



Towards a more precise therapy in cancer: Exploring epigenetic complexity

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Abstract

A plethora of preclinical evidences suggests that pharmacological targeting of epigenetic dysregulation is a potent strategy to combat human diseases. Nevertheless, the implementation of epidrugs in clinical practice is very scarce and mainly limited to haematological malignancies. In this review, we discuss cutting-edge strategies to foster the chemical design, the biological rationale and the clinical trial development of epidrugs. Specifically, we focus on the development of dual hybrids to exploit multitargeting of key epigenetic molecules deregulated in cancer; the study of epigenetic-synthetic lethality interactions as a mechanism to address loss-of-function mutations, and the combination of epidrugs with other therapies such as immunotherapy to avoid acquired chemoresistance and increase therapy sensitivity. By exploring these challenges, among others, the field of epigenetic chemical biology will increase its potential for clinical benefit, and more effective strategies targeting the aberrant epigenome in cancer are likely to be developed both in haematological and solid tumours.

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Keywords

Epidrugs, DNA methylation, Histone modifications, Dual inhibitors, Multitargeting, Synthetic lethality, Chemoresistance, Immunotherapy.

Abbreviations

BET, bromo- and extra-terminal domain proteins; DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDMT, histone demethylase; HMT, histone methyltransferase; MBD, methyl-binding domains proteins; ROS, reactive oxygen species.

Introduction

For the last two decades, epigenetic dysregulation has been recognized as a key factor contributing to human disorders. This is boosting an increasing number of studies into the field of epigenetic drug discovery [1,2]. Epidrugs, defined as small-molecule inhibitors that target either the epigenome or enzymes with epigenetic activity, have been developed for the three categories of epigenetic regulators (*writers*, *readers* and *erasers*). Although *writers* are responsible for adding chemical groups to histones or DNA (e.g., histone acetyltransferases (HATs), histone methyltransferases (HMTs) or DNA methyltransferases (DNMTs); *erasers* remove them (e.g., histone deacetylases (HDACs) or histone demethylases (HDMTs)). In addition, epigenetic modifications are recognized by a set of *reader* domains that are recruited to specific epigenetic marks and act as effector proteins (e.g., methyl-binding domains proteins or bromo- and extra-terminal (BETs) domain proteins). At present, the FDA-approved epidrugs include therapies with the following HDAC inhibitors: Vorinostat and Romidepsin for refractory cutaneous T cell lymphoma, belinostat for peripheral T cell lymphoma or panobinostat for multiple myeloma. Approvals also included the DNMT inhibitor Decitabine which is administrated in patients with haematological malignancies, such as myelodysplastic syndromes, acute myeloid leukaemia, and chronic myelomonocytic leukaemia. After the first- and second-generations of epidrugs, possibilities for epidrug

development are now being explored in erasers, and BET and methyl-binding domain proteins inhibitors are undergoing clinical evaluation for efficacy in different cancer settings [2].

Despite their promise, there are many challenges to be resolved for efficient use of epidrugs in the treatment of human cancer, including the lack of specificity of epidrugs, disappointing success in solid tumours and the acquisition of drug chemoresistance leading to higher risk of tumour relapse. Herein, we review the cutting-edge approaches in the field of chemical biology and molecular biology that are currently being taken to improve the translation of epidrugs into clinical practice. Although still in its infancy, the interesting concept of epidrugs multitargeting, the potential of the epigenetic-based synthetic lethality strategies and the use of epidrugs in combination with other therapies (such as immunotherapy) are introduced as alternatives

