Epigenetic therapy and chemosensitization in solid malignancy

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Abstract

Epigenetic modifications result in dynamic shifts between transcriptionally active and suppressed states. The potentially reversible nature of epigenetic changes underlies the concept of epigenetic therapy, which serves to reprogram cancer cells as opposed to inducing cytotoxicity that occurs with standard chemotherapeutics.

There are numerous enzymes involved in epigenetic changes and each can be potentially targetable. Although many investigations have evaluated the clinical potential of the various epigenetic therapies, currently only histone deacetylase inhibitors and DNA methyltransferase inhibitors are approved for use in specific hematologic malignancies.

Use of epigenetic therapy coincident with cytotoxic or targeted systemic therapy appears to derive a benefit due to chemosensitization. Trials demonstrating efficacy from combination therapy have been performed in various diseases such as NSCLC, ovarian cancer and breast cancer. Furthermore, there are patient subsets in certain solid tumors in which epigenetic therapy provide durable response, such as patients with NSCLC and specific hypermethylation patterns. The encouraging results from combination therapy identified in these trials built upon prior investigations and have provided a foundation for ensuing trials seeking to evaluate epigenetic therapy.

Introduction

The remarkable diversity of malignant processes underlies the difficulty in elucidating pathophysiology of and treatment for cancer. As eloquently outlined by Hanahan and Weinberg, a number of traits are acquired in the transformation from normal cell to neoplastic process, including sustained growth promoting signaling, circumventing apoptosis, immune evasion, and suppression of tumor suppressor genes [1]. These changes may occur via somatic mutations; alternatively, they can arise from epigenetic modification. For instance, cancer cells can achieve sustained proliferative stimulation via mutation in phosphatase and tensin homolog (PTEN), resulting in loss of function and amplification of PI3K signaling; similarly, PTEN expression can be inhibited by promoter methylation, which is a form of epigenetic modification [1].

Initially, epigenetic changes were found to be integral to malignant processes through a series of gene expression and DNA methylation studies [2]. Many of the early studies did not establish mechanism or pathways, but did identify a potential correlation between epigenetic modifications and cancer. With improved understanding of epigenetics, it has become clear that genetic and epigenetic changes are concomitantly involved in cancer initiation, promotion and progression. Affirming this concept is the fact that there have been identified a number of genetic lesions in epigenetic regulators in nearly all tumor types [3,4]. The ensuing aberrant signaling from these epigenetic regulators can then further promote gene expression alterations through modification of histone structure.

Epigenetics encompasses the heritable phenotype that arises from covalent modifications in histones and DNA without alterations of the DNA sequence itself [5,6]. Signals that initiate epigenetic changes may be an environmental cue, internal stimuli or developmental signals. Following the initial input, signal transduction incites a protein or noncoding RNA to establish chromatin interaction at a specific location, followed by a sustained chromatin state [5]. The chromatin-DNA interaction influences chromatin configuration; the presence of DNA in nucleosome-depleted regions is associated with gene expression while tightly bound DNA in the nucleosome structure leads to gene repression [7,8]. Nucleosomes consist of DNA wrapped around eight core histone proteins (2 each of H2A, H2B, H3 and H4), and post-translational modification of these core histones includes histone acetylation, methylation, ubiquitination, sumoylation, and phosphorylation; each can change the nucleosome structure, resulting
in modulation of DNA gene expression (see Fig. 1) [8–11]. Additional changes promoting gene transcription or gene repression arise from DNA methylation and the collective set of enzymes, which regulates open and closed chromatin conformation. Together, these changes allow regulation of cellular physiology such as transcription, DNA damage repair, DNA replication, genomic imprinting and X chromosome inactivation [6,11].

Enhanced understanding of epigenetic processes over the last several decades has identified a role for epigenetics in normal cellular mechanisms but also a significant role in cancer. This review encompasses epigenetics in the context of various malignant processes and therapeutic targets. Additionally, there have been an increasing number of clinical trials evaluating epigenetic therapy; earlier trials provided lessons for subsequent studies, and concepts such as combination therapy and chemosensitization have also arose. Finally, a discussion of future directions in epigenetics will be included.

