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Review Article

Epigenetic Drug Interventions in Breast Cancer: A Narrative Review of Current Research and Future Directions

Sarah Bibi Mungly[1], Evelyn Priya Peter[2], Ling-Wei Hii[3, 4], Chun-Wai Mai[5], Felicia Fei-Lei Chung[2, 3*]

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[1]Department of Biological Sciences, School of Medical and Life Sciences, Sunway University, Bandar Sunway, 47500, Subang Jaya, Selangor, Malaysia; 18026864@imail.sunway.edu.my (SBM)

[2]Department of Medical Sciences, School of Medical and Life Sciences, Sunway University, Bandar Sunway, 47500, Subang Jaya, Selangor, Malaysia; 22111587@imail.sunway.edu.my (EPP)

[3]Center for Cancer and Stem Cell Research, Institute for Research, Development and Innovation (IRDI), IMU University, Kuala Lumpur, Malaysia; lingweihii@gmail.com (LWH)

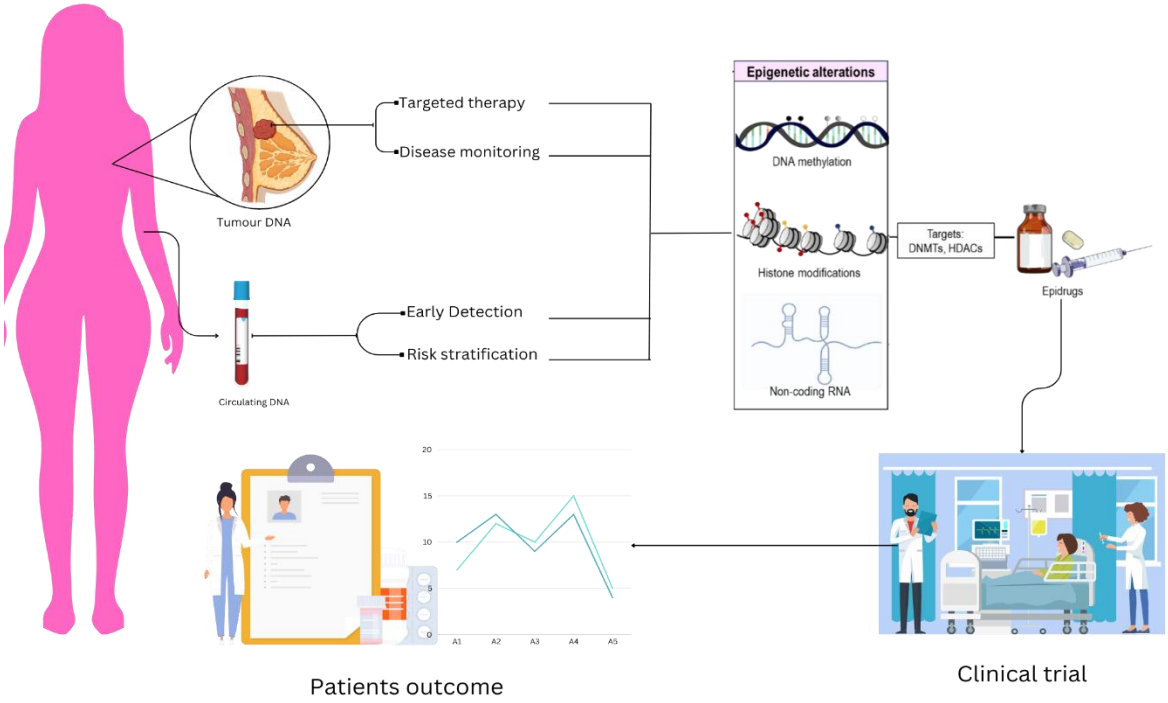
[4]Home Pharmacy Sdn Bhd (Alpro OPPS), 43300 Seri Kembangan, Selangor

[5]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, Cheras, 56000, Kuala Lumpur, Malaysia; MaiCW@ucsiuniversity.edu.my (CWM)

*Corresponding author: Felicia Fei-Lei Chung; Department of Medical Sciences, School of Medical and Life Sciences, Sunway University, Bandar Sunway, 47500, Subang Jaya, Selangor, Malaysia; feliciacfl@sunway.edu.my (FF-LC)

Abstract: Breast cancer is a life-threatening disease known for its extensive molecular heterogeneity. The study of the breast cancer epigenome has revealed potential avenues for improving breast cancer treatment risk stratification, early detection, and treatment. With renewed interest in epigenetic-modifying pharmaceutical agents, namely DNA methyltransferase inhibitors (DNMTi), histone deacetylase inhibitors (HDACi), bromodomain and extra-terminal inhibitors (BETi), and enhancer of zeste homolog 2 inhibitors (EZH2i), there have been extensive preclinical and clinical studies to evaluate the safety and efficacy of these agents as potential treatments for breast cancer. In this review, we summarise and present the preclinical and clinical evidence for epigenetic drugs in treating breast cancer. We review the challenges associated with the translation of these findings into improved patient outcomes, namely the optimisation of dosage and treatment regimens, and the emergence of resistance. These challenges have been widely recognised in the field and are of utmost importance for the successful implementation of personalised medicine. While there is strong evidence that epigenetic alterations, consisting of changes in DNA methylation, histone modifications, and non-coding RNAs, play a crucial role in breast cancer initiation and development, additional research is warranted to elucidate the safety

profile of long-term interventions involving epigenetic drugs and to validate the role of epigenetic markers in disease diagnosis, prognosis, and personalised treatment.



Graphical abstract caption: Multiple studies have demonstrated that the tumour epigenome bears extensive differences compared to normal or non-invasive adjacent tissue and that epigenetic alterations in circulating DNA may be used to improve risk stratification, early detection, and disease monitoring strategies. However, clinical studies investigating the use of epidrug as a single agent or as components of combination therapy focus on DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) and have demonstrated limited utility in improving patient outcomes. Further investigations into the epigenetic alterations involved in breast cancer are warranted to validate the role of epigenetic markers in disease diagnosis, prognosis, and personalised treatment. This figure was constructed using images from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/) and assets from Freepik.com and Biorender.com.

