 306–312. [CrossRef] [PubMed]
46. Tiffen, J.; Gallagher, S.J.; Hersey, P. EZH2: An emerging role in melanoma biology and strategies for targeted therapy. *Pigment Cell Melanoma Res.* **2015**, *28*, 21–30. [CrossRef] [PubMed]
47. 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]
48. Tiffen, J.C.; Gunatilake, D.; Gallagher, S.J.; Gowrishankar, K.; Heinemann, A.; Cullinane, C.; Dutton-Regester, K.; Pupo, G.M.; Strbenac, D.; Yang, J.Y.; *et al*. Targeting activating mutations of EZH2 leads to potent cell growth inhibition in human melanoma by derepression of tumor suppressor genes. *Oncotarget* **2015**. [PubMed]
49. Barsotti, A.M.; Ryskin, M.; Zhong, W.; Zhang, W.G.; Giannakou, A.; Loreth, C.; Diesl, V.; Follettie, M.; Golas, J.; Lee, M.; *et al*. Epigenetic reprogramming by tumor-derived EZH2 gain-of-function mutations promotes aggressive 3D cell morphologies and enhances melanoma tumor growth. *Oncotarget* **2015**, *6*, 2928–2938. [CrossRef] [PubMed]

50. Zingg, D.; Debbache, J.; Schaefer, S.M.; Tuncer, E.; Frommel, S.C.; Cheng, P.; Arenas-Ramirez, N.; Haeusel, J.; Zhang, Y.; Bonalli, M.; *et al*. The epigenetic modifier EZH2 controls melanoma growth and metastasis through silencing of distinct tumour suppressors. *Nat. Commun.* **2015**, *6*, 6051. [CrossRef] [PubMed]
51. Dawson, M.A.; Kouzarides, T.; Huntly, B.J. Targeting epigenetic readers in cancer. *N. Engl. J. Med.* **2012**, *367*, 647–657. [CrossRef] [PubMed]
52. Segura, M.F.; Fontanals-Cirera, B.; Gaziel-Sovran, A.; Guijarro, M.V.; Hanniford, D.; Zhang, G.; Gonzalez-Gomez, P.; Morante, M.; Jubierre, L.; Zhang, W.; *et al*. BRD4 sustains melanoma proliferation and represents a new target for epigenetic therapy. *Cancer Res.* **2013**, *73*, 6264–6276. [CrossRef] [PubMed]
53. Vardabasso, C.; Gaspar-Maia, A.; Hasson, D.; Punzeler, S.; Valle-Garcia, D.; Straub, T.; Keilhauer, E.C.; Strub, T.; Dong, J.; Panda, T.; *et al*. Histone variant H2A.Z.2 mediates proliferation and drug sensitivity of malignant melanoma. *Mol. Cell* **2015**, *59*, 75–88. [CrossRef] [PubMed]
54. Gallagher, S.J.; Mijatov, B.; Gunatilake, D.; Gowrishankar, K.; Tiffen, J.; James, W.; Jin, L.; Pupo, G.; Cullinane, C.; McArthur, G.A.; *et al*. Control of NF-κB activity in human melanoma by bromodomain and extra-terminal protein inhibitor I-BET151. *Pigment Cell Melanoma Res.* **2014**, *27*, 1126–1137. [CrossRef] [PubMed]
55. Gallagher, S.J.; Mijatov, B.; Gunatilake, D.; Tiffen, J.C.; Gowrishankar, K.; Jin, L.; Pupo, G.M.; Cullinane, C.; Prinjha, R.K.; Smithers, N.; *et al*. The epigenetic regulator I-BET151 induces BIM-dependent apoptosis and cell cycle arrest of human melanoma cells. *J. Investig. Dermatol.* **2014**, *134*, 2795–2805. [CrossRef] [PubMed]
56. Campos, E.I.; Cheung, K.J., Jr.; Murray, A.; Li, S.; Li, G. The novel tumour suppressor gene ING1 is overexpressed in human melanoma cell lines. *Br. J. Dermatol.* **2002**, *146*, 574–580. [CrossRef] [PubMed]
57. Mehrotra, A.; Mehta, G.; Aras, S.; Trivedi, A.; de la Serna, I.L. SWI/SNF chromatin remodeling enzymes in melanocyte differentiation and melanoma. *Crit. Rev. Eukaryot. Gene Expr.* **2014**, *24*, 151–161. [CrossRef] [PubMed]
58. Bitler, B.G.; Aird, K.M.; Garipov, A.; Li, H.; Amatangelo, M.; Kossenkov, A.V.; Schultz, D.C.; Liu, Q.; Shih Ie, M.; Conejo-Garcia, J.R.; *et al*. Synthetic lethality by targeting EZH2 methyltransferase activity in arid1a-mutated cancers. *Nat. Med.* **2015**, *21*, 231–238. [CrossRef] [PubMed]
59. Keenen, B.; Qi, H.; Saladi, S.V.; Yeung, M.; de la Serna, I.L. Heterogeneous SWI/SNF chromatin remodeling complexes promote expression of microphthalmia-associated transcription factor target genes in melanoma. *Oncogene* **2010**, *29*, 81–92. [CrossRef] [PubMed]
60. Qadeer, Z.A.; Harcharik, S.; Valle-Garcia, D.; Chen, C.; Birge, M.B.; Vardabasso, C.; Duarte, L.F.; Bernstein, E. Decreased expression of the chromatin remodeler atrx associates with melanoma progression. *J. Investig. Dermatol.* **2014**, *134*, 1768–1772. [CrossRef] [PubMed]
61. Vardabasso, C.; Hasson, D.; Ratnakumar, K.; Chung, C.Y.; Duarte, L.F.; Bernstein, E. Histone variants: Emerging players in cancer biology. *Cell. Mol. Life Sci.* **2014**, *71*, 379–404. [CrossRef] [PubMed]
62. Kapoor, A.; Goldberg, M.S.; Cumberland, L.K.; Ratnakumar, K.; Segura, M.F.; Emanuel, P.O.; Menendez, S.; Vardabasso, C.; Leroy, G.; Vidal, C.I.; *et al*. The histone variant MACROH2A suppresses melanoma progression through regulation of CDK8. *Nature* **2010**, *468*, 1105–1109. [CrossRef] [PubMed]