**Epigenetics and malignancy**

Epigenetic gene silencing is a common feature in tumor cells and typically arises from DNA hypermethylation at tumor suppressor genes. The targeted gene regulatory regions (promoters or enhancers) undergo DNA hypermethylation at cytosine residues within CpG-islands [12]. In general, CpG sequences are dispersed throughout the genome with relatively low frequency and are typically heavily methylated. In contrast, CpG-islands contain a high frequency of the CpG dinucleotide; these islands are present in gene promoter regions and are unmethylated to allow gene expression. However, in malignant processes, enzymes including DNA methyltransferase (DNMT) can inhibit tumor suppressor gene expression via CpG island hypermethylation, such as hypermethylation of the MLH1 promoter in the sporadic form of microsatellite unstable colorectal cancer [13]. Regions of DNA methylation are interpreted by methyl-CpG binding proteins (MBP), which promote a transcriptionally inert chromatin environment via recruitment of histone-modification genes such as histone deacetylase (HDAC) or histone methyltransferase (HMT) containing complexes [13–15]. Therefore, regions of CpG island methylation are also characterized by histone hypoacetylation (histones H3 and H4) and histone methylation (H3K9-me) [6]. The polycomb repressor complexes (PRC), PRC1 and PRC2, are also critical for numerous cellular processes and function to silence transcription via H3K27 methylation (PRC2) and DNA condensation (PRC1) [10,11]. Interestingly, not all methylation events promote a transcriptionally inert state; while H3K9-me3 is associated with compact heterochromatin, trimethylation of lysine 4 in histone 3 (H3K4me3) promotes induction of gene expression [12]. Furthermore, certain signatures are associated with both an activating and repressive pattern. In embryonic stem cells, concomitant methylation of histone 3 at lysine 4 (H3K4me3) and lysine 27 (H3K27) promotes a bivalent domain in which gene expression is suppressed but readily able to switch to an activated state [11]. Histone 3 tail acetylation (H3K14Ac and H3K27Ac), carried out by histone lysine (K) acetyltransferases (HAT, also known as KAT), neutralizes the positively charged lysine and weakens the lysine-DNA interaction, thereby promoting a transcriptionally active chromatin structure [14].

Other enzymes involved in histone and DNA modification include, kinases, histone demethylases (HDM), ten eleven translocation protein 1–3 and phosphatases. Focused studies have also elucidated a role for microRNAs (miRNAs) in facilitating epigenetic signaling. These small, non-coding RNAs exist as post-transcriptional regulators of gene expression and fall under the category of epigenetic regulators given their modification of gene expression without alteration of DNA sequence [10]. For instance, among the miRNAs evaluated, miRNA-29 and miR-101 have been associated with gene promoter hypermethylation [7].

The combination of DNA methylation at promoter or enhancer regions, histone modifications and non-coding RNA interactions collectively determine gene expression [11]. Compared to changes in the linear DNA sequence, shifts between transcriptionally active and suppressed states from epigenetic modifications are heritable in somatic cells but potentially reversible. This concept drives the aim of epigenetic therapy, which serves to reprogram cancer cells as opposed to inducing cytotoxicity that occurs with standard chemotherapeutics. Therapies targeting epigenetic changes have been developed in order to reverse or block the deviant epigenetic modifications that occur in tumor cells. These agents are targeted to the various enzymes responsible for epigenetic modification and gene expression such as DNMTs, KATs, HDACs, HMTs, HDMs, non-coding RNAs and kinases; among them include 5-azacitidine.
certain epigenetic agents [i.e. 5-azacytidine, 5-aza-deoxycytidine (DAC)] in specific disease processes such as myelodysplastic syndrome (MDS) [16,17].

**Therapeutic targets and therapies**

Aberrancy in epigenetic regulation can occur via alteration of any number of enzymes involved in the intricate network of epigenetic pathways. However, histone acetylation and methylation are the most commonly affected pathways [3]. Histone lysine demethylases have been found to be aberrantly functioning in a number of malignant processes, although currently there are targeted therapeutics that have only demonstrated preclinical efficacy (see Table 1) [11]. Similarly, although overexpression of various HMTs (e.g. protein arginine methyltransferase 5 (PRMT5)) has shown an oncogenic role, and PRMT-5 specific inhibitors demonstrate antitumor effect in preclinical models (mantle cell lymphoma), there are no clinically efficacious therapies approved for use in malignancy [11]. Potential exists for therapies that modify KATs and miRNA, but additional investigation is necessary for approval of epigenetic therapies in these categories. Currently, HDAC inhibitors and DNMT inhibitors are the classes of drugs approved for use in various malignancies by the FDA [14].

Dosing and schedule are particularly important in order to achieve an epigenetic effect; inappropriate frequency of administration or high drug concentrations can produce cell toxicity as opposed to cell reprogramming [14]. In conjunction, the synergistic effect with other therapeutic agents is contingent upon a prolonged reprogramming of the aberrant pathway. Conceptually, therapies aimed at epigenetic pathways target proteins that establish epigenetic changes (writers) such as DNMTs, that recognize changes to histones or DNA and facilitate interaction with additional protein complexes (readers), and those that remove the epigenetic modifications (erasers) such as HDAC (see Fig. 2) [17].

**MicroRNAs**

Through gene suppression and activation, various microRNAs influence cellular processes such as cell growth, differentiation and apoptosis; for example, miR-21 is upregulated in pancreatic cancer and has been shown to target apoptosis related genes such as PTEN [10]. Countering oncogenic miRNA changes can be achieved by various therapeutic agents including metformin, which has been shown to reverse the suppression of miR-101, miR-200, let-7 and miR-26a in models of pancreatic cancer [18]. The resultant miRNA upregulation promotes decreased pancreatic cancer cell survival and increased sensitivity to gemcitabine. These observations identify the importance of miRNAs in epigenetics and suggest a potential therapeutic target for various solid malignancies.

