Drugging the Epigenome: Overcoming Resistance to Targeted and Immunotherapies in Melanoma

Ailish Hanly[1], Frederick Gibson[1], Sarah Nocco[1], Samantha Rogers[1], Muzhou Wu[1] and Rhoda M. Alani[1]

This past decade has seen tremendous advances in understanding the molecular pathogenesis of melanoma and the development of novel effective therapies for melanoma. Targeted therapies and immunotherapies that extend survival of patients with advanced disease have been developed; however, the vast majority of patients experience relapse and therapeutic resistance over time. Moreover, cellular plasticity has been demonstrated to be a driver of therapeutic resistance mechanisms in melanoma and other cancers, largely functioning through epigenetic mechanisms, suggesting that targeting of the cancer epigenetic landscape may prove a worthwhile endeavor to ensure durable treatment responses and cures. Here, we review the epigenetic alterations that characterize melanoma development, progression, and resistance to targeted therapies as well as epigenetic therapies currently in use and under development for melanoma and other cancers. We further assess the landscape of epigenetic therapies in clinical trials for melanoma and provide a framework for future advances in epigenetic therapies to circumvent the development of therapeutic resistance in melanoma.

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INTRODUCTION

Melanoma is among the most lethal forms of skin cancer, accounting for the vast majority of skin cancer deaths in the United States each year (Miller et al., 2019). Over the past decade, significant advances in understanding the molecular basis for melanoma onset and progression have allowed for the development of novel therapies, which have dramatically improved patient outcomes, including targeted BRAF inhibitors (BRAFis), MAPK/extracellular signal–regulated kinase inhibitors (MEKis), and immunotherapies. Although targeted therapies elicit rapid antitumor responses in the majority of patients with *BRAF*-mutant melanoma, nearly all patients develop drug resistance and disease progression within a year of treatment initiation (Sullivan and Flaherty, 2013). Immunotherapies have also demonstrated significant responses; however, patients frequently show intrinsic or acquired resistance to such therapies. Accumulating data suggest that epigenetic alterations are involved in melanoma development, progression, and tumor cell plasticity; moreover, epigenetic changes are implicated as critical mediators of melanoma therapeutic resistance. In this review, we provide an overview of epigenetic alterations in melanoma progression and resistance to targeted therapies and immune checkpoint inhibition. We further review the current state of epigenetic therapies in preclinical and clinical settings for melanoma and share promising future applications of such agents.

EPIGENETICS AND CANCER

Epigenetics refers to reversible changes of gene expression, which are heritable and not associated with DNA sequence changes. The most widely studied epigenetic mechanisms include DNA methylation, histone tail modifications, and regulation through noncoding RNAs. To coordinate biological processes and regulate gene expression, the epigenome interacts with various cellular elements, including noncoding RNAs and transcription factors, and is widely viewed as a biological rheostat responding to cell signaling pathways and extracellular stimuli to rapidly influence cell functions.

Although cancer is primarily a genetic disease, mutational events occur at too low a frequency to account for the efficient malignant transformation and therapeutic resistance observed in most malignancies that lack defects in DNA repair (Shen and Laird, 2013). Epigenetic changes were first implicated in tumorigenesis over 40 years ago with the observation that DNA methyltransferase (DNMT) activity is associated with malignant transformation (Holliday, 1979). Today, the epigenome is known to allow genetically identical cells to cycle between diverse, stable phenotypes and acquire oncogenic traits (Shen and Laird, 2013) and therapeutic resistance (Boumahdi and de Sauvage, 2020; Strub et al., 2020) in melanoma and other cancers (Feinberg and Tycko, 2004; Moran et al., 2018).

[1]*Department of Dermatology, Boston University School of Medicine| Boston Medical Center, Boston, Massachusetts, USA*

Correspondence: Rhoda M. Alani, Department of Dermatology, Boston University School of Medicine, 609 Albany Street, J-507, Boston, Massachusetts 02118-2515, USA. E-mail: alani@bu.edu

Abbreviations: BRAFi, BRAF inhibitor; DNMT, DNA methyltransferase; DNMTi, DNA methyltransferase inhibitor; dsRNA, double-stranded RNA; EZH2, enhancer of zeste homolog 2; EZH2i, enhancer of zeste homolog 2 inhibitor; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; MEKi, MAPK/extracellular signal–regulated kinase inhibitor; PTM, post-translational modification; SIRT, sirtuin; TMZ, temozolomide

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DNA modifications

DNMT enzymes catalyze the transfer of a methyl group to a cytosine residue on a CpG dinucleotide, which allows for binding of methyl-CpG-binding domain proteins and transcriptional silencing (Figure 1a). In the mammalian genome, CpG dinucleotides occur in long repetitive sequences or in CpG islands associated with gene promoters (Smith et al., 2010), and both DNA hypermethylation and hypomethylation have been implicated in oncogenesis (Rodríguez-Paredes and Esteller, 2011).