63. Draker, R.; Ng, M.K.; Sarcinella, E.; Ignatchenko, V.; Kislinger, T.; Cheung, P. A combination of H2A.Z and H4 acetylation recruits BRD2 to chromatin during transcriptional activation. *PLoS Genet.* **2012**, *8*, e1003047. [CrossRef] [PubMed]
64. Duarte, L.F.; Young, A.R.; Wang, Z.; Wu, H.A.; Panda, T.; Kou, Y.; Kapoor, A.; Hasson, D.; Mills, N.R.; Ma'ayan, A.; *et al*. Histone H3.3 and its proteolytically processed form drive a cellular senescence programme. *Nat. Commun.* **2014**, *5*, 5210. [CrossRef] [PubMed]
65. Hartman, M.L.; Czyz, M. Anti-apoptotic proteins on guard of melanoma cell survival. *Cancer Lett.* **2013**, *331*, 24–34. [CrossRef] [PubMed]
66. Galluzzi, L.; Vitale, I.; Abrams, J.M.; Alnemri, E.S.; Baehrecke, E.H.; Blagosklonny, M.V.; Dawson, T.M.; Dawson, V.L.; El-Deiry, W.S.; Fulda, S.; *et al*. Molecular definitions of cell death subroutines: Recommendations of the nomenclature committee on cell death 2012. *Cell Death Differ.* **2012**, *19*, 107–120. [CrossRef] [PubMed]
67. Llambi, F.; Green, D.R. Apoptosis and oncogenesis: Give and take in the BCL-2 family. *Curr. Opin. Genet. Dev.* **2011**, *21*, 12–20. [CrossRef] [PubMed]

68. Delbridge, A.R.; Strasser, A. The BCL-2 protein family, BH3-mimetics and cancer therapy. *Cell Death Differ.* **2015**, *22*, 1071–1080. [CrossRef] [PubMed]
69. Chipuk, J.E.; Green, D.R. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? *Trends Cell Biol.* **2008**, *18*, 157–164. [CrossRef] [PubMed]
70. Mohana-Kumaran, N.; Hill, D.S.; Allen, J.D.; Haass, N.K. Targeting the intrinsic apoptosis pathway as a strategy for melanoma therapy. *Pigment Cell Melanoma Res.* **2014**, *27*, 525–539. [CrossRef] [PubMed]
71. Hussein, M.R.; Haemel, A.K.; Wood, G.S. Apoptosis and melanoma: Molecular mechanisms. *J. Pathol.* **2003**, *199*, 275–288. [CrossRef] [PubMed]
72. Tang, L.; Tron, V.A.; Reed, J.C.; Mah, K.J.; Krajewska, M.; Li, G.; Zhou, X.; Ho, V.C.; Trotter, M.J. Expression of apoptosis regulators in cutaneous malignant melanoma. *Clin. Cancer Res.* **1998**, *4*, 1865–1871. [PubMed]
73. Selzer, E.; Schlagbauer-Wadl, H.; Okamoto, I.; Pehamberger, H.; Potter, R.; Jansen, B. Expression of BCL-2 family members in human melanocytes, in melanoma metastases and in melanoma cell lines. *Melanoma Res.* **1998**, *8*, 197–203. [CrossRef] [PubMed]
74. Zhuang, L.; Lee, C.S.; Scolyer, R.A.; McCarthy, S.W.; Zhang, X.D.; Thompson, J.F.; Hersey, P. MCL-1, BCL-XL and STAT3 expression are associated with progression of melanoma whereas BCL-2, AP-2 and mitf levels decrease during progression of melanoma. *Mod. Pathol.* **2007**, *20*, 416–426. [CrossRef] [PubMed]
75. Boisvert-Adamo, K.; Longmate, W.; Abel, E.V.; Aplin, A.E. Mcl-1 is required for melanoma cell resistance to anoikis. *Mol. Cancer Res.* **2009**, *7*, 549–556. [CrossRef] [PubMed]
76. Bergamaschi, D. Is MCL-1l the new anti-apoptotic effector of B-RAFV(600e) in melanoma? *Exp. Dermatol.* **2014**, *23*, 94. [CrossRef] [PubMed]
77. McKee, C.S.; Hill, D.S.; Redfern, C.P.; Armstrong, J.L.; Lovat, P.E. Oncogenic BRAF signalling increases mcl-1 expression in cutaneous metastatic melanoma. *Exp. Dermatol.* **2013**, *22*, 767–769. [CrossRef] [PubMed]
78. Wang, Y.F.; Jiang, C.C.; Kiejda, K.A.; Gillespie, S.; Zhang, X.D.; Hersey, P. Apoptosis induction in human melanoma cells by inhibition of mek is caspase-independent and mediated by the BCL-2 family members puma, bim, and mcl-1. *Clin. Cancer Res.* **2007**, *13*, 4934–4942. [CrossRef] [PubMed]
79. Skvara, H.; Thallinger, C.; Wacheck, V.; Monia, B.P.; Pehamberger, H.; Jansen, B.; Selzer, E. MCL-1 blocks radiation-induced apoptosis and inhibits clonogenic cell death. *Anticancer Res.* **2005**, *25*, 2697–2703. [PubMed]
80. Jiang, C.C.; Lai, F.; Tay, K.H.; Croft, A.; Rizos, H.; Becker, T.M.; Yang, F.; Liu, H.; Thorne, R.F.; Hersey, P.; *et al*. Apoptosis of human melanoma cells induced by inhibition of B-RAFV600E involves preferential splicing of bims. *Cell Death Dis.* **2010**, *1*, e69. [CrossRef]
81. Goldstein, N.B.; Johannes, W.U.; Gadeliya, A.V.; Green, M.R.; Fujita, M.; Norris, D.A.; Shellman, Y.G. Active N-RAS and B-RAF inhibit anoikis by downregulating bim expression in melanocytic cells. *J. Investig. Dermatol.* **2009**, *129*, 432–437. [CrossRef] [PubMed]
82. Cartlidge, R.A.; Thomas, G.R.; Cagnol, S.; Jong, K.A.; Molton, S.A.; Finch, A.J.; McMahon, M. Oncogenic BRAF(v600e) inhibits bim expression to promote melanoma cell survival. *Pigment Cell Melanoma Res.* **2008**, *21*, 534–544. [CrossRef] [PubMed]
83. VanBrocklin, M.W.; Verhaegen, M.; Soengas, M.S.; Holmen, S.L. Mitogen-activated protein kinase inhibition induces translocation of bmf to promote apoptosis in melanoma. *Cancer Res.* **2009**, *69*, 1985–1994. [CrossRef] [PubMed]