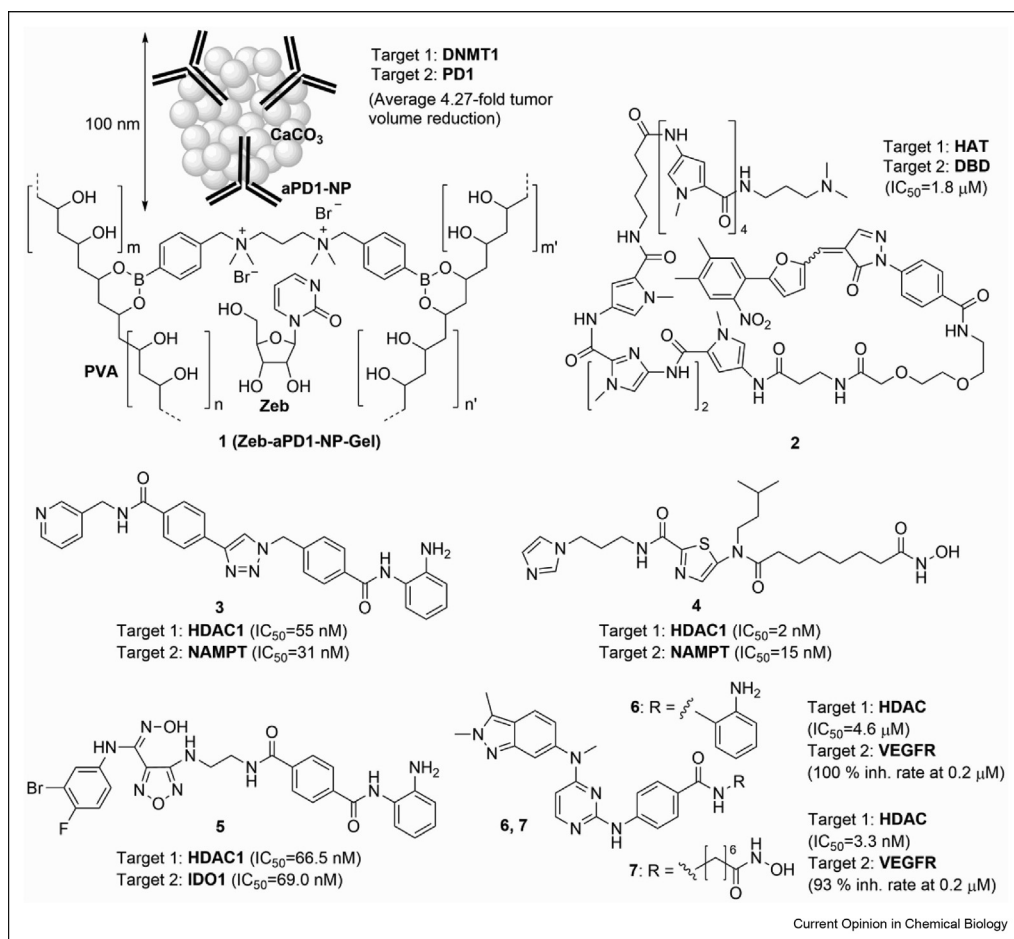
for optimizing the clinical translation of epigenetic therapy.

From the magic bullet paradigm to multitarget epigenetic inhibitors

Multitarget therapeutic strategies can involve separate molecules that give rise to well-known combined therapies. An alternative strategy consists of incorporating two biologically active units directed to their respective therapeutic targets. These moieties are connected by a spacer component bound to both units by covalent bonds. This strategy has yielded dual inhibitors, designed as ‘inhibitor(1)—spacer—inhibitor(2)’ compounds, which in turn act against epigenetic and nonepigenetic enzymes [3]. In the last three years, several additional examples have been reported (Figures 1 and 2).

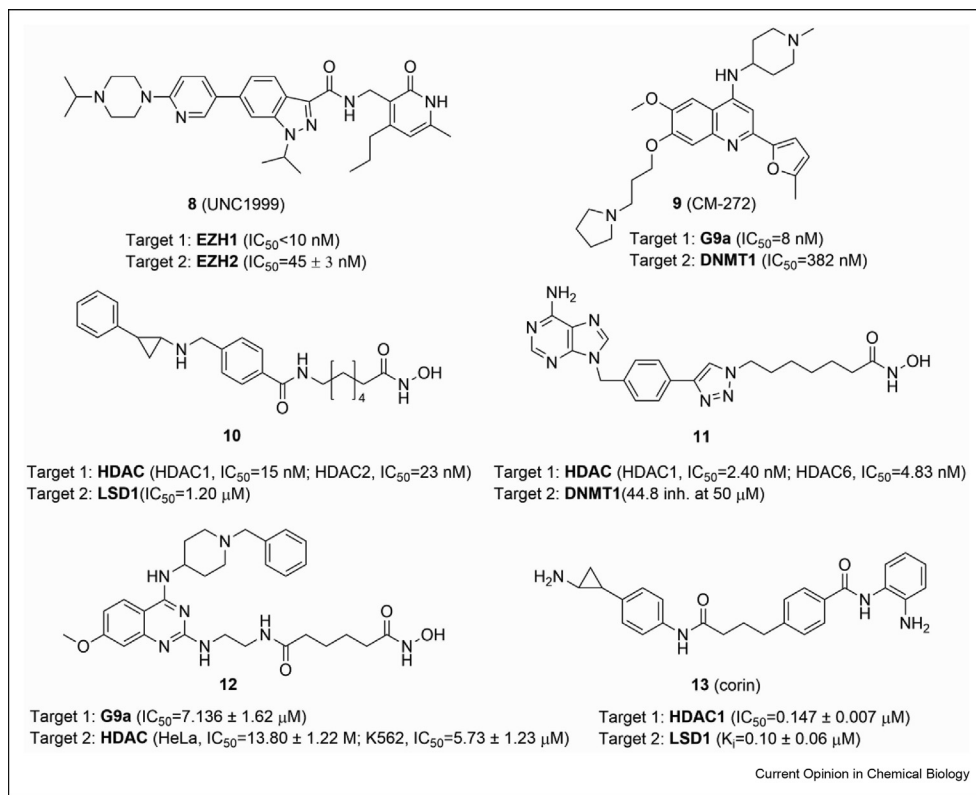
As far as the design, chemical synthesis and biological validation of novel epigenetic/nonepigenetic dual