DNA hypomethylation was the first epigenetic mark to be associated with human cancers (Feinberg and Vogelstein, 1983). Global DNA hypomethylation may result in genomic instability (Rodríguez-Paredes and Esteller, 2011) and has been observed in virtually all cancers, including melanoma (Chatterjee et al., 2018; Ehrlich, 2009) where it is associated with increased immunotherapeutic resistance in patients (Jung et al., 2019) and promotion of cell proliferation, angiogenesis, metastasis, and poor patient outcomes (Van Tongelen et al., 2017; Vizoso et al., 2015; Wang et al., 2016).

DNA hypermethylation in melanoma promotes transcriptional silencing of over 70 genes involved in tumorigenesis (Schinke et al., 2010; Sigalotti et al., 2010), including tumor suppressors *PTEN*, *RASSF1A*, and *p16INK4a/14ARF* (Jones and Baylin, 2002; Micevic et al., 2017), as well as genes associated with DNA repair (Moran et al., 2018).

Histone modifications

The nucleosome is the basic functional unit of chromatin and consists of DNA wrapped around a complex of histones (Dawson and Kouzarides, 2012). Histones may be modified by covalent post-translational modifications (PTMs), including methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation. These PTMs may affect chromatin structure or recruit histone modifiers leading to altered gene transcription in cancers (Arrowsmith et al., 2012). Lysine and arginine residues on histones may be monomethylated, dimethylated, or trimethylated with specific methyl marks signaling transcriptional activation or repression. For example, trimethylation of lysine 9 and 27 of histone H3 (H3K9me3 and H3K27me3) is associated with transcriptional silencing, whereas H3K4me3 is associated with transcriptional activation (Arrowsmith et al., 2012). H3K4me2 and H3K27me3 marks are increased in melanomas and may serve as biomarkers for cancer cells with stem cell–like properties (Kampilafkos et al., 2015).

Enhancer of zeste homolog 2 (EZH2) (Figure 1b), a histone methyltransferase that is the core subunit of PRC2, promotes