84. Tsai, J.; Lee, J.T.; Wang, W.; Zhang, J.; Cho, H.; Mamo, S.; Bremer, R.; Gillette, S.; Kong, J.; Haass, N.K.; *et al*. Discovery of a selective inhibitor of oncogenic B-RAF kinase with potent antimelanoma activity. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 3041–3046. [CrossRef] [PubMed]
85. Hingorani, S.R.; Jacobetz, M.A.; Robertson, G.P.; Herlyn, M.; Tuveson, D.A. Suppression of BRAF(v599e) in human melanoma abrogates transformation. *Cancer Res.* **2003**, *63*, 5198–5202. [PubMed]
86. Basile, K.J.; Aplin, A.E. Downregulation of noxa by RAF/MEK inhibition counteracts cell death response in mutant B-RAF melanoma cells. *Am. J. Cancer Res.* **2012**, *2*, 726–735. [PubMed]
87. Zhang, X.D.; Gillespie, S.K.; Borrow, J.M.; Hersey, P. The histone deacetylase inhibitor suberic bishydroxamate regulates the expression of multiple apoptotic mediators and induces mitochondria-dependent apoptosis of melanoma cells. *Mol. Cancer Ther.* **2004**, *3*, 425–435. [PubMed]

88. Lai, F.; Jin, L.; Gallagher, S.; Mijatov, B.; Zhang, X.D.; Hersey, P. Histone deacetylases (HDACS) as mediators of resistance to apoptosis in melanoma and as targets for combination therapy with selective BRAF inhibitors. *Adv. Pharmacol.* **2012**, *65*, 27–43. [PubMed]
89. Lai, F.; Guo, S.T.; Jin, L.; Jiang, C.C.; Wang, C.Y.; Croft, A.; Chi, M.N.; Tseng, H.Y.; Farrelly, M.; Atmadibrata, B.; *et al*. Cotargeting histone deacetylases and oncogenic BRAF synergistically kills human melanoma cells by necrosis independently of RIPK1 and RIPK3. *Cell Death Dis.* **2013**, *4*, e655. [CrossRef] [PubMed]
90. Dickinson, M.; Johnstone, R.W.; Prince, H.M. Histone deacetylase inhibitors: Potential targets responsible for their anti-cancer effect. *Investig. New Drugs* **2010**, *28*, S3–S20. [CrossRef] [PubMed]
91. Peart, M.J.; Tainton, K.M.; Ruefli, A.A.; Dear, A.E.; Sedelies, K.A.; O'Reilly, L.A.; Waterhouse, N.J.; Trapani, J.A.; Johnstone, R.W. Novel mechanisms of apoptosis induced by histone deacetylase inhibitors. *Cancer Res.* **2003**, *63*, 4460–4471. [PubMed]
92. Zhang, X.D.; Gillespie, S.K.; Borrow, J.M.; Hersey, P. The histone deacetylase inhibitor suberic bishydroxamate: A potential sensitizer of melanoma to TNF-related apoptosis-inducing ligand (trail) induced apoptosis. *Biochem. Pharmacol.* **2003**, *66*, 1537–1545. [CrossRef]
93. Atadja, P.; Hsu, M.; Kwon, P.; Trogani, N.; Bhalla, K.; Remiszewski, S. Molecular and cellular basis for the anti-proliferative effects of the hdac inhibitor LAQ824. *Novartis Found. Symp.* **2004**. [CrossRef]
94. Facchetti, F.; Previdi, S.; Ballarini, M.; Minucci, S.; Perego, P.; La Porta, C.A. Modulation of pro- and anti-apoptotic factors in human melanoma cells exposed to histone deacetylase inhibitors. *Apoptosis* **2004**, *9*, 573–582. [CrossRef] [PubMed]
95. Heinemann, A.; Cullinane, C.; De Paoli-Iseppi, R.; Wilmott, J.S.; Gunatilake, D.; Madore, J.; Strbenac, D.; Yang, J.Y.; Gowrishankar, K.; Tiffen, J.C.; *et al*. Combining BET and HDAC inhibitors synergistically induces apoptosis of melanoma and suppresses AKT and YAP signaling. *Oncotarget* **2015**, *6*, 21507–21521. [CrossRef] [PubMed]
96. Maggio, S.C.; Rosato, R.R.; Kramer, L.B.; Dai, Y.; Rahmani, M.; Paik, D.S.; Czarnik, A.C.; Payne, S.G.; Spiegel, S.; Grant, S. The histone deacetylase inhibitor MS-275 interacts synergistically with fludarabine to induce apoptosis in human leukemia cells. *Cancer Res.* **2004**, *64*, 2590–2600. [PubMed]
97. Bandyopadhyay, D.; Mishra, A.; Medrano, E.E. Overexpression of histone deacetylase 1 confers resistance to sodium butyrate-mediated apoptosis in melanoma cells through a P53-mediated pathway. *Cancer Res.* **2004**, *64*, 7706–7710. [CrossRef] [PubMed]
98. Gui, C.Y.; Ngo, L.; Xu, W.S.; Richon, V.M.; Marks, P.A. Histone deacetylase (HDAC) inhibitor activation of P21WAF1 involves changes in promoter-associated proteins, including HDAC1. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 1241–1246. [CrossRef] [PubMed]
99. Venza, I.; Visalli, M.; Oteri, R.; Teti, D.; Venza, M. Class I-specific histone deacetylase inhibitor MS-275 overrides trail-resistance in melanoma cells by downregulating c-flip. *Int. Immunopharmacol.* **2014**, *21*, 439–446. [CrossRef] [PubMed]
100. Paoluzzi, L.; Hanniford, D.; Sokolova, E.; Dolgalev, I.; Heguy, A.; Osman, I.; Darvishian, F.; Wang, J.; Bradner, J.E.; Hernando, E. Preclinical testing supports combined bet and BRAF inhibition as a promising therapeutic strategy for melanoma. *ASCO Meet. Abstr.* **2014**, *32*, 9072.