**Histone acetyltransferase**

KAT facilitates the transfer of an acetyl group (from acetyl coenzyme A (CoA)) to lysine located on a histone. These enzymes are subdivided into nuclear proteins that acetylate histones and other chromatin-associated proteins (type A KAT) and those that mostly acetylate histones in the cytoplasm to promote nuclear localization (type B KAT) [6]. KAT inhibitors are generally compounds that resemble acetyl CoA and are sub classified as synthetic peptides, small molecules, or natural product KAT inhibitors. Synthetic and naturally occurring KAT inhibitors are limited by cellular impermeability while permeability issues do not restrict small molecule KAT inhibitors. However, KAT inhibitors as drug targets for malignant processes are further limited by the large, complex multi-protein structure containing KAT, and therefore, additional work is required for KAT inhibitors to become cogent options as therapeutic intervention for cancer [6].

**Histone deacetylase**

HDACs function to remove acetyl groups from histones and are divided into four categories based on homology to yeast HDACs...
Class I (HDAC 1, 2, 3, 8), IIa (HDAC 4, 5, 7, 9), IIb (HDAC 6, 10) and IV (HDAC 11) encompass 11 different HDACs, and possess a zinc cofactor while class III HDACs utilize a nicotinamide adenine dinucleotide (NAD) cofactor [19]. Among the categories, class I HDACs demonstrate a propensity for nuclear location and therefore regulate acetylation of histones associated with DNA; in contrast, other classes are located in both the nucleus and cytoplasm and therefore demonstrate broad regulation of protein acetylation status [20]. In general, HDAC inhibitors exploit the HDAC cofactors, blocking zinc chelation in class I, II and IV HDACs, but do not possess activity against class III HDAC [19]. The resultant inhibition of HDACs generates pleiotropic effects, which include inducing DNA damage while inhibiting processes of DNA repair. Additionally, HDAC inhibitors alter chromatin structure to allow expression of suppressed genes such as cell cycle regulators (p21) with ensuing cell-cycle arrest and pro-apoptotic genes resulting in promotion of apoptosis [6,21]. Indeed, promotion of tumor cell apoptosis is the most common biologic effect identified [19]. Adding additional complexity is the fact that lysine de-acetylation inhibition may be indiscriminate, encompassing not only histones but also tubulin, HSP90 and proteins unrelated to the targeted pathway (such as p53) that may influence cell fate [4,14,22]. HDAC inhibitors include hydroxamic acids (vorinostat, dacinostat, panobinostat, belinostat), which function as pan-HDAC inhibitors, benzamides (entinostat, EX527), and cyclic tetrapeptides (romidepsin) [19,22].

The zinc-dependent HDAC inhibitors have been utilized in trials and have shown synergistic efficacy with standard chemotherapeutic agents in patients with myeloid and advanced solid malignancies [23–25]. In these dose escalation trials, patient toxicity included neutropenia, thrombocytopenia and respiratory distress. At high doses, HDAC inhibitors can induce DNA damage resulting in a cytotoxic effect, which highlights the importance of correct dosing in order to generate desired therapeutic outcome [9]. Markers of HDAC inhibitor efficacy include histone acetylation in peripheral blood mononuclear cells (PBMC), enzyme activity, identifying specific (target) gene re-activation and evaluation of circulating cell-free DNA; however, the optimal measure of efficacy remains tumor effect [14]. Therapeutic effectiveness has been demonstrated in cutaneous T-cell lymphoma and peripheral T-cell lymphoma; in fact, vorinostat and romidepsin are both FDA approved for use in these disease processes [26,27]. Although promising results as monotherapy in hematologic diseases, HDAC inhibitors utilized as single agents to combat solid tumors has revealed suboptimal results [19].

**DNA methyltransferase**

DNA methylation occurs when DNMTs facilitate transfer of a methyl group from a methyl donor (i.e. S-adenosyl-L-methionine) to the 5′ location of cytosine in a CpG sequence [14]. There are numerous methyltransferase enzymes including DNMT1, which is responsible for propagation of DNA methylation patterns during DNA replication, and DNMT3a and 3b, which serve to methylate DNA de novo and are present in embryonic stem cells, germ cells and to a lesser degree in somatic tissues [14]. The CpG dinucleotides can be gathered in sequential repeats within or around gene promoters such that methylation of these repetitive sequences (CpG islands) alters gene transcription. While DNA methylation is necessary during embryogenesis and for tissue-specific gene expression, erroneous DNA methylation is an established hallmark of cancer [14,28]. The consequence of DNA hypermethylation is potentiation of a suppressive chromatin environment and ensuing inhibition of gene expression [14]. Affected genes regulate DNA repair, apoptosis, angiogenesis, drug resistance and metastatic capability; all of which can contribute to cancer initiation, promotion or progression [15,29]. In fact, in the setting of colorectal tumorigenesis, DNA methylation errors appear to be an early event, identified in the pre-malignant stage [7,9]. Additionally, DNA methylation of specific genes can provide prognostic information. For instance, the correlation between poor prognosis in NSCLC and hypermethylation of CDKN2A and CDH13, or response to chemotherapy and hypermethylation of MGMT in glioma [7].

DNMT inhibitors include AZA, DAC and DAC combined with guanosine (guadecitabine or SGI-110). Guadecitabine is a DNMT pro-drug that is broken down to decitabine and overcomes the limitation of short half-life seen with current DNMT inhibitors. These cytidine analogues are incorporated into DNA and function to irreversibly inhibit the various DNMT enzymes as well as promote their degradation [17]. Each have shown efficacy in reprogramming tumor cells as evidenced by reactivation of suppressed genes that were inhibited as a consequence of CpG-island hypermethylation.