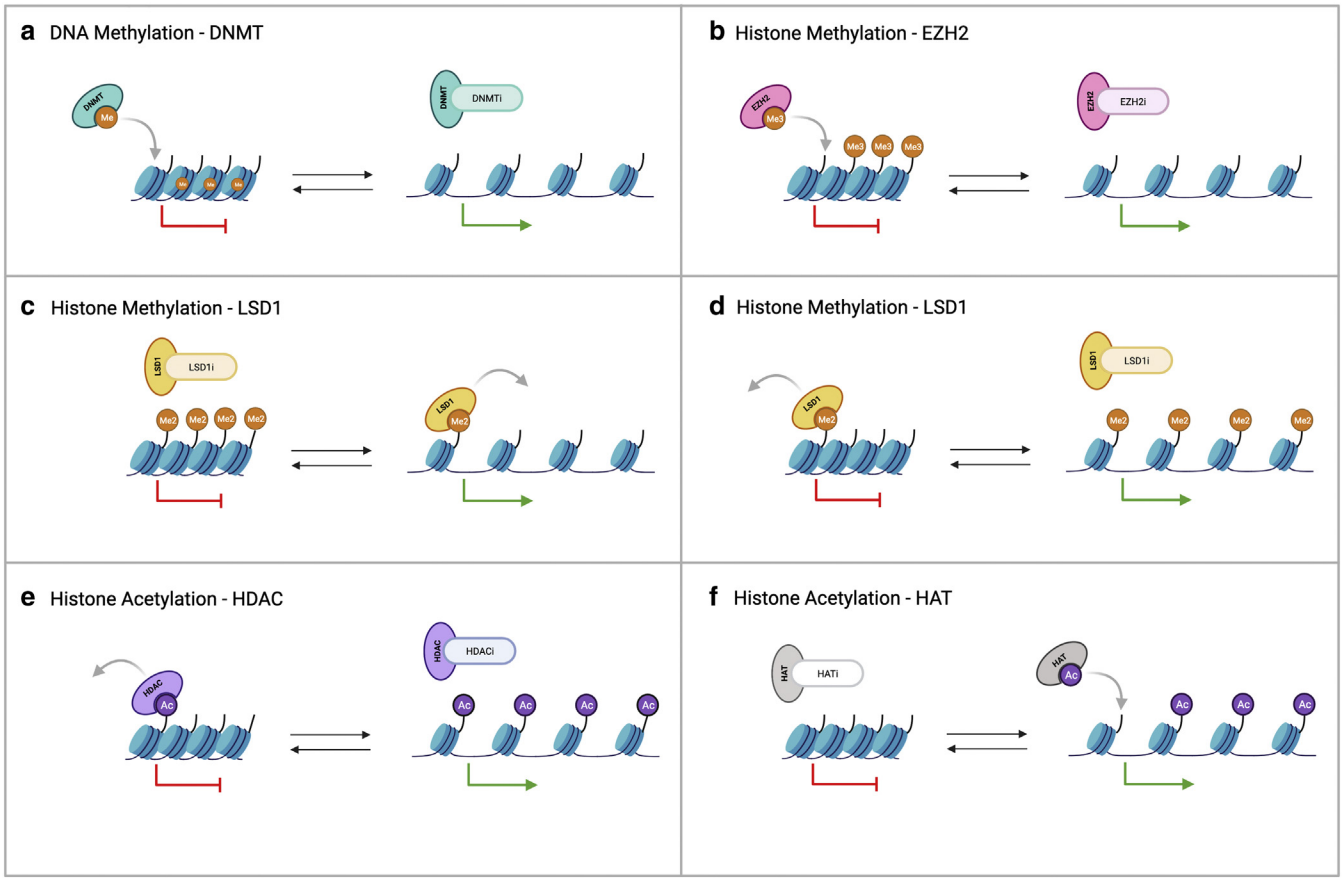

Figure 1. Targeting DNA methylation and histone modifications in melanoma. (**a**) DNA methylation and gene silencing can be targeted via inhibitors of DNMT enzymes. (**b**) Histone methylation can be blocked by EZH2 inhibitors, (**c, d**) leading to transcriptional activation and LSD1 inhibition, which can rarely function as (**c**) transcriptional repressors by removing activating monomethyl and dimethyl groups from H3K9 or, more commonly, as (**d**) transcriptional activators by removing monomethyl and dimethyl repressive methylation marks on H3K4. (**e**) Histone deacetylation can be inhibited by drugs targeting HDACs, leading to transcriptional activation, and (**f**) histone acetylation can be targeted by HAT inhibitors, leading to the inhibition of transcription. DNMT, DNA methyltransferase; EZH2, enhancer of zeste homolog 2; H3K4, H3 lysine 4; H3K9, histone 3 lysine 9; HAT, histone acetyltransferase.

tumor growth across a variety of cancer types (Bachmann et al., 2006; Mahmoud et al., 2016). EZH2 promotes trimethylation of histone H3 lysine 27 (H3K27me3), resulting in transcriptional silencing (Mahmoud et al., 2016) of genes, including tumor suppressors, HLA classes I and II, and other components of the immune system (Emran et al., 2019). EZH2 also interacts with DNMTs, inducing hypermethylation of promoter CpG islands (Moran et al., 2018). EZH2 shares several upstream signaling pathways with DNMTs, including BRAFV600E (Emran et al., 2019), and EZH2 activity has been associated with drug resistance in melanomas and prostate cancers (Boumahdi and de Sauvage, 2020; Zingg et al., 2017).

Enhanced activity of LSD1 (Figure 1c and d), the first histone demethylase to be identified (Shi et al., 2004), has been observed in several cancers, including melanoma (Maiques-Diaz and Somervaille, 2016; Yu et al., 2018). LSD1 is a core component of the CoREST repressor complex (Laugesen and Helin, 2014) and can demethylate histone and nonhistone substrates, including the tumor suppressor and transcriptional coactivator p53 (Huang et al., 2007). When associated with the histone deacetylases (HDACs) (HDAC1/2) in the CoREST complex, LSD1 primarily demethylates lysine 4 of histone H3, leading to transcriptional silencing (Laugesen and Helin, 2014; Shi et al., 2004). LSD1 also demethylates H3K9me3 in melanoma, promoting tumor cell growth (Yu et al., 2018). Inhibition of LSD1 activity enhances tumor immunogenicity and T-cell infiltration in melanoma, which resensitizes checkpoint blockade–refractory tumors to anti–PD-1 therapy (Sheng et al., 2018), whereas inhibition of the CoREST complex inhibits melanoma cell growth and resistance to targeted therapies (Kalin et al., 2018; Wu et al., 2020[1]).