Figure 1



Dual inhibitors involving epigenetic and nonepigenetic targets (2017–2019). DBD, DNA-binding domain; DNMT1, DNA methyltransferase 1; HAT, histone acetyltransferase; HDAC1, histone deacetylase 1; IDO1, indoleamine 2,3-dioxygenase 1; NAMPT, nicotinamide phosphoribosyltransferase; PD1, programmed cell death protein 1; VEGFR, vascular endothelial growth factor receptor.

Figure 2



Dual inhibitors involving solely epigenetic targets (2017–2019). DNMT1, DNA methyltransferase 1; EZH1, enhancer zeste homologue 1; EZH2, enhancer zeste homologue 2; HDAC1, histone deacetylase 1; LSD1, lysine specific demethylase 1; G9a (EHMT2), histone–lysine N-methyltransferase 2.

therapeutic agents are concerned (Figure 1), an interesting novel approach consists of incorporating into the same chemical entity several bioactive components not connected by covalent bonds. Thus, Ruan et al. [4] synthesized a reactive oxygen species (ROS)–responsive polymer by condensation of polyvinyl alcohol with a boronic diacid that creates a N1-(4-boronobenzyl)-N3-(4-boronophenyl)-N1,N1,N3,N3-tetramethylpropane-1,3-diaminium (TSPBA) cross-linker, which can be decomposed *in vivo* in the presence of ROS. This hydrogel was combined with pH-sensitive $CaCO_3$ nanoparticles (NPs) to generate a combined ROS/ H^+ biosensitive depot that can encapsulate bioactive species to be released into tumour microenvironments. These latter molecules were Zebularine [5], a known demethylating reagent, and an antibody against programmed death-1 (PD1) receptor [6], which is expressed in immune cells, including $CD8^+$ T cells. The combined Zebularine-polyvinyl alcohol-TSPBA-aPD1- $CaCO_3$ -NP chemical entity **1** demonstrated its efficacy in the inhibition of tumour growth and in prolonging the survival time of B16F10 melanoma bearing tissues [6]. This approach establishes a link between combined and dual therapies in cancer, as well as between epigenetic and immunotherapies (*later discussed in this review*).

Other recent developments in dual epigenetic/non-epigenetic inhibitors are gathered in Figure 1. Inhibitor **2** is quite different to other dual covalent bioactive molecules because it combines a DNA-binding domain consisting of a poly(1H-pyrrole) oligomer and a HAT inhibitor [7]. This dual molecule results in a sequence-specific inhibitor that shows promising inhibitory properties and antiproliferative effects in the upregulation of p53 genes thus initiating p53-dependent apoptosis. Many recently reported molecules include HDACs as epigenetic targets. Both hydroxamic acids and amides derived from ortho-phenylenediamines, well-known chelating groups for Zn(II)-dependent HDACs [2], have been reported. Dual inhibitor **3** includes two groups that simultaneously inhibit nicotinamide phosphoribosyltransferase and HDAC1, thus combining highly relevant metabolic and epigenetic targets [8]. The former inhibitory moiety consists of a substituted thiourea and the latter includes the orthophenylenediamine Zn(II)-chelating group. Both units are covalently connected by a very short para-phenylene unit. This molecule showed excellent activity and efficiently induced cell apoptosis and autophagy. In addition, it showed promising *in vivo* antitumour activity in the HCT116 xenograft model [8]. In the same vein,