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**Fig. 2.** Four R’s: a chromatin remodeling phenomenon.
tion [7,15,30]. In turn, clinical efficacy of AZA and DAC at appropriate (low) doses, led to approval by the FDA for use in MDS [16].

Trials

Many of the trials investigating epigenetic therapy in solid tumors evaluate patients receiving epigenetic therapy in combination with either cytotoxic chemotherapy or immunotherapy, typically in the setting of advanced disease refractory to standard anticancer treatment [14]. Interestingly, the most promising results in patients with solid tumors receiving epigenetic therapy have been as adjunct therapy. Conceptually, this may be due to oncogenic signaling leaving an ‘epigenetic imprint’ such that targeting the aberrant oncogenic pathway as well as the epigenetic imprint may be necessary [11]. For instance, in breast cancer cells, loss of estrogen receptor (ER) signaling results in recruitment of PRC2 complex and HDAC1 to the promoter region of ER responsive genes, such as progesterone receptor (PR) [31]. The ensuing promoter hypermethylation ensures that restoration of PR signaling necessitates reestablished ER signaling and promoter demethylation. Thus, the complexities associated with epigenetic pathway crosstalk, such as DNA hypermethylation and histone modification, and the interaction with canonical oncogenic signaling underscores the difficulty in targeting these pathways in solid tumors.

Initial lessons

One concept that has been repeatedly demonstrated is that use of demethylating agents and HDAC inhibitors is less effective when used at higher, cytotoxic doses [4]. The demethylating agents were initially used at cytotoxic doses and were associated with significant toxic side effects [30]. Further, cell death from cytotoxicity did not produce durable responses; an essential shift occurred when the lower doses of DNMT inhibitors used in MDS revealed durable single-agent responses [30]. This reprogramming effect at lower doses was not only identified in DNMT inhibitors, but also HDAC inhibitors (T-cell lymphoma), which provided the foundation for implementing low-dose epigenetic agents combined with chemotherapeutics (NSCLC, breast cancer) [4,30]. An additional consideration is implementation of these therapies when patients remain ‘salvageable’ from a therapeutic standpoint. Because tumor cell reprogramming requires a longer duration to become clinically evident, patient disease extent should not be so extensive to preclude any opportunity for effective therapeutic response [4]. In conjunction, evaluation of response to therapy has proven difficult given the propensity for disease stabilization without perceived reduction in tumor volume. For this reason, as opposed to standard response evaluation criteria in solid tumors (RECIST), disease stability, or alternative measures of therapeutic efficacy should be ascertained such as novel markers in addition to overall survival [30].

Combination therapy

Circumstances in which epigenetic aberrancy results in a driver mutation allows for epigenetic monotherapy. However, this occurrence in solid tumors is exceedingly rare as evidence by the lack of benefit from HDAC inhibitor monotherapy in thymic cancer, glioblastoma, renal cell carcinoma, hepatocellular carcinoma, ovarian cancer and other tumor types [4]. Rather, use of epigenetic therapy coincident with other systemic therapy (cytotoxic or targeted) appears to derive the greatest benefit. Even in circumstances where patients possess tumors refractory to chemotherapeutics, combination with epigenetic therapy can promote or reinstate chemosensitivity. This concept arose from preclinical models and has been studied extensively across many solid tumor types. For instance, enhancer of zeste homolog 2 (EZH2), an HMT that catalyzes methylation of H3K27 resulting in suppression of gene expression, is frequently overexpressed in cancer and elevated expression levels appear to correlate with poor prognosis, including patients with renal cell carcinoma (RCC) [32]. In turn, Adelaiye and colleagues sought to improve therapy in patients with RCC refractory to sunitinib therapy. The authors utilized cell lines, mouse model (patient derived xenografts) and resected specimens from patients with RCC and found that tumors resistant to sunitinib therapy exhibited high levels of expression of EZH2 and concordant increase in the histone mark H3K27me3 [33]. They utilized an EZH2 siRNA to demonstrate that knockdown of EZH2 expression decreased RCC cell viability and improved therapeutic efficacy of sunitinib treatment, in vitro. Preclinical studies such as this have been performed in many solid tumor subtypes and provide a foundation for subsequent trials. Although combination therapy of EZH2 inhibitors with sunitinib in RCC has not yet reached clinical trial, other combination therapies in various malignant processes have proven effective (see Table 2). For example, preclinical studies had demonstrated inhibitory growth effect on cancer cells of hydralazine and valproic acid in vitro [34], and also the ability to overcome chemotherapy resistance in refractory solid tumors in a phase II study [35]. Therefore, Coronel and colleagues sought to investigate the effect of hydralazine (DNMT inhibitor) and valproic acid (HDAC inhibitor) in 36 women with stage IVB cervical cancer [36]. This randomized, double blind, phase III trial demonstrated improved PFS (10 months versus 6 months, p = 0.0384) in patients receiving epigenetic therapy in combination with standard chemotherapy (topotecan, cisplatin) compared to chemotherapy alone. Further, 24% of patients in the chemotherapy plus epigenetic therapy arm achieved partial response (PR) versus 5% in the chemotherapy arm. The authors suggested the improved outcome was related to re-expression of tumor suppressor genes as well as chemosensitization.