101. Saladi, S.V.; Wong, P.G.; Trivedi, A.R.; Marathe, H.G.; Keenen, B.; Aras, S.; Liew, Z.Q.; Setaluri, V.; de la Serna, I.L. Brg1 promotes survival of uv-irradiated melanoma cells by cooperating with mitf to activate the melanoma inhibitor of apoptosis gene. *Pigment Cell Melanoma Res.* **2013**, *26*, 377–391. [CrossRef] [PubMed]
102. Grazia, G.; Penna, I.; Perotti, V.; Anichini, A.; Tassi, E. Towards combinatorial targeted therapy in melanoma: From pre-clinical evidence to clinical application (review). *Int. J. Oncol.* **2014**, *45*, 929–949. [CrossRef] [PubMed]
103. Poulikakos, P.I.; Rosen, N. Mutant BRAF melanomas—Dependence and resistance. *Cancer Cell* **2011**, *19*, 11–15. [CrossRef] [PubMed]
104. Poulikakos, P.I.; Persaud, Y.; Janakiraman, M.; Kong, X.; Ng, C.; Moriceau, G.; Shi, H.; Atefi, M.; Titz, B.; Gabay, M.T.; *et al*. Raf inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(v600e). *Nature* **2011**, *480*, 387–390. [CrossRef] [PubMed]

105. Su, F.; Bradley, W.D.; Wang, Q.; Yang, H.; Xu, L.; Higgins, B.; Kolinsky, K.; Packman, K.; Kim, M.J.; Trunzer, K.; *et al*. Resistance to selective BRAF inhibition can be mediated by modest upstream pathway activation. *Cancer Res.* **2012**, *72*, 969–978. [CrossRef] [PubMed]
106. Bali, P.; Pranpat, M.; Bradner, J.; Balasis, M.; Fiskus, W.; Guo, F.; Rocha, K.; Kumaraswamy, S.; Boyapalle, S.; Atadja, P.; *et al*. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: A novel basis for antileukemia activity of histone deacetylase inhibitors. *J. Biol. Chem.* **2005**, *280*, 26729–26734. [CrossRef] [PubMed]
107. Dar, A.A.; Nosrati, M.; Bezrookove, V.; de Semir, D.; Majid, S.; Thummala, S.; Sun, V.; Tong, S.; Leong, S.P.; Minor, D.; *et al*. The role of BPTF in melanoma progression and in response to BRAF-targeted therapy. *J. Natl. Cancer Inst.* **2015**, *107*. [CrossRef] [PubMed]
108. Perna, D.; Karreth, F.A.; Rust, A.G.; Perez-Mancera, P.A.; Rashid, M.; Iorio, F.; Alifrangis, C.; Arends, M.J.; Bosenberg, M.W.; Bollag, G.; *et al*. BRAF inhibitor resistance mediated by the AKT pathway in an oncogenic BRAF mouse melanoma model. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E536–E545. [CrossRef] [PubMed]
109. Ye, Y.; Jin, L.; Wilmott, J.S.; Hu, W.L.; Yosufi, B.; Thorne, R.F.; Liu, T.; Rizos, H.; Yan, X.G.; Dong, L.; *et al*. PI(4,5)P2 5-phosphatase a regulates PI3K/AKT signalling and has a tumour suppressive role in human melanoma. *Nat. Commun.* **2013**, *4*, 1508. [CrossRef] [PubMed]
110. Qian, C.; Lai, C.J.; Bao, R.; Wang, D.G.; Wang, J.; Xu, G.X.; Atoyan, R.; Qu, H.; Yin, L.; Samson, M.; *et al*. Cancer network disruption by a single molecule inhibitor targeting both histone deacetylase activity and phosphatidylinositol 3-kinase signaling. *Clin. Cancer Res.* **2012**, *18*, 4104–4113. [CrossRef] [PubMed]
111. Saijo, K.; Katoh, T.; Shimodaira, H.; Oda, A.; Takahashi, O.; Ishioka, C. Romidepsin (FK228) and its analogs directly inhibit phosphatidylinositol 3-kinase activity and potently induce apoptosis as histone deacetylase/phosphatidylinositol 3-kinase dual inhibitors. *Cancer Sci.* **2012**, *103*, 1994–2001. [CrossRef] [PubMed]
112. Nazarian, R.; Shi, H.; Wang, Q.; Kong, X.; Koya, R.C.; Lee, H.; Chen, Z.; Lee, M.K.; Attar, N.; Sazegar, H.; *et al*. Melanomas acquire resistance to B-RAF(v600e) inhibition by RTK or N-RAS upregulation. *Nature* **2010**, *468*, 973–977. [CrossRef] [PubMed]
113. 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] [PubMed]
114. Wilson, T.R.; Fridlyand, J.; Yan, Y.; Penuel, E.; Burton, L.; Chan, E.; Peng, J.; Lin, E.; Wang, Y.; Sosman, J.; *et al*. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. *Nature* **2012**, *487*, 505–509. [CrossRef] [PubMed]
115. Abel, E.V.; Basile, K.J.; Kugel, C.H., 3rd; Witkiewicz, A.K.; Le, K.; Amaravadi, R.K.; Karakousis, G.C.; Xu, X.; Xu, W.; Schuchter, L.M.; *et al*. Melanoma adapts to RAF/MEK inhibitors through FOXD3-mediated upregulation of ERBB3. *J. Clin. Investig.* **2013**, *123*, 2155–2168. [CrossRef] [PubMed]
116. Wang, J.; Huang, S.K.; Marzese, D.M.; Hsu, S.C.; Kawas, N.P.; Chong, K.K.; Long, G.V.; Menzies, A.M.; Scolyer, R.A.; Izraely, S.; *et al*. Epigenetic changes of egfr have an important role in BRAF inhibitor-resistant cutaneous melanomas. *J. Investig. Dermatol.* **2014**, *135*, 532–541. [CrossRef] [PubMed]
117. Basile, K.J.; Abel, E.V.; Aplin, A.E. Adaptive upregulation of FOXD3 and resistance to PLX4032/4720-induced cell death in mutant B-RAF melanoma cells. *Oncogene* **2012**, *31*, 2471–2479. [CrossRef] [PubMed]
118. Weiss, M.B.; Abel, E.V.; Dadpey, N.; Aplin, A.E. FOXD3 modulates migration through direct transcriptional repression of twist1 in melanoma. *Mol. Cancer Res.* **2014**, *12*, 1314–1323. [CrossRef] [PubMed]
119. Abel, E.V.; Aplin, A.E. FOXD3 is a mutant B-RAF-regulated inhibitor of G(1)-S progression in melanoma cells. *Cancer Res.* **2010**, *70*, 2891–2900. [CrossRef] [PubMed]
120. Matsukawa, S.; Miwata, K.; Asashima, M.; Michiue, T. The requirement of histone modification by PRDM12 and KDM4A for the development of pre-placodal ectoderm and neural crest in xenopus. *Dev. Biol.* **2015**, *399*, 164–176. [CrossRef] [PubMed]

121. Dugo, M.; Nicolini, G.; Tragni, G.; Bersani, I.; Tomassetti, A.; Colonna, V.; del Vecchio, M.; de Braud, F.; Canevari, S.; Anichini, A.; *et al*. A melanoma subtype with intrinsic resistance to BRAF inhibition identified by receptor tyrosine kinases gene-driven classification. *Oncotarget* **2015**, *6*, 5118–5133. [CrossRef] [PubMed]
122. Sensi, M.; Catani, M.; Castellano, G.; Nicolini, G.; Alciato, F.; Tragni, G.; de Santis, G.; Bersani, I.; Avanzi, G.; Tomassetti, A.; *et al*. Human cutaneous melanomas lacking mitf and melanocyte differentiation antigens express a functional axl receptor kinase. *J. Investig. Dermatol.* **2011**, *131*, 2448–2457. [CrossRef] [PubMed]