compound **4** shows a design based on the hydroxamic group as the chelating group of HDAC1. This dual inhibitor appears to be more potent than **3**, and also showed promising results with the HCT116 xenograft model [9]. Compound **5** also contains the amide HDAC1-inhibitory group, whereas the second bioactive moiety consists of an heterocyclic oxime that binds indoleamine 2,3-dioxygenase 1 [10]. This latter haem-containing dioxygenase catalyses the transformation of L-Tip into N-formylkynurenines, which results in tryptophan depletion and subsequent inhibition of the proliferation of T lymphocytes [10]. Therefore, dual inhibitor **5** also connects epigenetic and immunotherapeutic targets. This compound inhibited both enzymes in the nanomolar range (Figure 1) and showed excellent *in vivo* antitumour activity in the murine LLC tumour model [10]. Finally, compounds **6** and **7** demonstrated their inhibitory potency of class I HDACs and vascular endothelial growth factor (VEGFR) [11]. Inhibition of the first epigenetic target was accomplished in **6** by a moiety analogous to the known HDAC inhibitor MS-275 [12], whereas the second target is incorporated in **6** and **7** by means of a combined 2-methyl-2H-imidazole/pyrimidine polyheterocycle similar to that which is present in approved VEGFR inhibitor Pazopanib [13]. Both compounds exhibited HDACi and VEGFRi activities comparable with those found for MS-275 and pazopanib, aside additional activities not present in the separate units. In addition, compound **5** showed good pharmacokinetic profiles and oral bioavailability (72%), as well as a promising antitumour efficacy in the HT-29 xenograft model.

Among dual epigenetic inhibitors, recent examples include a dual inhibitor of the HMTs enhancer zeste homologues 2 and 1 (EHZ2 and EHZ1) triggering trimethylation of the Lys-27 residue of histone 3 (H3K27). Overexpression of EZH2 and subsequent hypermethylation of H3K27 is present in the progression of PRC2-dependent tumours. Bioavailable compound **8** (also known as UNC1999) [14] inhibits both EZH2 and EZH1 (Figure 2). Most interestingly, it induced antimyeloma *in vitro* activity in combination with proteasome inhibitor Bortezomib [15]. A potentially interesting combination of epigenetic targets associates methylation processes in histones and DNA. In particular, the inhibition of HMT G9a (also known as EHMT2) methylates H3K9, which is overexpressed in many tumours, decreases cancer cell proliferation and hampers the development of metastasis [16,17]. 4-Aminoquinoline **9** (CM-272) is a promising dual G9a/DNMT1 inhibitor, with potencies in the nanomolar range for both targets [16,17]. In addition, significantly prolonged survival of acute myeloid leukaemia, Acute lymphoblastic leukemia (ALL) and Diffuse large B-cell lymphoma (DLBCL) xenogenic models was described.