Keywords: Epigenetics; breast cancer; epidrugs; clinical trials; DNMTi; HDACi; DNA methylation

1. Introduction

1.1. Molecular Profiles of Breast Cancer

Breast cancer stands as the most prevalent malignancy affecting women and ranks as among the leading causes of cancer-related mortality worldwide. Although incidence rates of breast cancer vary by country, the global estimate for newly diagnosed cases exceeds 2 million each year[1]. Despite advances in early detection and treatment, breast cancer continues to be a major cause of cancer-related deaths among women[2]. Breast cancer prognosis is determined by a variety of factors, including the stage of illness upon diagnosis, tumour subtype as defined by the varying presence of hormone receptors, HER2 status, and histological features[3]. At present, the molecular classification of breast cancers identifies patients who would likely benefit from targeted therapy such as anti-oestrogen hormone therapy and anti-HER2 therapy[4,5]. While this has led to more effective and individualised treatments for a subset of breast cancer patients, there is currently no targeted therapy for the triple-negative breast cancer (TNBC) molecular subtype, for which systemic chemotherapy remains the primary treatment modality as it is insensitive to endocrine therapy and anti-HER2 targeted therapy[6]. Thus, there remains an urgent need for novel approaches for targeting treatment-refractory breast cancers.

Recent advances in molecular profiling technology have led to an improved understanding of the molecular landscape of breast cancers, further shedding light on the molecular heterogeneity of this disease in terms of their genomes, epigenomes, and metabolomes[7,8]. As it becomes increasingly clear that the development and progression of breast cancer are driven by both epigenetic and genetic factors, this review aims to provide a comprehensive overview of available information on the epigenetic abnormalities linked to breast cancer and provide insights into their therapeutic implications. We will review evidence of potential druggable epigenetic targets in breast cancer that may be applied to improve the current treatment strategies and address the clinical significance of epigenetic changes in breast cancer. This review includes evidence from preclinical and clinical studies exploring the efficacy and safety of epigenetic-based medicines, summarising the current understanding of epigenetic mechanisms involved in breast cancer and their therapeutic potential.

1.2. Epigenetic Alterations in Breast Cancer

Epigenetic alterations have long been linked to cancer development and progression[9–12]. Tumour profiling studies have described extensive differences in the epigenome between normal and tumour samples, while tumour samples exhibit marked heterogeneity[13,14]. Additionally, epigenetic biomarkers have been described for their potential utility in breast cancer risk stratification[15], diagnosis[16], prognosis[17] and therapy response[18]. As epigenetic changes in cancers may influence the expression of genes involved in cell motility, invasion, and angiogenesis[19], it stands to reason that agents which can reverse these epigenetic changes will also restore normal gene expression patterns,

suppress oncogenic signalling pathways, and make cancer cells more susceptible to conventional therapy[20].

Changes in the epigenome can be classified into three different categories, namely alterations in the DNA, histone, or non-coding RNA[21,22]. Each mode of epigenetic regulation has an important effect on gene regulation and is tightly regulated by epigenetic-modifying enzymes (Table 1). Various factors can alter epigenetic patterns, including age[23–27], tobacco smoking[25], exposure to environmental pollutants[21], probiotics[28,29], and endocrine-disrupting chemicals[30]. In this review, we explore the current state of the science in targeting epigenetic alterations in breast cancer for improved breast cancer therapy.

Table 1. Summary of the three main modes of epigenetic regulation and the related epigenetic-modifying enzymes. Epigenetic regulators are denoted using their UniProt protein names, while alternate gene names are indicated in parentheses if applicable.

Mode of epigenetic regulation	Epigenetic regulators	Effect on gene expression	References
DNA methylation	DNA methyltransferases: DNMT1, DNMT3A, DNMT3B Methylcytosine dioxygenases (demethylases): TET1, TET2, TET3. Methylated DNA binding proteins: MECP2, MBD1, MBD2, MBD4, KAISO (*ZBTB33*), ZBTB4, ZBT38 (*ZBTB38*), UHRF1, UHRF2	Silences gene expression by adding a methyl group to the DNA, primarily to cytosine bases located on CpGs	[31 32]
Histone acetylation	Class I HDACs: HDAC1, HDAC2, HDAC3, and HDAC8 Class II HDACs: HDAC4, HDAC 5, HDAC6, HDAC7, HDAC 9, and HDAC 10 Class III HDACs: SIRT1-7 Class IV HDACs: HDAC11 GNAT family acetyltransferases (HATs): KAT2A, KAT2B MYST family HATs: KAT5, KAT6A, KAT6B, KAT7, KAT8 p300/CBP family HATs: EP300, CBP	Enhances gene expression by adding an acetyl group to histones	[31,33–35]

	Others: NCOA1, NCOA2, NCOA3 Type B HATs: HAT1		
Histone methylation	Histone lysine methyltransferases (KMTs): EZH1, EZH2, SUV39H1, SUV39H2, EHMT2, SETDB1, Histone lysine demethylases (KDMs): KDM1A Type I arginine methyltransferases (PRMTs): PRMT-1, 2, 3, 4, 6 and 8 Type II PRMTs: PRMT-5 and 9 Type III PRMT: PRMT7 Arginine demethylases: JMJD6, PADI4	Can either activate or repress gene expression and is dependent on context	
Non-coding RNA	Various RNA molecules such as microRNAs and long non-coding RNA (lncRNA)	Regulate gene expression at transcriptional and post-transcriptional levels	[36–39]

2. Strategies for Targeting the Epigenome

2.1. Targeting Epigenetic Alterations in Breast Cancer for Improved Breast Cancer Therapy

Since the discovery that epigenetic alterations may be reversible, the epigenome has emerged as a promising therapeutic target for cancer treatment[40]. The goal of epigenetic therapies is to target cancer-specific changes in the epigenome and to reverse these changes or to restore gene expression patterns in cancer cells to patterns reminiscent of normal tissue, thus inhibiting cancer cell growth or survival[41]. Targeting epigenetic alterations in breast cancer may be achieved through two different techniques: 1) using small molecule inhibitors to target the enzymes involved in epigenetic modification in breast cancer cells; and 2) using immune-based therapies to facilitate the reprogramming of the epigenetic patterns in breast cancer cells.

2.1.1. Findings from clinical and preclinical studies evaluating the activity of epidrugs as single agents

Epigenetic drugs (epidrugs) are chemical compounds that can alter the chromatin structure by modulating the activity of enzymes related to epigenetic maintenance[42].

Currently, there are a total of 11 epidrugs approved for use in clinical settings. These epidrugs can be classified into the following categories based on their interaction in the epigenome: DNA methyltransferase inhibitors (DNMTis), histone deacetylase inhibitors (HDACis)[22], Bromodomain and extra-terminal inhibitors (BETis)[43] and enhancers of zeste homolog 2 inhibitors (EZH2is)[44].