Brock and colleagues evaluated the DNA methylation profile of primary tumors and mediastinal lymph nodes in patients with NSCLC and found several prognostic markers [37]. In particular, there were four gene targets that were silenced secondary to hypermethylation (CDKN2a, CDH13, APC, and RASSF1a) that demonstrated strong concordance with disease recurrence and death [37]. Methylation of two of the four target genes in patients with stage I NSCLC translated to a considerably worse prognosis (similar to patients with stage III disease). In turn, Juergens et al. sought to reverse the epigenetic silencing of these genes using combination epigenetic therapy (DNMT inhibitor + HDAC inhibitor) in patients with progressive, metastatic NSCLC [20]. This phase II study included 45 patients that received azacitidine and entinostat (class I HDAC inhibitor) for a median of 2 treatment cycles. Treatment was relatively well tolerated with 28% of patients experiencing grade 3 or 4 toxicity (most fatigue or anemia). One patient experienced a complete response of 14 months duration and a second patient had a partial response lasting 8 months. There were ten patients with stable disease for at least 12 weeks including one without progression for 18 months and another for 14 months. Twenty-two experienced progressive disease after 2 cycles of therapy while 11 did not complete one cycle of therapy due to withdrawal from study, decline in performance status or early progression. Interestingly, the patient with complete response underwent evaluation of epigenetic signatures and was found to have target gene hypermethylation highly consistent with an early recurrence pattern (methylation of CDKN2a, CDH13, APC, and RASSF1a). This patient failed multiple chemotherapeutic regimens prior to combination epigenetic therapy. While this patient demonstrated complete response to a combination of azacitidine and entinostat, she unfortunately developed a second distinct pri-
mary and endured relapse with fatal progression of the second NSCLC. Similarly, the patient with partial response also possessed hypermethylation of 3 of 4 target genes; the patient had a marked and sustained response (22 months) to the epigenetic therapy for his NSCLC. Furthermore, there were 19 patients that received systemic therapy following completion of epigenetic therapy. Remarkably, 4 demonstrated significant objective response to the adjunct chemotherapy, indicating likely chemosensitization by the epigenetic treatment in this heavily pretreated population. Although there was disease stability or response in only 12/45 (27%), durable survival (>2 years) was achieved in 5/45 (11%) and this study depicted a subset of patients in which epigenetic therapy may promote a durable response.

A study by Witt et al. identified a specific subset of patients with advanced NSCLC that benefitted from combination epigenetic and systemic therapy [38]. Erlotinib, an epidermal growth factor receptor – tyrosine kinase inhibitor (EGFR-TKI), has demonstrated increased efficacy in patients with elevated E-cadherin and therefore, the combination of erlotinib with an HDAC inhibitor (entinostat) was trialed versus erlotinib alone in a randomized phase II study for 132 patients with advanced stage (IIIB/IV) NSCLC. Overall, there was no difference in PFS (1.88 months erlotinib versus 1.97 months erlotinib + entinostat, p = 0.98) or OS (6.7 months versus 8.9 months, p = 0.39). However, in subset analysis of patients with elevated E-cadherin expression, patients receiving the HDAC inhibitor with erlotinib experienced improved OS compared to the erlotinib only group (9.4 months versus 5.4 months, p = 0.03). Importantly, this study identified a population of patients (E-cadherin high expression) in which the HDAC inhibitor sensitized to TKI therapy.

In a separate study that evaluated patients with advanced NSCLC, Ramalingam et al. sought to determine the benefit from addition of vorinostat to carboplatin and paclitaxel [39]. Preclinical studies had demonstrated synergistic effects of HDAC inhibitors when combined with taxanes and platinum compounds, and a phase I study revealed PR in 10/19 patients with advanced stage NSCLC; these results provided an impetus for the ensuing clinical study. In this phase II randomized double-blind study evaluating patients with stage IIIB/IV NSCLC, a total of 62 patients received the HDAC inhibitor in addition to chemotherapy while 32 received chemotherapy with placebo. Response rate was 34% in the vorinostat arm and 12.5% in the chemotherapy arm (p = 0.02). Although not statistically significant, there appeared to be favorable PFS (6 months versus 4.1 months, p = 0.48) and OS (13 months versus 9.7 months, p = 0.17) for patients receiving vorinostat.

Entinostat demonstrates class I specificity for HDAC inhibition, which is unique in comparison to vorinostat and romidepsin (wider range of deacetylase inhibition). This epigenetic drug has also demonstrated restoration of hormone sensitivity in preclinical models of breast cancer [40]. For this reason, Yardley and colleagues combined entinostat with exemestane in patients possessing ER-positive breast cancer for the purpose of cancer cell sensitization to the antiestrogen therapy [40]. The phase II, randomized, double blind, placebo-controlled study included patients with locally advanced or metastatic breast cancer (ER+) that had progressed on prior endocrine therapy (NCT02115282). The ability of entinostat with DNMTi (DNMT inhibitor, PFS = progression free survival, OS = overall survival, SD = stable disease, cPR = clinical partial response, cCR = clinical complete response; EGFR = epidermal growth factor receptor, TKI = tyrosine kinase inhibitor).