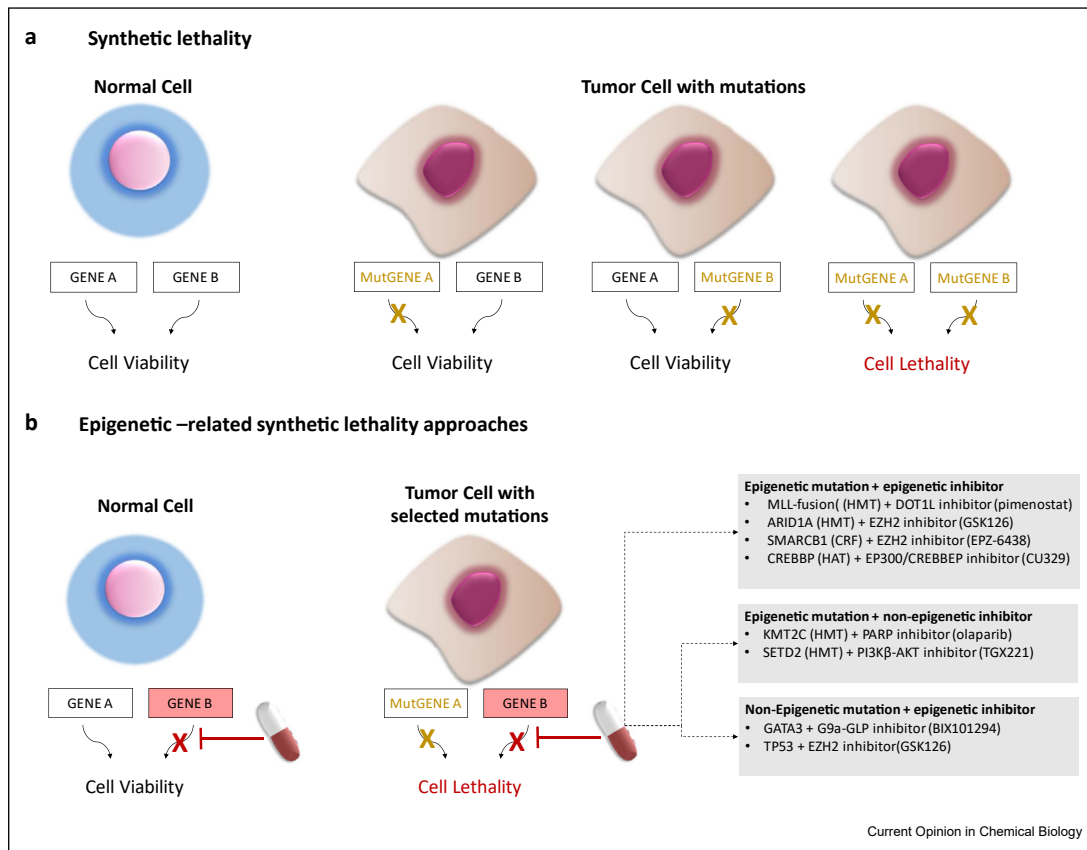
Other structural analogues [18] of **9** showed also promising dual G9a/DNMT1 inhibitory activity. Other dual inhibitors shown in Figure 2 involve HDACs as one of the classes of epigenetic targets. Compounds **10** [19], **11** [20] and **12** [21] incorporate hydroxamic acid units as HDAC-chelating groups, whereas **13** [22] possesses the alternative ortho-phenylenediamine unit as the preferred chelating group to HDAC1. Compound **10** also inhibits lysine specific demethylase 1 (LSD1), an enzyme that is present in the CoREST corepressor complex and demethylates mono- and di-methylated H3K4. The trans-aminocyclopropyl moiety of **10** is responsible for the inhibition of LSD1, with a potency within the low micromolecular range [19]. Compound **11** combines HDAC1,6 nM inhibition with micromolar binding to DNMT1 [20]. In contrast, compound **12** inhibits both HDACs and G9a, thus combining deacetylases and methyl transferases as dual targets [21]. Finally, compound **13** (Corin) inhibits HDAC1 and LSD1, thus targeting the CoREST complex by encompassing the chief features of Entinostat and Tranlycypromine [22], and showed very promising therapeutic potential in slowing tumour growth in a murine melanoma xenograft.

Targeting loss-of-function by synthetic lethality epigenetic approaches

Several examples of epigenetic therapy take advantage of the mutations found in all major classes of epigenetic proteins and have been explored as targets for therapy [23]. From a mechanistic consideration, mutations can be divided into two categories: loss- or gain-of function of the epigenetic enzyme. Although it is possible to inhibit gain-of-function of epigenetic enzymes (as example overexpression of the HMTs EZH2 or MLL), loss-of-function mutations are more difficult to target. An innovative approach based on the concept of synthetic lethality has been implemented in recent years opening up new strategies in drug development.

Synthetic lethality refers to a genetic interaction between two genes in which the loss of one of them has little effect on cellular viability, whereas loss of both genes leads to cellular lethality. This interaction provides a therapeutic opportunity by inhibiting the second partner (e.g., pharmacological inhibition) in those tumours with genetic mutations affecting the other partner. A normal cell without genetic mutations would tolerate the pharmacological inhibition, while it would be lethal for the tumour cell without the two functional genes (Figure 3a). After the first FDA-approved synthetic lethal drug therapy pairing BRCA1/2 mutations with PARP inhibitor olaparib treatment in ovarian cancer [24], studies of synthetic lethal pairs involving epigenetic-related synthetic lethal genes have been

Figure 3



Epigenetic synthetic lethality. **(a)** Synthetic lethality refers to a genetic interaction between two genes (e.g., gene A and gene B) in which the loss of gene A or gene B has little effect on cellular viability, whereas loss of both genes leads to cellular lethality. **(b)** Epigenetic-related synthetic approaches as therapeutic opportunity in tumours with specific mutations. The pharmacological inhibition of one partner of the pair (e.g., pharmacological inhibition of gene B in tumours harbouring gene A mutations) could result in cellular lethality. A normal cell without genetic mutations would tolerate the pharmacological inhibition of gene B. CRF, chromatin remodelling factor; HAT, histone acetyltransferase; HMT, histone methyltransferase.

conducted, including synthetic lethality between epigenetic mutations and epigenetic inhibitors, epigenetic mutations and non-epigenetic inhibitors and oncogene mutations and epigenetic inhibitors [25] (Figure 3b).