Mechanistic *in vitro* studies aimed at elucidating the cellular mechanisms underlying epidrug-induced cell cytotoxicity have revealed that epidrugs can induce diverse cellular effects, as would be expected due to the broad influence of epigenetic regulation in gene expression (Figure 1). A preclinical study by Laranjeira *et al*.[45] reported that the DNMTis azacytidine, decitabine, and 5-aza-4'-thio-2'-deoxycytidine (aza-T-dCyd) exhibit inhibitory effects on DNMT1, leading to the induction of DNA damage, apoptosis, and subsequent cell death in MCF7 human breast cancer cells. Aza-T-dCyd demonstrated superior performance in terms of inhibiting DNMT1 and exerted cytotoxic effects while inhibiting growth compared to decitabine and azacytidine. The suppression of DNMT1 led to diverse cellular effects, such as DNA demethylation, increased expression of p21, and initiation of the Chk1-Ser345 pathway and subsequent cell cycle arrest. 5'aza-C treatment was also shown to induce strong upregulation of immune gene sets involved in interferon signalling, antigen processing and presentation, and cytokines/chemokines[46]. In *in vitro* models of TNBCs, inhibition of DNMT1, whether through RNAi-induced silencing, inhibition of GSK3β-mediated phosphorylation[47], or treatment with DNMT1 inhibitor 5'aza 2'-deoxycytidine[48], or HDACis trichostatin A[48] and vorinostat[49] resulted in the re-expression of functional estrogen receptor alpha expression and cellular reprogramming to a less aggressive phenotype (Figure 1).

Similarly, treatment with the HDACi entinostat resulted in increased activation of the E-cadherin promoter in *in vitro* models of TNBCs, leading to a reversal of epithelial to mesenchymal transition, a key step in the metastatic process[50]. The pan-HDAC inhibitor, Panobinostat, was found to suppress proliferation, migration, and invasion while inducing apoptosis in cell line models of breast cancer by upregulating the expression of APC-2/APCL, which is a key regulator of the Wnt/β-catenin pathway[51]. Particularly useful in the context of breast cancer, treatment with entinostat led to the downregulation of HER2 in models of aromatase inhibitor-resistant breast cancers, which resulted in cellular reprogramming and the reduction in tumour growth rate, tumour-initiating cell characteristics and mammosphere formation[52].

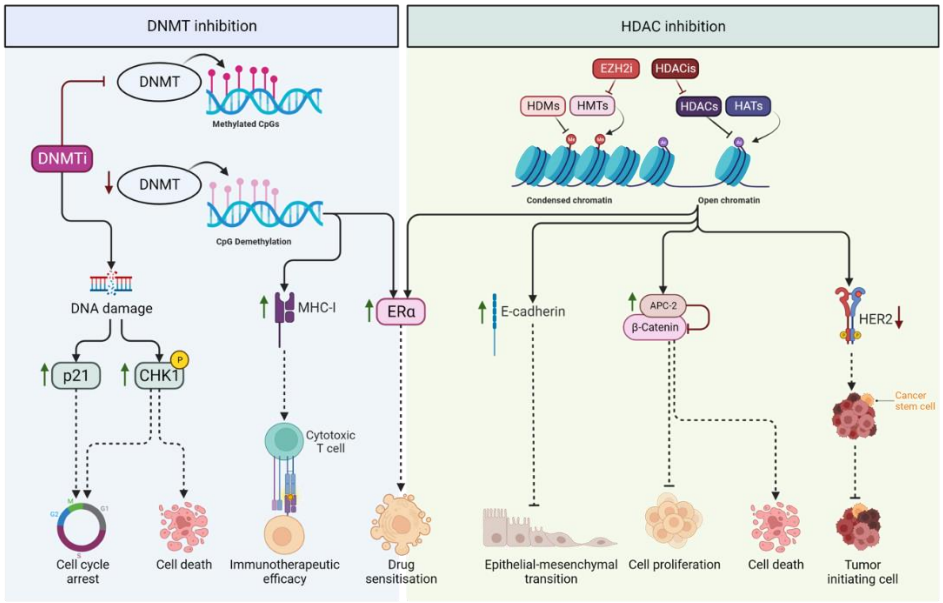


Figure 1. Cellular mechanisms influenced by treatment with DNMTis and HDACis, and their effects on cellular behaviour. DNMT inhibition resulted in DNA damage of BC cells and upregulated p21 and CHK1 resulting in cell cycle arrest and cell death[45]. The inhibition of DNMT also leads to upregulation of ERα, resulting in improved chemosensitivity to aromatase inhibitors and anti-oestrogens[52]. Increased expression of MHC-I has also been shown to occur upon DNMTi treatment, improving cellular response to immunotherapy[53]. On the other hand, inhibition of HDACs has been shown to result in the upregulation of E-Cadherin[50], ERα, β-catenin and APC2[51], resulting in the inhibition of EMT, reduced cell proliferation, and increased cell death. The use of HDACi also reduces the expression of HER2, reducing the observed population of tumour-initiating cells[52]. Arrows denote activating interactions, while T-ended lines denote inhibition. Solid lines denote direct interactions, while dotted lines denote indirect interactions with intermediate steps. Created with BioRender.com.

Despite extensive evidence for the potential utility of epidrugs as single agents in breast cancer treatment, the translation of these findings in clinical trials for breast cancer treatment has been limited (Table 2A and 2B). Currently, two DNMTis, azacitidine and decitabine, have been approved for the treatment of specific forms of myelodysplastic syndromes (MDS), including chronic myelomonocytic leukaemia (CMML) and acute myeloid leukaemia (AML)[54]. The inhibitors of class I and class II HDACs, belinostat and vorinostat, were respectively approved for treating refractory peripheral T-cell lymphoma[55], and for patients with cutaneous T-cell Lymphoma, multiple myeloma or melanoma[56]. Panobinostat, a pan-HDAC inhibitor, which was previously approved by the FDA for patients with relapsed or refractory multiple myeloma[57], was removed from the market due to a lack of adequate and well-controlled clinical studies to verify the product's clinical benefit. At the time of writing, none of the epidrugs has been approved for breast cancer treatment. Other classes of epidrugs include BETis, such as JQ1 and OTX015, which are currently under investigation as potential treatments for various types of cancer, including breast cancer[58], and EZH2is which block the activity of EZH2, a histone methyltransferase[59].

Table 2A. A summary of preclinical and recently active or completed (2013 to present) clinical studies evaluating the utility of HDACi as treatment modalities for breast cancer.