123. Huang, X.; Gao, L.; Wang, S.; Lee, C.K.; Ordentlich, P.; Liu, B. Hdac inhibitor SNDX-275 induces apoptosis in ERBB2-overexpressing breast cancer cells via down-regulation of ERBB3 expression. *Cancer Res.* **2009**, *69*, 8403–8411. [CrossRef] [PubMed]
124. Chou, C.W.; Wu, M.S.; Huang, W.C.; Chen, C.C. Hdac inhibition decreases the expression of EGFR in colorectal cancer cells. *PLoS ONE* **2011**, *6*, e18087. [CrossRef] [PubMed]
125. Wedel, S.; Hudak, L.; Seibel, J.M.; Juengel, E.; Oppermann, E.; Haferkamp, A.; Blaheta, R.A. Critical analysis of simultaneous blockage of histone deacetylase and multiple receptor tyrosine kinase in the treatment of prostate cancer. *Prostate* **2011**, *71*, 722–735. [CrossRef] [PubMed]
126. Chen, M.C.; Chen, C.H.; Wang, J.C.; Tsai, A.C.; Liou, J.P.; Pan, S.L.; Teng, C.M. The HDAC inhibitor, MPT0E028, enhances erlotinib-induced cell death in EGFR-TKI-resistant NSCLC cells. *Cell Death Dis.* **2013**, *4*, e810. [CrossRef] [PubMed]
127. Bruzzese, F.; Leone, A.; Rocco, M.; Carbone, C.; Piro, G.; Caraglia, M.; di Gennaro, E.; Budillon, A. Hdac inhibitor vorinostat enhances the antitumor effect of gefitinib in squamous cell carcinoma of head and neck by modulating erbb receptor expression and reverting emt. *J. Cell. Physiol.* **2011**, *226*, 2378–2390. [CrossRef] [PubMed]
128. Liffers, K.; Kolbe, K.; Westphal, M.; Lamszus, K.; Schulte, A. Histone deacetylase inhibitors resensitize EGFR/EGFRVIII-overexpressing, erlotinib-resistant glioblastoma cells to tyrosine kinase inhibition. *Target. Oncol.* **2015**. [CrossRef] [PubMed]
129. Zhang, Y.W.; Staal, B.; Dykema, K.J.; Furge, K.A.; Vande Woude, G.F. Cancer-type regulation of MIG-6 expression by inhibitors of methylation and histone deacetylation. *PLoS ONE* **2012**, *7*, e38955. [CrossRef] [PubMed]
130. Tu, C.Y.; Chen, C.H.; Hsia, T.C.; Hsu, M.H.; Wei, Y.L.; Yu, M.C.; Chen, W.S.; Hsu, K.W.; Yeh, M.H.; Liu, L.C.; *et al*. Trichostatin a suppresses EGFR expression through induction of microrna-7 in an HDAC-independent manner in lapatinib-treated cells. *Biomed. Res. Int.* **2014**, *2014*, 168949. [CrossRef] [PubMed]
131. Wouters, J.; Stas, M.; Gremeaux, L.; Govaere, O.; van den Broeck, A.; Maes, H.; Agostinis, P.; Roskams, T.; van den Oord, J.J.; Vankelecom, H. The human melanoma side population displays molecular and functional characteristics of enriched chemoresistance and tumorigenesis. *PLoS ONE* **2013**, *8*, e76550. [CrossRef] [PubMed]
132. Roesch, A.; Fukunaga-Kalabis, M.; Schmidt, E.C.; Zabierowski, S.E.; Brafford, P.A.; Vultur, A.; Basu, D.; Gimotty, P.; Vogt, T.; Herlyn, M. A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell* **2010**, *141*, 583–594. [CrossRef] [PubMed]
133. Yuan, P.; Ito, K.; Perez-Lorenzo, R.; Del Guzzo, C.; Lee, J.H.; Shen, C.H.; Bosenberg, M.W.; McMahon, M.; Cantley, L.C.; Zheng, B. Phenformin enhances the therapeutic benefit of BRAF(v600e) inhibition in melanoma. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 18226–18231. [CrossRef] [PubMed]
134. Roesch, A.; Vultur, A.; Bogeski, I.; Wang, H.; Zimmermann, K.M.; Speicher, D.; Korbel, C.; Laschke, M.W.; Gimotty, P.A.; Philipp, S.E.; *et al*. Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B(high) cells. *Cancer Cell* **2013**, *23*, 811–825. [CrossRef] [PubMed]
135. Burgess, A.; Ruefli, A.; Beamish, H.; Warrener, R.; Saunders, N.; Johnstone, R.; Gabrielli, B. Histone deacetylase inhibitors specifically kill nonproliferating tumour cells. *Oncogene* **2004**, *23*, 6693–6701. [CrossRef] [PubMed]
136. Hartman, M.L.; Czyz, M. Mitf in melanoma: Mechanisms behind its expression and activity. *Cell. Mol. Life Sci.* **2014**, *72*, 1249–1260. [CrossRef] [PubMed]

137. 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]
138. Gallagher, S.J.; Rambow, F.; Kumasaka, M.; Champeval, D.; Bellacosa, A.; Delmas, V.; Larue, L. β-catenin inhibits melanocyte migration but induces melanoma metastasis. *Oncogene* **2013**, *32*, 2230–2238. [CrossRef] [PubMed]
139. Kim, J.E.; Leung, E.; Baguley, B.C.; Finlay, G.J. Heterogeneity of expression of epithelial-mesenchymal transition markers in melanocytes and melanoma cell lines. *Front. Genet.* **2013**, *4*, 97. [CrossRef] [PubMed]
140. Verfaillie, A.; Imrichova, H.; Atak, Z.K.; Dewaele, M.; Rambow, F.; Hulselmans, G.; Christiaens, V.; Svetlichnyy, D.; Luciani, F.; van den Mooter, L.; *et al*. Decoding the regulatory landscape of melanoma reveals teads as regulators of the invasive cell state. *Nat. Commun.* **2015**, *6*, 6683. [CrossRef] [PubMed]
141. Yokoyama, S.; Feige, E.; Poling, L.L.; Levy, C.; Widlund, H.R.; Khaled, M.; Kung, A.L.; Fisher, D.E. Pharmacologic suppression of mitf expression via hdac inhibitors in the melanocyte lineage. *Pigment Cell Melanoma Res.* **2008**, *21*, 457–463. [CrossRef] [PubMed]
142. Genovese, G.; Ghosh, P.; Li, H.; Rettino, A.; Sioletic, S.; Cittadini, A.; Sgambato, A. The tumor suppressor HINT1 regulates MITF and β-catenin transcriptional activity in melanoma cells. *Cell Cycle* **2012**, *11*, 2206–2215. [CrossRef] [PubMed]
143. Nishimura, K.; Hirokawa, Y.S.; Mizutani, H.; Shiraishi, T. Reduced heterochromatin protein 1-β (hp1β) expression is correlated with increased invasive activity in human melanoma cells. *Anticancer Res.* **2006**, *26*, 4349–4356. [PubMed]
144. De la Serna, I.L.; Ohkawa, Y.; Higashi, C.; Dutta, C.; Osias, J.; Kommajosyula, N.; Tachibana, T.; Imbalzano, A.N. The microphthalmia-associated transcription factor requires swi/snf enzymes to activate melanocyte-specific genes. *J. Biol. Chem.* **2006**, *281*, 20233–20241.