One pioneer example of epigenetic-related synthetic lethality entering in clinical trials was the use of inhibitors of the HMT DOT1L, such as pinometostat, in the treatment of MLL-fusion leukaemia [26]. The MLL-fusion results in DOT1L recruitment and epigenetic-mediated activation of well-known drivers of tumorigenesis (e.g., HOXA9, MEIS1) together with proteins involved in protection of MLL fusion proteins from autophagic degradation (e.g., LAMP5) [27]. The pinometostat inhibition effect on DOT1L selectively killed MLL-fusion leukaemia cells but not normal cells without the MLL genetic aberrations throughout downregulating LAMP5 and enhancing the selective autophagic degradation of MLL oncoproteins [27].

Additional examples of synthetic lethality between epigenetic alterations and epigenetic inhibitors exist, specifically involving mutations in chromatin remodelling proteins. Members of the SWI/SNF complex are frequently mutated in cancer, including mutations in the tumour suppressors ARID1A or SMARCB1 [23]. PRC2 is another crucial chromatin complex that includes the HMT EZH2, and its deregulation is associated with multiple cancers [28]. To note that there is an epigenetic antagonism between SWI/SNF and PRC2 complexes, which has been the focus of synthetic lethal strategies. In this way, pharmacological inhibition of EZH2 is a synthetic lethal strategy in tumours harbouring SWI/SNF mutations [28,29]. Treatment with GSK126, a specific inhibitor of EZH2, decrease tumorigenesis is preclinical models of ovarian tumours with ARID1A mutations [29]. Recently, an elegant work performed by Meyer et al. [30] described relevant applications of synthetic lethality approaches involving HAT activities in lymphomas. Inactivating mutations of the CREBBP and

EP300 HATs are mutually exclusive genetic alterations in diffuse large B cell lymphoma and follicular lymphoma. However, treatments with small molecule inhibitors that are selective for CREBBP and EP300 (i.e., the bromo-domain inhibitor CCS1477 and the preclinical HAT domain inhibitor CU329) abolished the EP300-dependency in CREBBP mutants and resulted in diminished tumour proliferation in murine models [30].

Nonepigenetic inhibitors have been also explored in synthetic lethality therapies involving tumours with epigenetic alterations. PARP inhibitors have synthetic lethal effects with epigenetic enzymes in cancer. The HMT KMT2C (also known as MLL3) gene has a high frequency in bladder cancer. The reduction of KMT2C activity results in decreased H3K27ac-dependent expression of genes from the DNA repair pathways (particularly in the homologous recombination pathway) associated with higher endogenous DNA damage and genomic instability in the tumour cells, and consequently increased sensitivity to the PARP inhibitor Olaparib in epithelial carcinomas [31]. Similarly, a molecular interaction between the HMT SETD2, which catalyses the methylation at H3K36 enriched at promoters with active transcription, and PI3K β kinase has been explored in preclinical models of renal cell carcinoma [32]. Treatment with inhibitors of PI3K β /AKT pathway causes synthetic lethality with SET2D loss-of-function and increased tumour inhibition in renal cancer cells [32].

Finally, a synthetic lethal interaction between an oncogene mutation (nonepigenetic) and the epigenetic drug has been described for the transcription factor GATA3 and the HMTs G9A and GLP (also known as EHMT2 and EHMT1, respectively) in breast cancer [33]. Interestingly, not only loss-of-function but also gain-of-function of oncogenes could be exploited in epigenetic-related synthetic lethality approaches. Treatment with EZH2 inhibitors results in decreased metastatic

potential in TP53-overexpressing prostate tumours and represents new therapeutic opportunities for treatment of advanced solid cancers [34].