Example of epidrugs	**Type of studies**				
	Preclinical studies		***Clinical studies***		
HDACis	**Reference**	**Key Findings**	**Study Code**	**Phase**	**Study design and findings**
Vorinostat (pan-HDACi)	[60–64]	Vorinostat induces cell cycle arrest, apoptosis and inhibits cell proliferation seen in both *in vitro* and *in vivo* studies	NCT00365599	2	21% of the patients showed stable disease for about 24 weeks while 19% had confirmed objective response.
			NCT00262834	2	No results were posted on the response or survival
		Vorinostat sensitises TNBC cell lines to cisplatin treatment by downregulation of NOTCH1	NCT00258349	1 & 2	Median times to progression was 1.5 months while patients were assessed by overall survival at three-month intervals within the first two years and at six-month intervals thereafter up to the end of the third year.
			NCT00368875	2	Vorinostat administered at the recommended phase II dose demonstrated promising efficacy with manageable toxicities in patients with metastatic breast cancer.
			NCT00262834	2	No results were posted on the response or survival
			NCT01153672	2	No results were posted on the response or survival
			NCT00574587	1 & 2	No results were posted on the response or survival
			NCT00616967	2	Vorinostat treatment resulted in a significant decrease in tumour content and methylation from baseline to day 15, but there was no significant difference in tissue CMI between treatment arms. D15 tumours from individuals who achieved pCR after receiving vorinostat had significantly lower CMI than those who failed to achieve pCR.
			NCT00838929	1	No results were posted on the response or survival

Panobinostat (LBH589) (pan-HDACi)	[51,65–69]	Panobinostat inhibits EMT by upregulation of CDH1 leading to the inhibition metastasis. Panobinostat downregulates the Wnt/B-catenin pathway by upregulating the regulator APCL even in TNBC cell lines	NCT00632489	1	No results were posted on the response or survival
			NCT01105312	1 & 2	The study found 43 treatment cycles with dose-limiting toxicities, particularly at the 30 mg dose level. Two patients showed partial responses and one had stable disease, but five experienced disease progression. The patient with a partial response remained on study for six cycles, with a time to progression of 5.1 months. All patients with evaluable disease were ER-positive and resistant to endocrine and chemotherapy, experiencing stable disease for 5.6 months.
			NCT00788931	1	No results were posted on the response or survival
			NCT00777049	2	No results were posted on the response or survival
			NCT02890069	1	No results were posted on the response or survival
			NCT03878524	1	No results were posted on the response or survival
Givinostat (pan-HDACi)	[70]	Givinostat was shown potential as a drug for reversing epithelial-mesenchymal transition (EMT) in mesenchymal mammary tumour organoids. This study identified givinostat through a morphological screening method as one of the drugs that can reverse EMT in claudin-low mammary tumours, a mesenchymal subtype of triple-negative breast cancer. The findings indicate that givinostat may have the ability to reprogram EMT and offer new therapeutic approaches for breast cancer treatment. Givinostat potentially inhibit EMT in claudin low aggressive breast tumour cell line	No records of clinical studies involving this compound related to breast cancer		

PCI-24781 (pan-HDACi)	[71]	PCI-24781 inhibits cell proliferation and metastasis by upregulating RGDS2 expression which is involved in Ca2+ influx	NCT04498520	1	No results were posted on the response or survival
JNJ-26481585 (pan-HDACi)	[72]	Combination of bromodomain extra terminal JQ1 and JNJ26481585 promotes apoptosis in rhabdomyosarcoma	No records of clinical studies involving this compound related to breast cancer		
SB-639 (pan-HDACi)	No studies related to breast cancer.		No records of clinical studies involving this compound related to breast cancer		
Romidepsin (HDACi class I)	[73–75]	Romidepsin has been shown to reduce tumour growth and perform cell cycle arrest and has been approved for cutaneous T-cell lymphoma. In comparison to Romidepsin, Thailandepsin A loaded into cross-linked micelles was more effective for inhibiting tumour growth in an animal model	NCT02393794	1 & 2	No results were posted on the response or survival
			NCT00098397	2	No results were posted on the response or survival
			NCT01638533	1	No results were posted on the response or survival
Valproic acid (pan-HDACi)	[76–80]	VPA downregulated the mitochondrial elongation factor 1 (MIEF1) by activation of the hippo pathway which leads to inhibition of cell proliferation in MCF7 and MDA-MB-231 andwas found to induce apoptosis and inhibit tumour sphere formation in a dose-dependent manner. VPA also induced H3 histone acetylation in a dose and time-dependent fashion	NCT01552434	1	No results were posted on the response or survival

		VPA in combination with capecitabine acts synergistically in provision of thymidine phosphorylase upregulation to inhibit proliferation and promote apoptosis of breast cancer cell line. Combinatorial treatment of VPA increased cytotoxicity of methotrexate in MCF7 but such effect was absent in MDA-MB-231Predictive model based on Ki-67 staining and MRI scanning can predict the response of BC patients towards adjuvant and neoadjuvant VPA treatment			
Phenylbutyrate (HDACi class I)	[81]	MCF-7 cell lines study reported sodium phenylbutrate at 3 µM in combination with cyclophosphamide is the best combinatorial treatment to induce apoptosis possibly through hypomethylation and sodium phenylbutyrate at 3 µM could solely reduce cell viability of the MCF-7 cell line phenylbutrate	No records of clinical studies involving this compound related to breast cancer		
Pivanex (pan-HDACi)	No studies related to breast cancer.		No records of clinical studies involving this compound related to breast cancer		
Entinostat (HDACi class I)	[50,52,82–86]	Entinostat reverses the gene repression of CDH1 to increase E-cadherin transcription and reduces cell migration in	NCT02833155	1	No results were posted on the response or survival
			NCT03473639	1	No results were posted on the response or survival

	TNBC cell lines. Combination entinostat reduces mammary sphere formation and tumour initiating cell markers in MCF-7 cells. Entinostat in synergy with lapatinib inhibits proliferation and induce apoptosis in vitro by inducing the expression of Bim-1 which supports apoptosis. Xenograft model also reported reduction in tumour volume on this combinatorial administration. In terms of immune regulation, entinostat reduces the immune suppressive capacity of granulocytic-MDSCs and increases the anti-tumour M1 macrophages in a tumour microenvironment population analysis	NCT02820961	1	No results were posted on the response or survival
		NCT02897778	1	No results were posted on the response or survival
		NCT02623751	1	No results were posted on the response or survival
		NCT02708680	2	The combination of entinostat and atezolizumab showed modest efficacy, with a median PFS of 1.68 months and an ORR of 12.5%. The clinical benefit rate (CBR) was 15.0%, suggesting some patients experienced stable disease for a significant duration. The median overall survival was 12.25 months, indicating a moderate extension of survival
		NCT03291886	2	No results were posted on the response or survival
		NCT00676663	2	The combination of Exemestane and entinostat demonstrated improved Progression-Free Survival, Objective Response Rate, Clinical Benefit Rate, and Overall Survival compared to Exemestane alone, indicating potential efficacy in the treatment of cancer
		NCT01594398	1	No results were posted on the response or survival
		NCT02909452	1	No results were posted on the response or survival
		NCT01349959	2	Treatment of patients of Triple Negative Breast Cancer (TNBC) and Hormone-Related Breast Cancer (HRBC with entinostat and azacitidine showed that the confirmed response rate in HRBC is relatively low, and there was no observed response in TNBC, suggesting limited efficacy in terms of tumour response. Furthermore, the clinical benefit rate, which includes stable disease for at least 6 months, is higher in HRBC compared to TNBC. However, the overall clinical benefit rate remains modest. The median overall survival is higher in HRBC compared to TNBC, indicating a potential survival benefit in HRBC. Both TNBC and HRBC