145. Vachtenheim, J.; Ondrusova, L.; Borovansky, J. SWI/SNF chromatin remodeling complex is critical for the expression of microphthalmia-associated transcription factor in melanoma cells. *Biochem. Biophys. Res. Commun.* **2010**, *392*, 454–459. [CrossRef] [PubMed]
146. Lin, H.; Wong, R.P.; Martinka, M.; Li, G. BRG1 expression is increased in human cutaneous melanoma. *Br. J. Dermatol.* **2010**, *163*, 502–510. [CrossRef] [PubMed]
147. Becker, T.M.; Haferkamp, S.; Dijkstra, M.K.; Scurr, L.L.; Frausto, M.; Diefenbach, E.; Scolyer, R.A.; Reisman, D.N.; Mann, G.J.; Kefford, R.F.; *et al*. The chromatin remodelling factor brg1 is a novel binding partner of the tumor suppressor p16ink4a. *Mol. Cancer* **2009**, *8*, 4. [CrossRef] [PubMed]
148. Lin, H.; Wong, R.P.; Martinka, M.; Li, G. Loss of snf5 expression correlates with poor patient survival in melanoma. *Clin. Cancer Res.* **2009**, *15*, 6404–6411. [CrossRef] [PubMed]
149. Mehrotra, A.; Saladi, S.V.; Trivedi, A.R.; Aras, S.; Qi, H.; Jayanthy, A.; Setaluri, V.; de la Serna, I.L. Modulation of brahma expression by the mitogen-activated protein kinase/extracellular signal regulated kinase pathway is associated with changes in melanoma proliferation. *Arch. Biochem. Biophys.* **2014**, *563*, 125–135. [CrossRef] [PubMed]
150. Hersey, P.; Kakavand, H.; Wilmott, J.; van der Westhuizen, A.; Gallagher, S.; Gowrishankar, K.; Scolyer, R. How anti-PD1 treatments are changing the management of melanoma. *Melanoma Manag.* **2014**, *1*, 165–172. [CrossRef]
151. Spranger, S.; Bao, R.; Gajewski, T.F. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* **2015**, *523*, 231–235. [CrossRef] [PubMed]
152. Jazirehi, A.R.; Nazarian, R.; Torres-Collado, A.X.; Economou, J.S. Aberrant apoptotic machinery confers melanoma dual resistance to braf(v600e) inhibitor and immune effector cells: Immunosensitization by a histone deacetylase inhibitor. *Am. J. Clin. Exp. Immunol.* **2014**, *3*, 43–56. [PubMed]
153. Hersey, P.; Zhang, X.D. Treatment combinations targeting apoptosis to improve immunotherapy of melanoma. *Cancer Immunol. Immunother.* **2009**, *58*, 1749–1759. [CrossRef] [PubMed]
154. Jazirehi, A.R.; Arle, D. Epigenetic regulation of the TRAIL/Apo2L apoptotic pathway by histone deacetylase inhibitors: An attractive approach to bypass melanoma immunotherapy resistance. *Am. J. Clin. Exp. Immunol.* **2013**, *2*, 55–74. [PubMed]

155. Lillehammer, T.; Engesaeter, B.O.; Prasmickaite, L.; Maelandsmo, G.M.; Fodstad, O.; Engebraaten, O. Combined treatment with Ad-HTRAIL and DTIC or SAHA is associated with increased mitochondrial-mediated apoptosis in human melanoma cell lines. *J. Gene Med.* **2007**, *9*, 440–451. [CrossRef] [PubMed]
156. Kroesen, M.; Gielen, P.; Brok, I.C.; Armandari, I.; Hoogerbrugge, P.M.; Adema, G.J. HDAC inhibitors and immunotherapy; a double edged sword? *Oncotarget* **2014**, *5*, 6558–6572. [CrossRef] [PubMed]
157. Wong, D.J.; Rao, A.; Avramis, E.; Matsunaga, D.R.; Komatsubara, K.M.; Atefi, M.S.; Escuin-Ordinas, H.; Chodon, T.; Koya, R.C.; Ribas, A.; *et al*. Exposure to a histone deacetylase inhibitor has detrimental effects on human lymphocyte viability and function. *Cancer Immunol. Res.* **2014**, *2*, 459–468. [CrossRef] [PubMed]
158. Leoni, F.; Fossati, G.; Lewis, E.C.; Lee, J.K.; Porro, G.; Pagani, P.; Modena, D.; Moras, M.L.; Pozzi, P.; Reznikov, L.L.; *et al*. The histone deacetylase inhibitor ITF2357 reduces production of pro-inflammatory cytokines *in vitro* and systemic inflammation *in vivo*. *Mol. Med.* **2005**, *11*, 1–15. [CrossRef] [PubMed]
159. Leoni, F.; Zaliani, A.; Bertolini, G.; Porro, G.; Pagani, P.; Pozzi, P.; Dona, G.; Fossati, G.; Sozzani, S.; Azam, T.; *et al*. The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 2995–3000. [CrossRef] [PubMed]
160. Fraczek, J.; Vanhaecke, T.; Rogiers, V. Toxicological and metabolic considerations for histone deacetylase inhibitors. *Expert Opin. Drug Metab. Toxicol.* **2013**, *9*, 441–457. [CrossRef] [PubMed]
161. Galanis, E.; Jaeckle, K.A.; Maurer, M.J.; Reid, J.M.; Ames, M.M.; Hardwick, J.S.; Reilly, J.F.; Loboda, A.; Nebozhyn, M.; Fantin, V.R.; *et al*. Phase II trial of vorinostat in recurrent glioblastoma multiforme: A north central cancer treatment group study. *J. Clin. Oncol.* **2009**, *27*, 2052–2058. [CrossRef] [PubMed]