Epigenetic therapy in combination with other drugs to boost immune response or drug sensitivity

In the recent years, results from preclinical and phase I/II clinical trials support that, beyond their potential as monotherapies, epigenetic drugs could have important roles in combination with other anticancer therapies. Epidrugs, especially HDACi and DNMTi, have been tested in combination with: chemotherapy (e.g., vorinostat plus capecitabine and Cisplatin in unresectable gastric cancer [35]), radiotherapy (e.g., vorinostat plus pelvic radiotherapy in gastric cancer [36]), hormonal therapy (e.g., panobinostat plus bicalutamide in castration-resistant prostate cancer [37]) or targeted therapies (e.g., Abexinostat plus the tyrosine kinase inhibitor pazopanib in advanced renal cell carcinoma [38]). A synergistic effect to favour sensitization of the cancer cell to the giving therapy and for overcoming acquired chemoresistance has been observed.

Among the combinatorial possibilities, enhancing the anticancer efficacy of immunotherapy through combination with epigenetic drugs is receiving the most attention [39]. Positive results for the immunogenicity of tumour cells are described when epidrugs are administered together with immune checkpoint blockade therapy (anti-PD1/PDL1 therapy in combination with HDACi in non-small-cell lung cancer [40]) and adoptive cellular immunotherapy (e.g., HDACi plus CD19-CAR CTL therapy in non-Hodgkin's lymphoma [41]). A summary of the most recent examples of clinical trials is provided in Table 1. Although the mechanisms of action by which the epidrug therapies modulate the immune response still need further investigation, the reactivation of tumour-surface antigens, endogenous retroviruses and proteins for the major complex of histocompatibility

Table 1

Examples of current clinical trials involving epidrugs in combination with immunotherapy agents. BETi, bromodomain inhibitor; CRC, colorectal cancer; DNMTi, DNA methyltransferase inhibitor; HDACi, histone deacetylase inhibitor; HDMTi, histone demethylase inhibitor; HNSCC, head and neck squamous cell carcinoma; HMTi, histone methyltransferase inhibitor; SGC, salivary gland cancer; NSCLC, non-small-cell lung cancer; RCC, renal cell carcinoma; UB, urinary bladder cancer.

Clinical trial identifier	Clinical trial phase	Epigenetic drug	Immunotherapy agent	Cancer type	Status
NCT01928576	II	Azacytidine (DNMTi), entinostat (HDACi)	Nivolumab (anti-PD1)	NSCLC	Recruiting
NCT02638090	I/II	Vorinostat (HDACi)	Pembrolizumab (anti-PD1)	NSCLC	Recruiting
NCT02635061	I	ACY 241 (HDACi)	Nivolumab (anti-PD1)	NSCLC	Recruiting
NCT03179930	II	Entinostat (HDACi)	Pembrolizumab (anti-PD1)	Lymphoma	Recruiting
NCT02619253	I	Vorinostat (HDACi)	Pembrolizumab (anti-PD1)	RCC, UB	Recruiting
NCT02453620	I	Entinostat (HDACi)	Ipilimumab (anti-CTLA-4), nivolumab (anti-PD1)	Breast cancer	Recruiting

Table 1 (continued)