Histone acetylation largely determines chromatin states and subsequent transcriptional activity (Bolden et al., 2006). Histone lysine acetylation promotes transcriptionally active chromatin, whereas deacetylation promotes chromatin compaction (Bolden et al., 2006). HDACs catalyze the removal of acetyl groups from lysine residues of both histone and nonhistone proteins; are associated with gene silencing (Bolden et al., 2006); and regulate tumor cellular proliferation, differentiation, and immunogenicity (Hornig et al., 2016) (Figure 1e). Four main classes of HDACs have been identified with class I (zinc-dependent sirtuins [SIRTs]) and class III (NAD^+-dependent SIRTs) HDACs as being of particular interest in oncogenesis (Garcia-Peterson et al., 2017; Weichert, 2009). Expression of class I HDACs, including HDAC1, 2, and 3, has been implicated in the development of melanoma (Rothhammer and Bosserhoff, 2007). SIRT1 inhibition resensitizes *BRAF*-mutant melanomas to BRAFi therapy (Ohanna et al., 2014), whereas inhibition of SIRT2 promotes MAPK inhibitor resistance in *BRAF*-mutant melanoma (Bajpe et al., 2015). Targeting SIRTs in cancer is complex because many have been found to possess tumor-suppressor and tumor-promoter functions (Garcia-Peterson et al., 2017); however, targeting of HDACs largely promotes anticancer effects (Hornig et al., 2016).

Histone acetylation, catalyzed by histone acetyltransferases (HATs), promotes open chromatin states and increased gene transcription (Arrowsmith et al., 2012), which may be associated with cancer (Portela and Esteller, 2010) (Figure 1f). The p300/CBP HAT is of particular interest in cancer because it may acetylate lysine residues on all four histones and regulate numerous cancer-associated pathways, including TGF-β, p53, and pRb (Iyer et al., 2004). Inhibition of p300 HAT in melanoma inhibits the expression of cell cycle regulatory genes, resulting in cellular senescence (Bandyopadhyay et al., 2002; Kim et al., 2019).

EPIGENETICS AND THERAPEUTIC RESISTANCE IN MALIGNANT MELANOMA

As the role of the epigenome in the biology of cancer and its specific functions in the emergence of drug tolerance and resistance in melanoma becomes elucidated, the development of novel treatments targeting the epigenome is rapidly expanding and may provide promise for more durable therapeutic responses. Although the investigations of epigenetic agents as monotherapies are ongoing, no single epigenetic therapeutic agent has proven effective for melanoma to date; however, emerging evidence suggests that their greatest benefits may lie in use as adjunctive therapies with other classes of drugs (Table 1). Given the inherent and acquired resistance to targeted and immunotherapies seen in melanoma, the prospect of combining epigenetic therapies with such established treatments to overcome this resistance is particularly promising. Below, we review the current status of various classes of epigenetic agents and how they are being developed in melanoma.

Combination epigenetic therapies for melanoma

In *BRAF*-mutant melanomas, reactivation of the MAPK pathway is a crucial mechanism in the development of resistance to targeted therapies (Long et al., 2014), which is largely linked to changes in gene expression and transcriptional programs, as opposed to gene mutations (Arozarena and Wellbrock, 2019). As such, epigenetic alterations have emerged as key players in the ability of melanoma cells to achieve resistance to BRAFi/MEKi therapies and are being evaluated as targets to overcome resistance. In addition, epigenetic alterations have been found to induce changes in both cancer and immune cells that enhance antitumor cellular responses, suggesting the utility of epigenetic agents in combination with immunotherapies (Chiappinelli et al., 2016; Héninger et al., 2015). Indeed, preclinical work suggests the benefit of combining immunotherapies alongside numerous classes of epigenetic drugs, and combination treatment protocols exploring the use of epigenetic therapies and immune modulators are actively being investigated in clinical trials (Table 2).