			show a short median progression-free survival, suggesting limited efficacy in preventing disease progression. Limited information is available on the change in gene expression, particularly in TNBC. Data on circulating DNA were not reported, so its impact cannot be assessed. The confirmed response rate with the addition of hormone therapy is consistent with the primary analysis, indicating limited improvement with the combination. The feasibility of adding hormone therapy appears to be higher in HRBC, but the high percentage in TNBC may be anomalous or needs further investigation. There is a notable change in gene methylation in HRBC, suggesting a potential impact of the treatment on epigenetic modifications.
		NCT00828854	2	The results suggest a modest clinical benefit with a Clinical benefit rate of 15.4% and a median Progression-Free Survival of 3.9 months. The Objective response rate was relatively low at 3.9%. It's important to note that all participants experienced at least one adverse event, indicating the need for careful consideration of the safety profile.
		NCT00020579	1	No results were posted on the response or survival
		NCT01434303	1	No results were posted on the response or survival
		NCT02453620	1	No results were posted on the response or survival
		NCT03280563	1 & 2	No results were posted on the response or survival
		NCT02115282	3	The difference in median of the progression-free survival between the two arms is minimal, suggesting limited improvement with the addition of entinostat. Arm A shows a slightly longer median in overall survival, but the clinical significance should be carefully considered. The objective response rate is low in both arms, indicating modest tumour response to treatment.

Mocetinostat (MGCD0103) (pan-HDACi)	[87]	Mocetinostat inhibits HDAC2 which then leads to the increase of miR-182 expression resulting in downregulation of DNA damage response gene RAD51 in basal-type breast cancer	No records of clinical studies involving this compound related to breast cancer		
Chidamide (HDACi class I and IIb)	[88–92]	Chidamide in combination with doxorubicin (DOX) sensitises DOX-resistant breast cancer cell lines to DOX resulting in cell apoptosis and cell cycle arrest. In combinatorial treatment, chidamide sensitises fluzoparib-resistant cells to the PARPi, inducing cell cycle arrest and cell apoptosis in TNBC cell lines. Both *in vitro* and *in vivo* studies showed that chidamide in combination with BETi PF-1 significantly reduces cell viability via downregulation of p-STAT3 in TNBC compared to either drugs used as single agents. Chidamide affects glycolysis of TNBC by upregulation of miR-33a-5p.	NCT05276713	Not indicated	No results were posted on the response or survival
			NCT05400993	2	No results were posted on the response or survival
			NCT05390476	2	No results were posted on the response or survival
			NCT04999540	2	No results were posted on the response or survival
			NCT05191914	4	No results were posted on the response or survival
			NCT05747313	3	No results were posted on the response or survival
			NCT05186545	2	No results were posted on the response or survival
			NCT05047848	Not indicated	No results were posted on the response or survival
			NCT05411380	2	No results were posted on the response or survival
			NCT05464173	1 & 2	No results were posted on the response or survival
			NCT05632848	2	No results were posted on the response or survival
			NCT05890287	3	No results were posted on the response or survival
			NCT05400993	2	No results were posted on the response or survival
			NCT05390476	2	No results were posted on the response or survival
			NCT04999540	2	No results were posted on the response or survival
			NCT05191914	4	No results were posted on the response or survival
			NCT05747313	3	No results were posted on the response or survival

			NCT05186545	2	No results were posted on the response or survival
			NCT05047848	Not indicated	No results were posted on the response or survival
			NCT05411380	2	No results were posted on the response or survival
			NCT05464173	1 & 2	No results were posted on the response or survival
			NCT05632848	2	No results were posted on the response or survival
			NCT05890287	3	No results were posted on the response or survival
			NCT05586841	1	No results were posted on the response or survival
			NCT05633914	2	No results were posted on the response or survival
			NCT05808582	Not indicated	No results were posted on the response or survival
			NCT05749575	2	No results were posted on the response or survival
			NCT04192903	2	No results were posted on the response or survival
			NCT05085626	2	No results were posted on the response or survival
			NCT05438706	2	No results were posted on the response or survival
			NCT05253066	2 & 3	No results were posted on the response or survival
			NCT05335473	1 & 2	No results were posted on the response or survival
			NCT05806047	2	No results were posted on the response or survival
Abexinostat (pan-HDACi	[93]	Abexinostat induces differentiation in breast cancer stem cells and this effect is only seen in tumours that have high expression of X inactivating specific transcripts	No records of clinical studies involving this compound related to breast cancer		
Fimepinostat (pan-HDACi)	[70]	Fimepinostat upregulates E-cadherin gene expression thereby potentially reversing epithelial to mesenchymal transition.	NCT02307240	1	No results were posted on the response or survival

Rocilinostat (HDAC6 inhibitor)	[94,95]	Rocilinostat reduced invasiveness in 4T1 and MC4L2 cell lines. Pre-treatement with rocilinostat improves the response of cell towards immunotherapy with the reduction PD-1 expression.	No records of clinical studies involving this compound related to breast cancer		
Belinostat (pan-HDACi)	[96–98]	Belinostat upregulates CXCL1 in TNBC and high expression of CXCL1 potentially acts as a prognostic indicator for improved overall survival. Belinostat in synergy with 17-AAG, an HSP90 inhibitor induces cell cycle arrest, apoptosis and reduces cell migration and invasion in a TNBC cell line	NCT04315233	1	No results were posted on the response or survival
			NCT04703920	1	No results were posted on the response or survival

Table 2B. A summary of preclinical and recently active or completed (2013 to present) clinical studies evaluating the utility of DNMTi as treatment modalities for breast cancer.