162. Woods, D.M.; Woan, K.; Cheng, F.; Wang, H.; Perez-Villarroel, P.; Lee, C.; Lienlaf, M.; Atadja, P.; Seto, E.; Weber, J.; *et al*. The antimelanoma activity of the histone deacetylase inhibitor panobinostat (LBH589) is mediated by direct tumor cytotoxicity and increased tumor immunogenicity. *Melanoma Res.* **2013**, *23*, 341–348. [CrossRef] [PubMed]
163. Lisiero, D.N.; Soto, H.; Everson, R.G.; Liau, L.M.; Prins, R.M. The histone deacetylase inhibitor, LBH589, promotes the systemic cytokine and effector responses of adoptively transferred CD8+ T cells. *J. Immunother. Cancer* **2014**, *2*, 8. [CrossRef] [PubMed]
164. Cao, K.; Wang, G.; Li, W.; Zhang, L.; Wang, R.; Huang, Y.; Du, L.; Jiang, J.; Wu, C.; He, X.; *et al*. Histone deacetylase inhibitors prevent activation-induced cell death and promote anti-tumor immunity. *Oncogene* **2015**. [CrossRef] [PubMed]
165. Chan, D.V.; Gibson, H.M.; Aufiero, B.M.; Wilson, A.J.; Hafner, M.S.; Mi, Q.S.; Wong, H.K. Differential CTLA-4 expression in human CD4+ *versus* CD8+ T cells is associated with increased nfat1 and inhibition of CD4+ proliferation. *Genes Immun.* **2014**, *15*, 25–32. [CrossRef] [PubMed]
166. Pan, L.; Pan, H.; Jiang, H.; Du, J.; Wang, X.; Huang, B.; Lu, J. HDAC4 inhibits the transcriptional activation of mda-7/IL-24 induced by Sp1. *Cell. Mol. Immunol.* **2010**, *7*, 221–226. [CrossRef] [PubMed]
167. Murakami, T.; Sato, A.; Chun, N.A.; Hara, M.; Naito, Y.; Kobayashi, Y.; Kano, Y.; Ohtsuki, M.; Furukawa, Y.; Kobayashi, E. Transcriptional modulation using HDACI depsipeptide promotes immune cell-mediated tumor destruction of murine B16 melanoma. *J. Investig. Dermatol.* **2008**, *128*, 1506–1516. [CrossRef] [PubMed]
168. Khan, A.N.; Magner, W.J.; Tomasi, T.B. An epigenetically altered tumor cell vaccine. *Cancer Immunol. Immunother.* **2004**, *53*, 748–754. [CrossRef] [PubMed]
169. Khan, A.N.; Magner, W.J.; Tomasi, T.B. An epigenetic vaccine model active in the prevention and treatment of melanoma. *J. Transl. Med.* **2007**, *5*, 64. [CrossRef] [PubMed]
170. Garrido, C.; Algarra, I.; Maleno, I.; Stefanski, J.; Collado, A.; Garrido, F.; Garcia-Lora, A.M. Alterations of HLA class I expression in human melanoma xenografts in immunodeficient mice occur frequently and are associated with higher tumorigenicity. *Cancer Immunol. Immunother.* **2010**, *59*, 13–26. [CrossRef] [PubMed]
171. Komatsu, Y.; Hayashi, H. Histone deacetylase inhibitors up-regulate the expression of cell surface MHC class-I molecules in B16/Bl6 cells. *J. Antibiot.* **1998**, *51*, 89–91. [CrossRef] [PubMed]
172. Setiadi, A.F.; Omilusik, K.; David, M.D.; Seipp, R.P.; Hartikainen, J.; Gopaul, R.; Choi, K.B.; Jefferies, W.A. Epigenetic enhancement of antigen processing and presentation promotes immune recognition of tumors. *Cancer Res.* **2008**, *68*, 9601–9607. [CrossRef] [PubMed]

173. Vo, D.D.; Prins, R.M.; Begley, J.L.; Donahue, T.R.; Morris, L.F.; Bruhn, K.W.; de la Rocha, P.; Yang, M.Y.; Mok, S.; Garban, H.J.; *et al*. Enhanced antitumor activity induced by adoptive T-cell transfer and adjunctive use of the histone deacetylase inhibitor LAQ824. *Cancer Res.* **2009**, *69*, 8693–8699. [CrossRef] [PubMed]
174. Khan, A.N.; Gregorie, C.J.; Tomasi, T.B. Histone deacetylase inhibitors induce TAP, LMP, tapasin genes and MHC class I antigen presentation by melanoma cells. *Cancer Immunol. Immunother.* **2008**, *57*, 647–654. [CrossRef] [PubMed]
175. Woan, K.V.; Lienlaf, M.; Perez-Villaroel, P.; Lee, C.; Cheng, F.; Knox, T.; Woods, D.M.; Barrios, K.; Powers, J.; Sahakian, E.; *et al*. Targeting histone deacetylase 6 mediates a dual anti-melanoma effect: Enhanced antitumor immunity and impaired cell proliferation. *Mol. Oncol.* **2015**, *9*, 1447–1457. [CrossRef] [PubMed]
176. Cronin, K.; Escobar, H.; Szekeres, K.; Reyes-Vargas, E.; Rockwood, A.L.; Lloyd, M.C.; Delgado, J.C.; Blanck, G. Regulation of HLA-DR peptide occupancy by histone deacetylase inhibitors. *Hum. Vaccines Immunother.* **2013**, *9*, 784–789. [CrossRef] [PubMed]
177. Holling, T.M.; Bergevoet, M.W.; Wilson, L.; van Eggermond, M.C.; Schooten, E.; Steenbergen, R.D.; Snijders, P.J.; Jager, M.J.; van den Elsen, P.J. A role for EZH2 in silencing of IFN-γ inducible MHC2TA transcription in uveal melanoma. *J. Immunol.* **2007**, *179*, 5317–5325. [CrossRef] [PubMed]
178. Abou El Hassan, M.; Yu, T.; Song, L.; Bremner, R. Polycomb repressive complex 2 confers BRG1 dependency on the ciita locus. *J. Immunol.* **2015**, *194*, 5007–5013. [CrossRef] [PubMed]
179. Arvey, A.; van der Veeken, J.; Samstein, R.M.; Feng, Y.; Stamatoyannopoulos, J.A.; Rudensky, A.Y. Inflammation-induced repression of chromatin bound by the transcription factor FOXP3 in regulatory T cells. *Nat. Immunol.* **2014**, *15*, 580–587. [CrossRef] [PubMed]