Clinical trial identifier	Clinical trial phase	Epigenetic drug	Immunotherapy agent	Cancer type	Status
NCT03552380	II	Entinostat (HDACi)	Ipilimumab (anti-CTLA-4), nivolumab (anti-PD1)	RCC	Recruiting
NCT03250273	II	Entinostat (HDACi)	Nivolumab (anti-PD1)	Cholangiocarcinoma, pancreatic cancer	Recruiting
NCT03278782	II	Romidepsin (HDACi)	Pembrolizumab (anti-PD1)	Lymphoma	Recruiting
NCT03150329	I	Vorinostat (HDACi)	Pembrolizumab (anti-PD1)	Lymphoma	Recruiting
NCT03220477	I	Guadecitabine (DNMTi) plus mocetinostat (HDACi)	Pembrolizumab (anti-PD1)	NSCLC	Recruiting
NCT03903458	I	Tinostamustine (HDACi)	Nivolumab (anti-PD1)	Cutaneous melanoma	Recruiting
NCT02546986	II	CC-486 (DNMTi) or placebo	Pembrolizumab (anti-PD1)	NSCLC	Active, not recruiting
NCT02437136	I/II	Entinostat (HDACi)	Pembrolizumab (anti-PD1)	NSCLC, cutaneous melanoma and CRC	Active, not recruiting
NCT02538510	I/II	Vorinostat (HDACi)	Pembrolizumab (anti-PD1)	HNSCC, SGC	Active, not recruiting
NCT02032810	I	Panobinostat (HDACi)	Ipilimumab (anti-CTLA-4)	Cutaneous melanoma	Active, not recruiting
NCT02909452	I	Entinostat (HDACi)	Pembrolizumab (anti-PD1)	Advanced solid tumours	Active, not recruiting
NCT02395627	II	Vorinostat (HDACi) plus tamoxifen (anti-oestrogen)	Pembrolizumab (anti-PD1)	Breast cancer	Active, not recruiting
NCT02512172	I	Romidepsin (HDACi), azacytidine (DNMTi)	Pembrolizumab (anti-PD1)	CRC	Active, not recruiting
NCT02697630	II	Entinostat (HDACi)	Pembrolizumab (anti-PD1)	Ocular melanoma	Active, not recruiting
NCT02915523	I/II	Entinostat (HDACi)	Avelumab (anti-PDL1)	Epithelial ovarian cancer, peritoneal cancer	Active, not recruiting
NCT02708680	II	Entinostat (HDACi)	Atezolizumab (anti-PDL1)	Breast cancer	Active, not recruiting
NCT02220842	I	Tazemetostat	Atezolizumab (anti-PDL1), obinutuzumab	Lymphoma	Active, not recruiting
NCT03525795	I/II	CPI-1205 (HMTi)	Ipilimumab (anti-CTLA-4)	Advanced solid tumours	Active, not recruiting
NCT02250326	II	CC-486 (DNMTi) or paclitaxel (targeted therapy)	Durvalumab (anti-PDL1)	NSCLC	Active, not recruiting
NCT02959437	I/II	Azacytidine (DNMTi) or INCB057643 (BETi) or INCB059872 (HDMTi)	Pembrolizumab (anti-PD1) plus Epacadostat	Advanced solid tumours	Active, not recruiting

HDACi, histone deacetylase inhibitor; DNMTi, DNA methyltransferase inhibitor; NSCLC, non-small-cell lung cancer; CRC, colorectal cancer; PD1, programmed cell death protein 1.

could be mediators of the increased tumour visibility to the host immune system [42]. In spite of its preliminary success, some frequent limitations of epidrugs also need to be solved in combinational therapy, including the reduction of the toxicity and the secondary effects. Improvements in the immunotherapy schemes will also benefit from systems biology approaches. In this regard, a recent mathematical model to predict synergies between BET inhibitors and anti-CTLA4 immunotherapy has been proposed for the optimization of combinatory therapy in breast cancer [43].

Conclusions

A simplified vision of epigenetic regulation and the use of inappropriate cohorts in clinical trials are undoubtedly limiting factors to the success of epidrugs as therapeutic agents. At present, we are envisioning an approach which moves “*from the simplest to the most complex*”. We are accepting that epigenetic modifications are not stand alone processes because several epigenetic proteins (and also not epigenetic) contribute to regulate chromatin accessibility, as do several nonepigenetic proteins.

Inhibition of a single epigenetic alteration can have global and local effects on chromatin conformation affecting multiple biological processes (e.g., not only gene regulation but also DNA repair or DNA recombination could be affected after treatments) and multiple biological pathways (e.g., the HAT CBP/EP300 acetylates lysine residues of histones H3 and H4, as well as the oncogene p53 [44]). Furthermore, the effect of targeting a specific mark could result in further changes in different modifications (e.g., treatments with HDAC inhibitors could increase acetylation of histones but also affect histone methylation [45]). Therefore, research in the field of systems biology applied to epigenetic complexes would allow us to exploit the benefits of targeting complexity, such as the aforementioned strategies for development of dual inhibitors.

Undoubtedly, it is not only a question of chemical drug design. Basic research to unravel the mechanisms of action of tumour cell progression and response to therapy (e.g., molecular pathways associated with tumoral evasion from the host immune system), the broader

characterisation of the genetic and epigenetic mutations acting as drivers in a tumour cell and a better stratification of the patients entering clinical trials (i.e., development of predictive biomarkers of response) would contribute to the development of more adequate clinical trials involving epigenetic drugs. In summary, the studies reviewed here suggest promising opportunities for targeting epigenetic alterations to make further advances in precision oncology.

Author contributions

All authors contributed to all aspects of the manuscript.

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Conflict of interest statement

M.B. discloses no conflicts of interest. F.P.C. and M.E. are consultants of Quimatrix.

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- * of special interest
- ** of outstanding interest

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