Example of epidrug	**Type of studies**				
DNMTis	Preclinical studies		Clinical studies		
			Study Code	Phase	Study design and result
5-Azacytidine	[99–104]	5-Azacytidine showed selective cytotoxicity towards breast cancer cell lines as the cytotoxicity was not observed in healthy breast cell line	NCT04891068	2	No results were posted on the response or survival
			NCT05381038	1&2	No results were posted on the response or survival
		Prior to chemotherapy, pre-treating breast cancer cell line with 5-Azacytidne and 6-mercaptonol is effective in reversing chemoresistance and more effective in fostering growth inhibition	NCT02223052	1	No results were posted on the response or survival
			NCT00748553	1&2	61.5% of participants responded to treatment (13 out of 16) which indicates a positive response rate, thus, suggesting a potential efficacy of the treatment in the phase 1 of the clinical trial. The second phase which consisted of testing the objective response rate of the participants gave a response rate above 50% is notable and suggests that a substantial proportion of patients were benefiting from the treatment.
		5-Azacytidne is able sensitise MCF-7 cell line to doxorubicin			
		5-Azacytidine able to inhibit the growth of tumour spheres as a neoadjuvant to radiation in MCF-7 cancer stem cells	NCT02811497	2	No results were posted on the response or survival

Decitabine	[105–109]	Decitabine could sensitise breast cancer cell line to taxol and anthracyclines based chemotherapy	NCT00030615	1	No results were posted on the response or survival
			NCT05673200	1	No results were posted on the response or survival
		Increased IFN-gamma release by T lymphocytes with co-culture of Decitabine treated 4T1 cell lines with showcased the DNMTi's capacity to improve immunogenicity	NCT04134884	1	No results were posted on the response or survival
		Decitabine reduces the circulating MDSCs as well as in spleen of animal models carrying 4T1 tumour			
		Decitabine induced DNMT degradation is dependent on the TRAF6 E3 ligase ubiquitination			
		High expression of the drug resistance gene, ABCB1 does not affect the tumour growth inhibition induced by Decitabine			

Guadecitabine	[53]	Guadecitabine upregulated MHC-I and MHC-II gene expression and in combination with ICI able to reduce tumour growth in animal model	No records of clinical studies involving this compound related to breast cancer
Zebularine	[110]	In a head and neck cancer cell line, zebularine induces cell apoptosis through the upregulation of caspase 3 and PARP proteins and the decrease in cell DNA synthesis persisted up till 1 week after the withdrawal of the drug.	No records of clinical studies involving this compound related to breast cancer

2.1.2. Utility of epigenetic drugs as components of combination therapy – evidence from preclinical studies

In addition to their use as monotherapies, there has been a substantial body of work evaluating the utility of epigenetic therapy as a component of combination therapy for the treatment of breast cancer. Preclinical studies evaluating the utility of epigenetic drugs in combination with immunotherapies[111,112], cytotoxic agents[98,108,113], radiotherapy[114,115], and other epigenetic drugs[116–119] have been conducted.

Burke *et al*.[112] investigated the therapeutic potential of combining anti-PD-1 immunotherapies with HDACi for treating bladder cancer in *in vitro* and *in vivo* models. The results demonstrated that systemic anti-PD-1 therapy in conjunction with local HDAC inhibitor therapy resulted in considerable immune-mediated tumour regression and long-lasting tumour immunity. The anti-tumour immune responses were dependent on Cytotoxic T-cells (CD8 T cells) and were Natural Killer cells (NK cells)-independent. The work established the viability of employing intravesical delivery of HDACi in an orthotopic bladder cancer model, and that immunological memory was persistent in mice with fully regressed tumours. The researchers reported that HDACi treatment caused gene deregulation in the bladder cancer cells. As a result of this gene deregulation, certain genes responsible for mediating immunorecognition, more specifically genes related to NKG2D ligands and HSP70 were upregulated. This means that the expression of these genes increased, leading to the production of proteins that help the T cells recognize the cancer cells. The observation of improved T cell recognition and killing suggests that HDACi may make tumour cells more vulnerable to T cell-mediated cytotoxicity. Similarly, Luker *et al*.[111] showed that the DNA methyltransferase inhibitor (guadecitabine) reduced the proliferation and accumulation of myeloid-derived suppressor cells, in turn improving T cell-dependent cytotoxicity in BALB/cJ mice. When paired with adoptive T cell therapy (AIT), it also overcame the T cell suppression brought on by arginase 1 and improved tumour suppression and survival. This shows that guadecitabine can improve the efficacy of immunotherapy for breast cancer by reversing tumour-induced immunosuppression. While preclinical findings are promising, it remains to be seen if the therapeutic potential of epidrugs can be recapitulated in human patients, and whether similar effects may be observed when paired with CAR T cell therapy or other modes of immunotherapy.

Preclinical evidence indicates that combinations of epidrugs can enhance the cytotoxic efficacy of chemotherapeutics. Vijayaraghavalu *et al*.[117] provided evidence that the sequential administration of decitabine and doxorubicin was more effective in overcoming drug resistance towards doxorubicin in breast cancer cells (MCF7 and MCF-7/ADR) when compared to simultaneous treatment. The pre-treatment administration of decitabine led to improved doxorubicin absorption and heightened cytotoxic effects. The combination of vorinostat and doxorubicin exhibited synergistic antiproliferative effects and resulted in the downregulation of genes (*CCL20*, *CTSL*, *HDGFL1*, *HSPA2*, *LOC342897*, *MAP7*, *MMP9*, *NNAT*, *NMB*, *RPL10L*, *STMN3*, *TKTL1*) associated with tumour promotion. Correspondingly, Hii *et al*. (2020) investigated the efficacy of HDAC inhibitor combinations with chemotherapeutics such as doxorubicin in targeting breast cancer stem cells (CSCs)[113]. They discovered that HDACi

increased breast CSCs' and non-CSCs' sensitivity to doxorubicin, suggesting a potential for increased therapeutic efficacy. Additionally, the synergistic effects of doxorubicin and HDACi were observed in a variety of breast cancer cell subtypes, indicating the efficacy of this combination in multiple breast cancer subtypes. These findings underscore the hypothesis that HDACi can induce a reprogramming of CSCs, which are typically relatively resistant to cytotoxic agents. Due to the interruption of CSC plasticity, non-CSCs are less likely to develop into drug-resistant CSCs.

Yu *et al.*[108] conducted a study that presented empirical evidence supporting the notion that the suppression of DNMTs via the E3 ligase TRAF6 functions as a mechanism of action for decitabine. Furthermore, the research findings revealed a substantial correlation between the suppression of DNMT expression and the responsiveness of triple-negative breast cancer (TNBC) cells to decitabine. Thus, in the context of managing patients diagnosed with triple-negative breast cancer (TNBC) who have demonstrated insufficient response to conventional chemotherapy, it is conceivable to employ DNMT protein levels as prospective indicators for forecasting the effectiveness of decitabine treatment[108]. However, Wawrsuzcazk *et al.*[64] demonstrated that the utility of HDACis in combination therapies is dependent on cellular context. In this study, which utilized an in vitro cell line model for TNBC with differential levels of Notch1 activity, they observed that combinations of valproic acid with cisplatin or vorinostat with cisplatin yielded additive interactions in cells with increased activity of Notch1. However, the cisplatin with vorinostat combination yielded an antagonistic interaction in cells with decreased Notch1 activity. Further studies are warranted to better elucidate the cellular determinants of the chemosensitizing effects of HDACis.