### Table 2

Select trials of combination epigenetic therapy and chemotherapy in advanced cancer. Primary cancer highlighted in bold.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient pathology</th>
<th>AIM</th>
<th>Epigenetic therapy</th>
<th>Chemotherapy</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronel et al. [36]</td>
<td>Stage IIB/IV Cervical Cancer (n = 36)</td>
<td>Evaluate efficacy of CTx + Epigenetic Tx versus CTx alone</td>
<td>Hydralazine (DNMTi), Valproic Acid (HDACi)</td>
<td>Topotecan, Cisplatin</td>
<td>10 months versus 6 months PFS, (p = 0.03)</td>
</tr>
<tr>
<td>Juergens et al. [20]</td>
<td>Progressive, metastatic NSCLC (n = 45)</td>
<td>Evaluate efficacy of DNMTi plus HDACi in chemotherapy refractory disease</td>
<td>Azacitidine (DNMTi), Entinostat (HDACi)</td>
<td>Variable following epigenetic tx</td>
<td>OS or PR in 12/45, durable response (&gt;2 years) in 5/45</td>
</tr>
<tr>
<td>Witta et al. [38]</td>
<td>Advanced stage (IIIB/IV) NSCLC progressed on prior therapy (n = 132)</td>
<td>Evaluate HDACi in reversing EGFR-TKI resistance in NSCLC</td>
<td>Entinostat (HDACi)</td>
<td>Erlotinib (EGFR-TKI)</td>
<td>E-cadherin high expression benefit (9.4 months versus 5.4 months OS)</td>
</tr>
<tr>
<td>Ramalingam et al. [39]</td>
<td>Advanced stage (IIIB/IV) NSCLC previously untreated (n = 94)</td>
<td>Evaluate HDACi in reversing EGFR-TKI resistance in NSCLC</td>
<td>Vorinostat (HDACi)</td>
<td>Carboplatin and Paclitaxel</td>
<td>RR 34% versus 12.5% (p = 0.02); OS 13 months versus 9.7 months (p = 0.17)</td>
</tr>
<tr>
<td>Yardley et al. [40]</td>
<td>Locally advanced or stage IV Breast Ca progressed on AI (n = 130)</td>
<td>Evaluate HDACi effect of sensitization to AI therapy</td>
<td>Entinostat (HDACi)</td>
<td>Exemestane (AI)</td>
<td>Median OS 28.1 versus 19.8 months (p = 0.036)</td>
</tr>
<tr>
<td>Fu et al. [41]</td>
<td>Advanced stage (IIIB/IV) platinum refractory Ovarian Ca (n = 30)</td>
<td>Evaluate ability of DNMTi in reversing platinum resistant or platinum refractory ovarian ca</td>
<td>Azacitidine (DNMTi)</td>
<td>Carboplatin</td>
<td>1 cCR, 3 cPR and 10 patients with SD</td>
</tr>
<tr>
<td>Matei et al. [42]</td>
<td>Advanced stage, heavily treated, platinum-res Ovarian Ca (n = 17)</td>
<td>Evaluate efficacy of DNMTi in reversing platinum resistant or platinum refractory ovarian ca</td>
<td>Decitabine (DNMTi)</td>
<td>Carboplatin</td>
<td>RR 35% (1 cCR, 3 cPR); Median PFS 10.2 months</td>
</tr>
</tbody>
</table>

NSCLC = non small cell lung cancer, CTx = chemotherapy, DNMTi = DNMT inhibitor, HDACi = HDAC inhibitor, PFS = progression free survival, OS = overall survival, SD = stable disease, cPR = clinical partial response, cCR = clinical complete response; EGFR = epidermal growth factor receptor, TKI = tyrosine kinase inhibitor.
experiencing progression after chemotherapy (TNBC) or chemotheraphy and endocrine therapy (hormone resistant breast cancer) [41]. Patients with TNBC (n = 13) or hormone resistant breast cancer (n = 27) received combination epigenetic therapy (AZA + entinostat) and when progression occurred, they were offered continuation therapy including epigenetic therapy and hormone therapy. There was minimal clinical efficacy overall in this trial (0/13 response in TNBC, 2/27 response hormone resistant), and on hormone expression analysis (ER), there were no TNBC patients with changes in ER expression. However, there were 2 patients in the hormone resistant group that experienced increased ER expression post- versus pre-therapy. These patients also demonstrated moderate disease stabilization when endocrine therapy was added to epigenetic therapy (8 months and 11 months), suggesting a potential subset that responds to endocrine therapy following epigenetic priming (although low patient numbers provide limited conclusions).