DNMT inhibitors. DNMT inhibitors (DNMTis), such as 5-azacytidine and 5-aza-2′-deoxycytidine (decitabine), were first synthesized in the 1960s and have been explored in clinical trials for a number of different malignancies, making them among the longest studied of epigenetic therapies (Christman, 2002; Sorm et al., 1964). These agents are cytidine analogs that block the catalytic activity of DNMTs, thus inhibiting DNA synthesis (Figure 1a) (Ahn et al., 2017). Perhaps the most promising potential application of DNMTis in the treatment of malignant melanoma is in combination

[1] Wu M, Hanly A, Gibson F, Kuang K, Kalin J, Nocco S, et al. The CoREST repressor complex mediates phenotype switching and therapy resistance in melanoma. bioRxiv 2020.

Table 1. Combination Treatment with Selected Epigenetic Therapies and Other Agents

Class of Combination Agent	Examples	Rationale	Refs
Epigenetic therapy: DNMT inhibitors			
BRAF inhibitors	Vemurafenib + decitabine	DNMT1 is upregulated by MAPK pathway and causes hypermethylation of *BRAFV600E*-mutant genes	Hou et al., 2012; Zakharia et al., 2017
Anti–PD-1 antibodies	Azacitidine + pembrolizumab (NCT02816021)	DNMT inhibition promotes PD-L1 expression	Chatterjee et al., 2018; Micevic et al., 2017
Anti–CTLA-4 antibodies	azacitidine + anti–CTLA-4	DNMT inhibition improves the recognition of tumor cells by T cells and upregulates viral defense response through cytoplasmic dsRNA sensing	Chiappinelli et al., 2015; Fonsatti et al., 2007; Triozzi et al., 2012
Alkylating agents	Decitabine + TMZ	Downregulation of MGMT, which is the mechanism by which melanoma cells achieve TMZ resistance	Tawbi et al., 2013
Epigenetic therapy: EZH2 inhibitors			
BRAF inhibitors	GSK2816126 + vemurafenib	*BRAF* mutations increase EZH2, leading to the downregulation of tumor suppressor genes	Yu et al., 2017; Zingg et al., 2015
Anti–CTLA-4 antibodies	GSK503 + anti–CTLA-4 antibodies	EZH2 silences immunogenicity in tumor cells	Goswami et al., 2018; Zingg et al., 2017, 2015
Epigenetic therapy: HDAC inhibitors			
BRAF inhibitors	panobinostat + encorafenib	HDACis reduce activity in RTK and PI3K signaling pathways	Emmons et al., 2019; Gallagher et al., 2018; Maertens et al., 2019
Anti–PD-1 antibodies	Nexturastat A + anti–PD-1 antibodies	HDACis increase the expression of PD-L1, enhancing T-cell activity	Knox et al., 2019
LSD1 inhibitors	Corin	Inhibiting the CoREST complex by cotargeting HDAC1/2 and LSD1 leads to growth inhibition	Kalin et al., 2018; Wu et al., 2020[1]
BET inhibitors	LBH598 + I-BET151	Caspase-dependent increase in apoptosis	Heinemann et al., 2015

Abbreviations: DNMT, DNA methyltransferase; dsRNA, double-stranded RNA; EZH2, enhancer of zeste homolog 2; Ref, reference; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; MGMT, O[6]-methylguanine-DNA methyltransferase; PI3K, phosphoinositide 3-kinase; TMZ, temozolomide.

with immunotherapy. In previous studies, the treatment of melanoma cell lines with decitabine resulted in improved recognition of melanoma cells by gp100-specific cytotoxic T cells through the upregulation of HLA class I antigens and ICAM-1 (Fonsatti et al., 2007). In addition, treatment of a murine melanoma model with low-dose decitabine led to increased macrophage effector and dendritic cell activation and decreased myeloid suppressor activity (Triozzi et al., 2012). Studies by Chiappinelli et al. (2015) showed that DNMTi therapies activated the viral defense response through cytoplasmic double-stranded RNA (dsRNA) sensing, thus potentiating the effects of anti-CTLA4 immune

Table 2. Current Clinical Trials with Epigenetic Agents and Immunotherapies in Cutaneous Melanoma