Aside from conventional chemotherapeutics, preclinical reports combining epidrugs with other cytotoxic agents have revealed additional promising avenues for applying epidrugs. For instance, Zuo *et al.*[98]looked at the effects of combining the HSP90 inhibitor 17-AAG with the HDAC6 inhibitor belinostat for the treatment of TNBC, and observed that TNBC cell migration, invasion, and proliferation were all reduced as a result of the combined treatment. The combined therapy raised acetylation rates of HSP90 and tubulin while decreasing HSP90 mRNA expression and HDAC6 protein abundance. This observation was accompanied by the suppression of Rho-mediated cell movement and the Hippo signalling pathway. In comparison to single-drug therapy, the combination therapy showed improved suppression of proliferation, migration, and invasion, suggesting its potential as an anti-metastatic treatment for TNBC. Vernier *et al.*[118] focused on the role of the transcription factor ERRα (Estrogen-Related Receptor Alpha) in regulating the expression of enzymes associated with the methionine cycle and DNA methylation. The regulation of key enzymes in the methionine cycle by ERRα has been found to play a significant role in the production of S-adenosylmethionine (SAM) which regulates the expression of DNA methyltransferase 1 (DNMT1), an essential enzyme involved in the process of DNA methylation. According to the study, blocking ERR with the inhibitor C29 alters the expression of genes involved in DNA methylation, increasing the expression of DNA-demethylating enzymes while decreasing the expression of DNA methylating enzymes. The role of ERRα extends to the regulation of key enzymes within the methionine cycle, such as MAT1A and MAT2A, thereby impacting the synthesis of SAM[74]. When ERR is

pharmacologically inhibited with C29, breast cancer cell lines exhibit altered intermediates of the methionine cycle and decreased levels of DNA methylation globally. The combination of treatment involving C29 and the DNA-demethylating drug 5-azadC has been observed to enhance the anti-tumour effects. This finding suggests that there may be potential therapeutic benefits associated with this combined approach. According to the findings, breast cancer cells had hypermethylated and repressed IRF4, a tumour suppressor gene. The co-administration of C29 and 5-azacytidine (5-azadC) has been found to induce demethylation and subsequent re-expression of the *IRF4* gene in breast cancer cells. This reactivation of *IRF4* has been observed to have notable antiproliferative effects specifically on breast cancer cells. In a mouse model, the efficacy of the combined treatment of C29 and 5-azadC was validated, showcasing its ability to suppress the growth of breast cancer tumours effectively. The results of this study provide further evidence for the association between ERRα activity and DNMT1 expression in individuals with breast cancer.

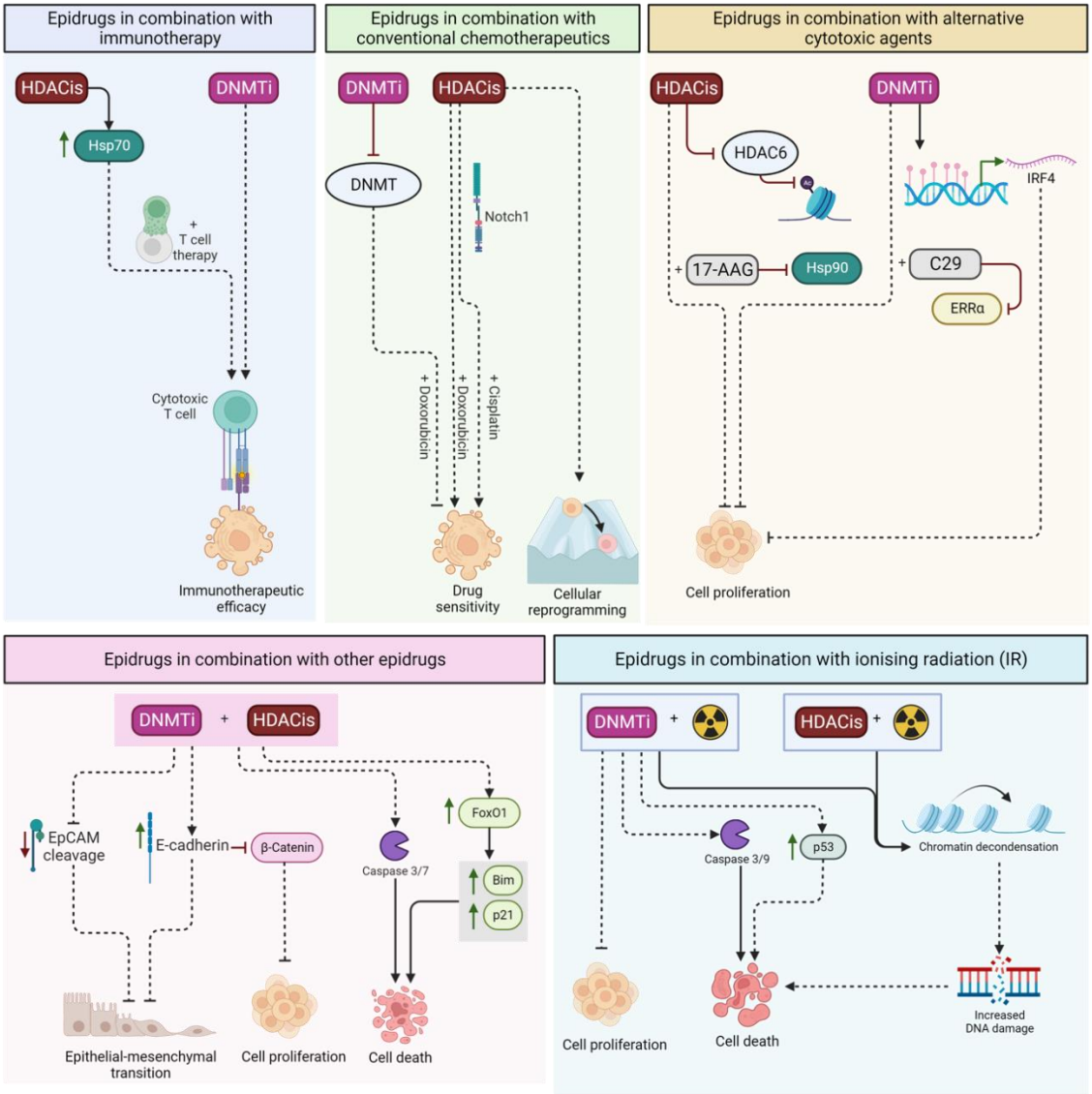


Figure 2. Reported cellular mechanisms that mediate the effect of epidrugs in combination with immunotherapeutics, conventional chemotherapeutics, cytotoxic agents, other epidrugs, and ionising radiation.