Chemosensitization was similarly the premise for the phase Ib/IIa clinical study of azacitidine in ovarian cancer performed by Fu and colleagues [42]. Previously, differences in PFS were noted between patients with late-stage ovarian cancer stratified by levels of methylation. Patients with higher levels of methylation fared worse than those with lower levels of methylation (<8 months versus >12 months, p < 0.001), and in preclinical studies, treatment of platinum resistant ovarian cancer cell lines induced re-expression of HMLH1 with consequent re-sensitization to platinum therapy [43,44]. Therefore, the phase Ib/IIa study was undertaken in order to identify the safety and efficacy of azacitidine combined with carboplatin in 30 patients with advanced ovarian cancer that had progressed on platinum therapy [42]. In this heavily pre-treated population (2/3 of patients received at least 3 types of systemic chemotherapy prior to the study), 1 patient demonstrated a complete clinical response (cCR), 3 achieved a partial clinical response (pPR) and 10 patients demonstrated disease stability. Median PFS was 3.7 months and median OS was 14 months with 6/30 patients (20%) achieving greater than 30 months survival. Although no biomarker was evident, DNMT inhibition appeared to reverse platinum resistance in select patients.

In the prior study, patients with platinum resistant (recurrence within 6 months of platinum therapy) ovarian cancer experienced increased response rate and overall survival compared to those with platinum refractory (progression while on platinum therapy) ovarian cancer [42]. A subsequent study by Matei et al. also evaluated heavily pretreated patients (median 5 regimens) with platinum resistant or platinum refractory ovarian cancer in a phase I/II study of low-dose decitabine (DNMT inhibitor) combined with carboplatin [45]. Repeated doses of decitabine were provided prior to platinum therapy, and peripheral blood samples demonstrated biologic activity as evidenced by DNA hypomethylation. There was one cCR and 5 cPR among 17 patients in the study (response rate = 35%), with 6 additional patients achieving stable disease for longer than 3 months. Median PFS was 10.2 months and median OS was 13.8 months. Through methylation studies, there appeared to be an association between extent of methylation and PFS, indicating treatment induced decreased in methylation was associated with reversal of platinum resistance.

Although there are certainly favorable results in various tumor types (NSCLC, ovarian, breast), other solid tumors, such as pancreatic ductal adenocarcinoma (PDA), have proven less successful. Unfortunately, promising pre-clinical studies of HDAC inhibition for PDA have not translated into similar efficacy in clinical trials investigating epigenetic therapy coincident with chemotherapy [32]. Despite this, the encouraging results from combination therapy studies yielding efficacious outcomes have provided a foundation for ensuing trials seeking to evaluate epigenetic therapy in conjunction with chemotherapeutics (see Table 3). For instance, in patients with advanced colorectal cancer who have failed at least first line irinotecan-based chemotherapy (with no limit on the number of lines failed), there is an ongoing phase I/II trial evaluating Guadecitabine (SGI-110) combined with irinotecan versus regorafenib [NCT01896856]. The aims of this trial are to identify the maximum tolerated dose of Guadecitabine and then to determine the efficacy of DNMT inhibition to re-sensitize patients to irinotecan [30]. The phase I component of the trial (n = 22) was reported at the AACR meeting in 2016. The various drug dosages appeared to be reasonably well tolerated, and of 22 heavily pre-treated patients, 12 patients experienced stable disease (for greater than 4 months) and one patient demonstrated a partial response [46].

Congruent with the concept of chemosensitization, epigenetic therapy is also being studied to determine the capability of ‘switching’ patients from an immunotherapy unresponsive to an immunotherapy responsive state. AZA treatment, for instance, has been shown to induce immune related gene upregulation in various solid tumors [47], including PD-L1 expression in patients with NSCLC, allowing for combination treatment with checkpoint inhibitors and prompting a subsequent clinical trial (NCT01928576) investigating the effects of epigenetic therapy as a sensitizing agent to immune checkpoint therapy [17,20,48].

Further, another clinical trial is evaluating the combination of HDAC inhibition (romidepsin) with or without DNMT inhibition (5-azacitidine) adjunct to MK-3475 (PD-1 inhibitor) in patients with micro-satellite stable (MSS) colorectal cancer. These investigators will evaluate the extent of tumor infiltrating lymphocytes and also determine the extent to which patients become responsive to targeted immunotherapy (NCT02512172).

**Future directions and conclusions**

The field of epigenetics is rapidly changing the approach to cancer therapy. Canonical oncogenic pathways arise from alterations in DNA, and the epigenetic changes can also promote tumor initiation, promotion or progression by further regulating gene expression. It is quite clear that both genetic mutation and epigenetic abnormalities drive development of cancer. Targeting both the traditional oncogenic pathways combined with epigenetic therapies will likely provide the most robust results in solid tumors. However, unlike genetic mutations, epigenetic changes are reversible in nature, and the reprogramming associated with therapy represents an exciting avenue for targeted cancer treatment.

Early successes in hematologic malignancies led to the approval of several epigenetic drugs in cancer, and allowed better understanding of epigenetic agents to treat malignant processes. These successes continue as evidenced by recent studies demonstrating combination vitamin C and DAC resulting in synergistic tumor cell death in multiple tumor cell lines [49]. The combination therapy produced robust upregulation (>120-fold) of genes involved in dsRNA recognition and viral defense, with ensuing enhanced apoptosis of cancer cells in comparison to either therapy alone. The authors then revealed that nearly 60% of patients with hematologic malignancies are vitamin C deficient (in comparison to 7% of US population) and proposed the novel combination of vitamin C and DNMT inhibitor for this group. In fact, there is currently a pilot study (NCT02877277) that is investigating whether vitamin C improves response to therapy with DNMT inhibitors in patients with myelodysplastic syndrome and acute myeloid leukemia. These results may then have subsequent implications in solid tumors.