Drug	Target	Cancer Type	Phase	Status	NCT Number
Entinostat Pembrolizumab	HDAC1 PD-1	Melanoma	II	Recruiting	NCT03765229
Tinostamustine Nivolumab	HDAC PD-1	Melanoma	I	Recruiting	NCT03903458
Abexinostat Pembrolizumab	HDAC PD-1	Advanced solid tumors	Ib	Recruiting	NCT03590054
Mocetinostat Ipilimumab Nivolumab	HDAC CTLA-4 PD-1	Melanoma	Ib	Recruiting	NCT03565406
Domatinostat Nivolumab Ipilimumab	HDAC PD-1 CTLA-4	Melanoma	Ib	Not yet recruiting	NCT04133948
Panobinostat Ipilimumab	HDAC CTLA-4	Melanoma	I	Active, not recruiting	NCT02032810
Azacitidine Pembrolizumab	DNMT PD-1	Melanoma	II	Recruiting	NCT02816021
HBI-8000 Nivolumab	HDAC PD-1	Melanoma, renal cell carcinoma, NSCLC	Ib/II	Recruiting	NCT02718066
Entinostat Pembrolizumab	HDAC1 PD-1	NSCLC, melanoma, colorectal cancer	Ib/II	Active, not recruiting	NCT02437136

Abbreviations: DNMT, DNA methyltransferase; HDAC, histone deacetylase; NCT, National Clinical Trial; NSCLC, non–small cell lung cancer.

checkpoint therapy, whereas others have shown (Chatterjee et al., 2018) that decitabine treatment of melanoma cells led to increased PD-L1. A phase II clinical trial is currently underway to assess the efficacy of combined immune checkpoint blockade (pembrolizumab) and azacitidine in patients with metastatic melanoma (NCT02816021).

By contrast, DNMTis have shown promise as melanoma therapies when combined with BRAFis. *BRAFV600E* knockdown in melanoma cells leads to a downregulation of DNMT expression, suggesting that *BRAF V600E* mutation promotes gene hypermethylation through the upregulation of DNMT (Hou et al., 2012). Zakharia et al. (2017) conducted a phase 1b study using combination treatment of oral vemurafenib with subcutaneous administration of the DNMTi, decitabine, in a small group of patients with metastatic melanoma and found delayed development of resistance and improved duration of treatment response in those treated with low-dose, long-term decitabine and vemurafenib, with no dose-limiting toxicities observed, and follow-up studies are underway. Other studies suggest that DNMTis may play a role in combatting resistance to standard chemotherapy regimens in melanoma, including treatment with the alkylating agent temozolomide (TMZ), to which patients develop resistance through epigenetic silencing of DNA mismatch repair genes such as *MLH1* (Tawbi et al., 2013), and a phase I/II study investigating patients with metastatic melanoma who received the combination of decitabine and TMZ found improved overall survival and limited toxicities (Tawbi et al., 2013). Similarly, sequential treatment of melanoma cell lines with decitabine followed by the platinum-based chemotherapy carboplatin resulted in greater apoptotic response and decreased cellular proliferation (Budden et al., 2018).

EZH2 inhibitors. EZH2 transcriptionally represses several genes associated with immune responses, including genes that encode proteins involved in antigen presentation (major histocompatibility complex class I/II molecules) and chemokines needed to attract tumor-infiltrating lymphocytes (Tiffen et al., 2016). The upregulation of EZH2 and the resulting immunosuppressive microenvironment has been implicated as a mechanism through which melanoma cells develop resistance to immunotherapies (Tiffen et al., 2016; Zingg et al., 2017). After anti–CTLA-4 or IL-2 immunotherapy, melanoma cells increase EZH2 expression and transcriptionally silence the genes involved in tumor cell immunogenicity (Zingg et al., 2017). In addition, EZH2 inhibition has been shown to reverse acquired immune resistance and promote antimelanoma effects when combined with immunotherapies (Zingg et al., 2017), suggesting that a combination of EZH2 inhibitor (EZH2i) and immunotherapy may allow for overcoming resistance to immunotherapies in melanoma and other cancers (Tiffen et al., 2016; Zingg et al., 2017), and several EZH2is have entered clinical trials for nonmelanoma malignancies (Kim and Roberts, 2016).

EZH2 has been shown to promote melanoma growth and metastasis (Mahmoud et al., 2016), and EZH2 inhibition has been shown to impair melanoma growth and invasion (Zingg et al., 2015). Moreover, dual inhibition of BRAF and EZH2 showed synergies versus BRAFi treatment alone (Yu et al., 2017), and clinical trials of an EZH2i, tazemetostat, in advanced solid tumors are currently underway (NCT03217253, NCT01897571).