180. DuPage, M.; Chopra, G.; Quiros, J.; Rosenthal, W.L.; Morar, M.M.; Holohan, D.; Zhang, R.; Turka, L.; Marson, A.; Bluestone, J.A. The chromatin-modifying enzyme EZH2 is critical for the maintenance of regulatory T cell identity after activation. *Immunity* **2015**, *42*, 227–238. [CrossRef] [PubMed]
181. Rao, M.; Chinnasamy, N.; Hong, J.A.; Zhang, Y.; Zhang, M.; Xi, S.; Liu, F.; Marquez, V.E.; Morgan, R.A.; Schrump, D.S. Inhibition of histone lysine methylation enhances cancer-testis antigen expression in lung cancer cells: Implications for adoptive immunotherapy of cancer. *Cancer Res.* **2011**, *71*, 4192–4204. [CrossRef] [PubMed]
182. Sanchez-Perez, L.; Kottke, T.; Diaz, R.M.; Ahmed, A.; Thompson, J.; Chong, H.; Melcher, A.; Holmen, S.; Daniels, G.; Vile, R.G. Potent selection of antigen loss variants of B16 melanoma following inflammatory killing of melanocytes *in vivo*. *Cancer Res.* **2005**, *65*, 2009–2017. [CrossRef] [PubMed]
183. Kirkwood, J.M.; Butterfield, L.H.; Tarhini, A.A.; Zarour, H.; Kalinski, P.; Ferrone, S. Immunotherapy of cancer in 2012. *CA Cancer J. Clin.* **2012**, *62*, 309–335. [CrossRef] [PubMed]
184. Weiser, T.S.; Guo, Z.S.; Ohnmacht, G.A.; Parkhurst, M.L.; Tong-On, P.; Marincola, F.M.; Fischette, M.R.; Yu, X.; Chen, G.A.; Hong, J.A.; *et al*. Sequential 5-Aza-2 deoxycytidine-depsipeptide FR901228 treatment induces apoptosis preferentially in cancer cells and facilitates their recognition by cytolytic T lymphocytes specific for NY-ESO-1. *J. Immunother.* **2001**, *24*, 151–161. [CrossRef] [PubMed]
185. Wischnewski, F.; Pantel, K.; Schwarzenbach, H. Promoter demethylation and histone acetylation mediate gene expression of mage-a1, -a2, -a3, and -a12 in human cancer cells. *Mol. Cancer Res.* **2006**, *4*, 339–349. [CrossRef] [PubMed]
186. Villagra, A.; Cheng, F.; Wang, H.W.; Suarez, I.; Glozak, M.; Maurin, M.; Nguyen, D.; Wright, K.L.; Atadja, P.W.; Bhalla, K.; *et al*. The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance. *Nat. Immunol.* **2009**, *10*, 92–100. [CrossRef] [PubMed]
187. Hellebrekers, D.M.; Castermans, K.; Vire, E.; Dings, R.P.; Hoebers, N.T.; Mayo, K.H.; Oude Egbrink, M.G.; Molema, G.; Fuks, F.; van Engeland, M.; *et al*. Epigenetic regulation of tumor endothelial cell anergy: Silencing of intercellular adhesion molecule-1 by histone modifications. *Cancer Res.* **2006**, *66*, 10770–10777. [CrossRef] [PubMed]
188. Woods, K.; Pasam, A.; Jayachandran, A.; Andrews, M.C.; Cebon, J. Effects of epithelial to mesenchymal transition on T cell targeting of melanoma cells. *Front. Oncol.* **2014**, *4*, 367. [CrossRef] [PubMed]
189. Gowrishankar, K.; Gunatilake, D.; Gallagher, S.J.; Tiffen, J.; Rizos, H.; Hersey, P. Inducible but not constitutive expression of Pd-L1 in human melanoma cells is dependent on activation of NF-κB. *PLoS ONE* **2015**, *10*, e0123410. [CrossRef] [PubMed]

190. Wang, H.W.; Cheng, F.D.; Xing, L.M.; Zhao, X.H.; Villagra, A.; Pinilla-Ibarz, J.; Tao, J.G. JQ 1, a selective bromodomain inhibitor, decreased the expression of the tolerogenic molecule PDL1 in antigen-presenting cells (APCs) and restores the responsiveness of anergic CD4+ T cells. *Blood* **2014**, *124*, 2749–2749.
191. Nicodeme, E.; Jeffrey, K.L.; Schaefer, U.; Beinke, S.; Dewell, S.; Chung, C.W.; Chandwani, R.; Marazzi, I.; Wilson, P.; Coste, H.; *et al*. Suppression of inflammation by a synthetic histone mimic. *Nature* **2010**, *468*, 1119–1123. [CrossRef] [PubMed]
192. Belkina, A.C.; Nikolajczyk, B.S.; Denis, G.V. Bet protein function is required for inflammation: BRD2 genetic disruption and bet inhibitor JQ1 impair mouse macrophage inflammatory responses. *J. Immunol.* **2013**, *190*, 3670–3678. [CrossRef] [PubMed]
193. Toniolo, P.A.; Liu, S.; Yeh, J.E.; Moraes-Vieira, P.M.; Walker, S.R.; Vafaizadeh, V.; Barbuto, J.A.; Frank, D.A. Inhibiting STAT5 by the BET bromodomain inhibitor JQ1 disrupts human dendritic cell maturation. *J. Immunol.* **2015**, *194*, 3180–3190. [CrossRef] [PubMed]
194. Herait, P.E.; Berthon, C.; Thieblemont, C.; Raffoux, E.; Magarotto, V.; Stathis, A.; Thomas, X.; Leleu, X.; Gomez-Roca, C.; Odore, E.; *et al*. Abstract ct231: Bet-bromodomain inhibitor otx015 shows clinically meaningful activity at nontoxic doses: Interim results of an ongoing Phase I trial in hematologic malignancies. *Cancer Res.* **2014**, *74*, CT231. [CrossRef]