However, the successes identified in hematologic disease are less reproducible in solid tumors, and likely requires improved identification of patient subsets that derive benefit from therapy.
Table 3
Trials in Epigenetic Therapy.

<table>
<thead>
<tr>
<th>Type</th>
<th>Group</th>
<th>Drug</th>
<th>Disease</th>
<th>Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monotherapy</td>
<td>DNMTi</td>
<td>Azacitidine, DAC</td>
<td>ABC, NSCLC</td>
<td>NCT01349959</td>
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<tr>
<td></td>
<td></td>
<td>SG1-11, Vorinostat</td>
<td>MDS, Ovarian, AML, Colon, HCC</td>
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<td></td>
<td></td>
<td>Romidepsin</td>
<td>Advanced CTCL</td>
<td>NCT0091539</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Valproic acid</td>
<td>CTC, Breast Cancer</td>
<td>NCT00607345</td>
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<td></td>
<td></td>
<td>Entinostat</td>
<td>Hodgkin’s lymphoma, kidney cancer, ABC</td>
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<tr>
<td></td>
<td></td>
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<td>Chronic myeloprolif neoplasms</td>
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<td>Hodgkin’s lymphoma</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Multiple myeloma</td>
<td>NCT01023308</td>
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<td>Combination therapy</td>
<td>Epi + Chemo</td>
<td>Vorinostat/5-FU/Leucovorin</td>
<td>CRC, CRC</td>
<td>NCT00942266</td>
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<td></td>
<td></td>
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<td>CRC</td>
<td>NCT01896856</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Valproic acid/Hyrdalazine/cisplatin</td>
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<tr>
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<td></td>
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<td>Ovarian Cancer</td>
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<td></td>
<td>AZA/Abrazane/Gemcitabine</td>
<td>Pancreatic Cancer</td>
<td>NCT01845805</td>
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<td>Epigenetic Priming</td>
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<td>Vorinostat + Radiotherapy</td>
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<td></td>
<td>Vorinostat/Gemcitabine/Paclitaxel/Sorafenib toyslate</td>
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<td>Epi + Immune</td>
<td>AZA/Romidepsin/PD-1 Ab</td>
<td>CRC</td>
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<td></td>
<td>Decitabine/Autologus dendritic cell vaccine and poly-ICLC</td>
<td>Pediatric high-grade glioma/MB/CNS pNT</td>
<td>NCT02332889</td>
</tr>
</tbody>
</table>


[20,38,45]. There have been promising initial results where the epigenetic agents serve as effectors for chemosensitization or induction of immune recognition [42,45,47,50]. With improved understanding of the targetable nature of various epigenetic aberrances, there has been an array of potential epigenetic agents evaluated in various preclinical and clinical stages. These compounds serve to reprogram cancer cells and have significant synergistic potential in combination with traditional agents. Additionally, epigenetic modifications also have the potential to provide biomarkers for prognostic and diagnostic detection. For instance, the methylation panel (CDKN2a, CDH13, APC, and RASSF1a) identified in patients with NSCLC predicts early recurrence but was also associated with response to DNMT inhibitor plus HDAC inhibitor therapy [20]. In conjunction, a recent study by Hulbert and colleagues revealed that promoter methylation of cancer-specific genes (SOX17, TAC1, HOXA7, CDO1, HOXA9, and ZFP42) identified in plasma and sputum improved diagnostic accuracy for early stage lung cancer [51]. Improved biomarkers of diagnosis and prognosis would, in turn, improve stratification of patients for a more personalized approach.

Further considerations in epigenetic therapy for solid tumors include circumventing issues with pharmacokinetics (i.e. short half-life, avoiding toxic doses), targeting of proteins involved in collaboration between epigenetic modifiers (e.g. MBD), and using small molecule inhibitors to target other components of the basic machinery such as erasers (e.g. lysine specific demethylase 1 (LSD1) or writers (e.g. PCG enzyme EZH2) [30,52]. In fact, small molecule inhibitors aimed towards inhibition of EZH2 have entered clinical trials for ovarian clear cell carcinomas (OCCC) and diffuse large B-cell lymphoma [53]. Remarkably, in ARID1A (component of chromatin remodeling complex) mutated OCCC cells, inhibition of EZH2 methyltransferase function results in synthetic lethality likely via induction of PI3K interacting protein 1 (PIK3IP1) and ensuing apoptosis [53]. ARID1A and EZH2 regulate PIK3IP1 expression in an antagonistic manner; therefore, loss of ARID1A necessitates inhibition of EZH2 to restore PIK3IP1 expression. Mutations in ARID1A occur in 50% of OCCC and these observations demonstrate a potential novel epigenetic therapeutic strategy. Findings such as these have significant implications for cancer therapy. Certain patient populations, with malignancy that is driven by aberrancy in epigenetic regulators, can be potentially treated with epigenetic therapy alone. These patients possess cancer that harbors a sort of ‘epigenetic addiction’ and with improved understanding of the oncogenic epigenetic pathways, improved therapeutic combinations will aim to achieve a more tailored approach with better prognostic outcomes.

Conflict of interest
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Dr. Sharma has nothing to disclose.
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Disclosures
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References