LSD1 inhibitors. Recent studies (Sheng et al., 2018) showed enhanced immune response to melanoma cells after ablation of LSD1, with subsequent increased expression of repetitive elements, including endogenous retroviral elements, and decreased expression of RNA-induced silencing complex components, leading to dsRNA stress and activation of the IFN-1 response. In addition, LSD1 ablation led to increased expression of PD-L1 on tumor cells and enhanced response to PD-1 blockade. A phase 1 trial of the LSD1 inhibitor SP-2577 (seclidemstat) is currently underway in patients with advanced solid tumors (NCT03895684). In addition, studies of the CoREST repressor complex in melanoma, of which LSD1 is a critical component, have shown enhanced antitumor immunity after CoREST inhibition by the small molecule, Corin (Xiong et al., 2020), whereas CoREST inhibition has also been shown to reactivate sensitivity to BRAFi in BRAFi-resistant melanomas in vitro and in vivo (Wu et al., 2020[1]), further supporting its specific role in acquired resistance to BRAFi therapies.

HDAC inhibitors. HDAC inhibitors (HDACis) have been shown to promote immunogenicity of melanoma cells, including through enhanced antigen processing and presentation (Khan et al., 2008) and enhanced expression of PD-L1, suggesting the potential role of dual treatment with HDACis and PD-1 inhibitors (Lienlaf et al., 2016; Woan et al., 2015; Woods et al., 2015), and recent studies (Knox et al., 2019) showed a shift to a hot, proinflammatory tumor microenvironment after melanoma treatment with the HDAC6 inhibitor Nexturastat A in combination with anti–PD-1 antibodies. These promising results suggest that dual treatment with HDACis and immunotherapies may have potent synergies. Clinical trials investigating combinational therapy with these two classes of drugs in melanoma are ongoing (NCT03590054, NCT03565406).

HDACis have also been shown to induce apoptosis and cell cycle arrest in melanoma cells (Pal-Bhadra et al., 2012; Venturelli et al., 2018), and BRAFi resistance is associated with increased expression of HDACs (Emmons et al., 2019). Treatment of melanoma cells with the HDACi suberic bishydroxamate has been shown to promote apoptosis (Zhang et al., 2004), whereas the pan-HDACi, LBH589, has been shown to induce apoptosis and G1 cell cycle arrest as well as increased immunogenicity in melanoma (Woods et al., 2013). Targeted inhibition of HDAC6 has also been shown to reduce the proliferation of melanoma cells and induced G1 arrest, without affecting apoptosis (Woan et al., 2015), whereas treatment of melanoma cells with the HDAC6 inhibitor ACY 1215 (ricolinostat) was shown to induce apoptosis and G0/G1 cell cycle arrest (Wang et al., 2018a).

With regard to melanoma resistance to BRAFis, HDAC8 was shown to contribute to the development of a drug-resistant phenotype in melanoma cells, whereas cotargeting of HDAC8 and BRAF in a mouse melanoma model resulted in synergistically decreased tumor growth (Emmons et al.,

2019). In addition, dual treatment of melanoma cells with panobinostat and the BRAFi, encorafenib, led to a synergistic reduction in RTK and phosphoinositide 3-kinase signaling in a subset of melanoma cell lines (Gallagher et al., 2018), whereas targeted inhibition of HDAC3 with entinostat enhanced the efficacy of BRAF/MEKis in *BRAF*-mutant melanomas (Maertens et al., 2019).

A major concern for the use of HDACi therapies has been their relative lack of selectivity and narrow therapeutic window. This limitation appears to have been overcome with the recent development of a novel specific inhibitor of the CoREST repressor complex, Corin (Figure 2), which showed dual-warhead activity versus LSD1 and HDAC1/2 (Kalin et al., 2018) and inhibits melanoma cell growth across a number of melanoma cell lines without significant effects on normal human melanocytes. Furthermore, treatment of BRAFi-resistant melanoma cells with Corin restored sensitivity of these tumor cells to treatment with BRAFi, suggesting that CoREST activity is a critical mediator of acquired resistance to BRAFi therapy in melanoma (Wu et al., 2020[1]).

HATs. Following the revelation that p300/CBP inhibition resulted in decreased melanoma proliferation (Bandyopadhyay et al., 2002), significant efforts were made to develop inhibitors of p300/CBP HAT activity (Lasko et al., 2017; Yan et al., 2013). Recently, a potent and selective inhibitor of p300/CBP, A485, has been shown to decrease melanoma cell proliferation and promote cellular senescence in a MITF-dependent fashion (Kim et al., 2019; Wang et al., 2018b). Of note, recent studies identified EP300 amplifications in over 16% of acral melanomas, suggesting a potential area of therapeutic focus for these compounds (Yeh et al., 2019).

CONCLUSION AND PERSPECTIVES

Despite the monumental advances in the treatment of melanoma over the past decade, the 5-year survival rate for the advanced disease remains low (Enninga et al., 2017). Investigations into the role of epigenetic influences in melanoma have increased dramatically in recent years, leading to important discoveries that have made us rethink the role of genetic versus of nongenetic alterations in cancer and their significance as therapeutic targets. Epigenetic alterations are becoming increasingly recognized as key mechanisms by which melanoma cells evade and develop resistance to treatment with targeted and immunotherapies and are therefore widely acknowledged as crucial targets in melanoma therapy. Inhibitors of DNMTs, EZH2, LSD1, HDACs, and HATs have shown antimelanoma effects both in vitro and in vivo, particularly when combined with BRAFi or immunomodulators; however, concerns regarding off-target effects of epigenetic therapies have significantly limited their utility in the clinic. The significant potential of targeted dual-acting epigenetic therapies to generate precise and effective responses at specific epigenetic modifying complexes while minimizing off-target effects should not be underestimated, and further research to optimize such therapies is of great importance for the field. Although there are currently no Food Drug and Administration–approved epigenetic therapies for melanoma, future studies will define the utility of novel potent and specific epigenetic therapies either as single or combination agents in melanoma, which are poised to revolutionize our approach to the management of patients with advanced disease.

Epigenetic alterations represent the major mechanisms of resistance for both targeted and immunotherapies in melanoma; however, combination therapies have proved ineffective in bypassing this major therapeutic hurdle, largely owing to significant toxicities and a limited therapeutic window. Further development and exploration of novel compounds that target the epigenome are essential to developing more effective treatments in melanoma; we propose that such novel therapies must be developed in a more directed manner to allow for specific inhibition of epigenetic complexes through strategic targeting of individual epigenetic complexes and that dual-action therapeutic agents will allow for significant advances in accomplishing these goals while minimizing toxicities. In addition, current research has only scratched the surface of the myriad complexes and interactions that influence the structure of the epigenome; we therefore expect that additional investigations will further clarify the implications of such complexes in the development of melanoma and other malignancies, which will lead to improved and effective targeted epigenetic therapies.

ORCIDs
Ailish Hanly: http://orcid.org/0000-0002-2242-423X
Frederick Gibson: http://orcid.org/0000-0002-4767-5393
Sarah Nocco: http://orcid.org/0000-0002-9719-3456
Samantha Rogers: http://orcid.org/0000-0003-1876-5944
Muzhou Wu: http://orcid.org/0000-0002-6322-0313

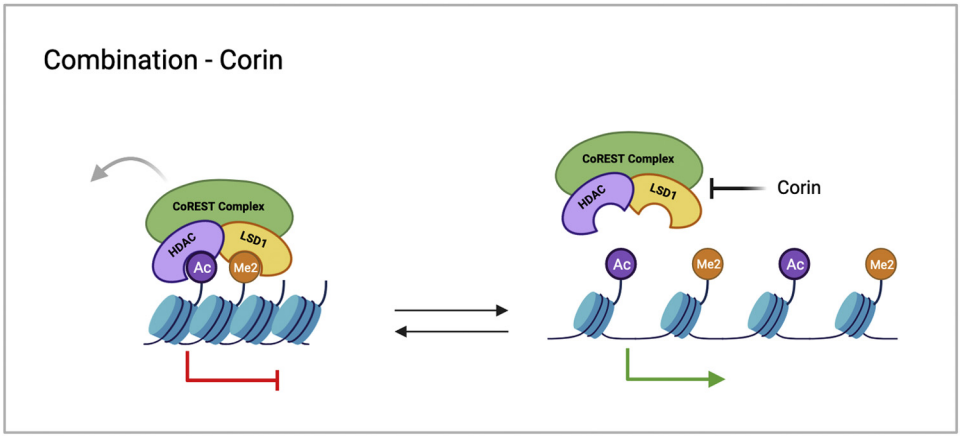

Figure 2. A novel inhibitor of the CoREST repressor complex, Corin, shows dual-warhead activity versus LSD1 and HDAC1/2, resulting in increased sensitivity and specificity for its epigenetic target (Kalin et al., 2018). HDAC, histone deacetylase.

Rhoda M. Alani: http://orcid.org/0000-0003-2741-2665

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

RMA and MW's interests were reviewed and are managed by the Boston University School of Medicine in accordance with their conflict of interest policies. The remaining authors state no conflict of interest.

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