Both HDACis and DNMTis have been shown to activate cytotoxic T cells, leading to enhanced immune response against cancer cells[111,112]. When used in conjunction with chemotherapeutic drugs such as doxorubicin and cisplatin, epidrugs have been shown to increase chemosensitivity. Combinations of HDACis with chemotherapeutics such as doxorubicin have been shown to alter cellular programming, reducing the viability and population of cancer stem cells[64,108,113,117]. Epidrugs combined with other cytotoxic agents such as Hsp90 inhibitor 17-AAG and C29 ERRα inhibitor also effectively reduce and inhibit cell growth, at least in part through the upregulation of the tumour suppressor IRF4[74,98,118]. Combining HDACi and DNMTi, although through different pathways, results in three main outcomes: inhibition of epithelial-mesenchymal transition through a reduction in EpCAM cleavage and increased E-cadherin expression, inhibition of cell proliferation through inhibition of β-catenin, and cell death resulting from increased caspase activity and FoxO1 signalling[116,119]. DNMTis, when combined with ionising radiation, has been shown to inhibit cell proliferation, and induce cell death through increased activation of caspases 3/9 and elevation of p53 expression. Additionally, the decondensation induced by both DNMTis and HDACis resulted in higher levels of ionising radiation-induced DNA damage, thus promoting cell death[114,115]. Arrows denote activating interactions, while T-ended lines denote inhibition. Solid lines denote direct interactions, while dotted lines denote indirect interactions with intermediate steps. Created with BioRender.com.

Su *et al*.[119] assessed the effectiveness of DNMT and HDACi in the treatment of triple-negative breast cancer (TNBC) and cell line models of epithelial-mesenchymal transition (EMT). They observed that the TNBC cell lines, which were reprogrammed by DNMT and HDACi to a less aggressive state, characterized by reduced cell proliferation, motility, invasion, and colony formation compared to untreated controls. When combined, guadecitabine and entinostat demonstrated improved effects in suppressing cell proliferation, motility, colony formation, stemness, and triggering apoptosis compared to each inhibitor alone. These drugs upregulated E-cadherin, an important marker in epithelial cells that inhibits tumour cell growth by antagonizing beta-catenin signalling. Moreover, the combination treatment of guadecitabine with entinostat resulted in the inhibition of EpCAM cleavage despite the increase in expression of full-length EpCAM, thus, suggesting that this treatment suppressed WNT signalling and reversed EMT, potentially offering new treatment strategies for TNBC. However, further clinical studies are necessary to evaluate the efficacy of these drugs in TNBC patients. A similar approach has been demonstrated in urothelial cancer cell lines by Wang *et al*.[116], where the simultaneous administration of DNMTis and HDACis demonstrated considerably higher cytotoxicity than either alone. The combined treatment enhanced sub-G1 populations and caspases 3/7 activation in cancer cells, indicating cell death which subsequently arrested cell cycles at various stages in different cell lines. The combined treatment downregulated genes involved in the Akt/FoxO signalling pathway, which govern cell survival and proliferation, increasing proapoptotic proteins and cell cycle regulators. Aside from the above-mentioned observations in breast cancer, epidrugs have also been studied as potential treatment options for other cancers. In a study conducted by Kim *et al*. (2014), the researchers aimed to explore the potential synergistic effects of combining the epigenetic drug 5-aza-dC with ionising radiation (IR) to enhance the radiosensitivity of colorectal cancer cell lines[115]. The implementation of combination therapy demonstrated superior efficacy when compared to the administration of IR or 5-aza-dC individually. The combination of treatments resulted in a notable suppression of cellular growth, in the tested HCT116 and SW480 cell lines, as well as a notable increase in the proportion of cells undergoing apoptosis, activation of caspases 3 and 9, cleavage of PARP1, and upregulation of p53. These findings collectively suggest that the combination therapy induces significant alterations in the apoptosis-associated

proteins, highlighting the potential of epidrugs in combination with IR as a therapeutic strategy for combating this malignancy. Similarly, Terry & Vallis[114]investigated the combination of epigenetic drugs with radiotherapy, specifically focusing on the effects of histone deacetylase (HDAC) inhibition and DNA demethylation on cellular response to radiation. The m researchers found that HDAC inhibition induced chromatin decondensation, leading to increased DNA damage when combined with radiation or exposure to a radiopharmaceutical 111In-DTPA-hEGF. DNA demethylation also increased DNA damage after radiation or exposure to 111In-DTPA-hEGF. Both HDAC inhibition and DNA demethylation decreased clonogenic survival when combined with radiation or 111In-DTPA-hEGF. The intracellular localization of 111In-DTPA-hEGF and chromatin condensation were also examined, and it was found that altering chromatin structure reduced DNA damage but did not significantly affect clonogenic survival. The study suggested that the sensitizing effects of HDAC inhibition are not due to changes in EGFR expression but are likely the result of chromatin decondensation resulting from the HDACi treatment, which increases the likelihood of radiation-induced DNA damage. The results have clinical relevance and highlight the potential of epigenetic modulators as radiosensitizers for targeted radiotherapy, particularly with specific radiopharmaceuticals. Overall, these findings provide insights into the interplay between chromatin structure, DNA damage, and cellular response to radiation-based treatments, offering new avenues for improving the efficacy of cancer treatments involving radiotherapy combined with epigenetic modulators.

2.1.3. Findings from clinical trials evaluating the utility of epigenetic drugs as components of combination therapy

Azacitidine is currently in phase 2 clinical trials as a component of combination therapies (NCT01349959, NCT02811497 and NCT00748553) and monotherapy (NCT04891068, see Table 2B for more details) for breast cancer, while decitabine is in Phase 1 clinical trials for both combined (NCT02957968, NCT05673200) and monotherapy (NCT00030615, see Table 2B for more information) of breast cancer (A summary is provided in Table 3). Among the HDACis known to be effective in cancer treatment, belinostat is currently being used in two different Phase 1 clinical trials as components of combined therapy for metastatic breast cancer (NCT04703920, NCT04315233), while panobinostat was in a Phase 2 clinical trial for Monotherapy of Locally Recurrent HER2-negative or Metastatic Breast Cancer (NCT00777049, see Table 2A for more details). Vorinostat has been studied in various phase 2 clinical trials as a component of combinational therapy of different subtypes of breast cancer (NCT00262834, NCT00258349, NCT00368875). Entinostat, a class I HDACi presented a promising outlook in a Phase 2 with improved PFS and OS[85,120]. However, such benefits were not reflected in Phase 3 trials which could be due to a larger population study and the incongruence in the ethnicity between the Phase 2 and Phase 3 trials[121].

Table 3. A non-exhaustive summary of epigenetic drugs undergoing clinical trials as components of drug combinations in Phase 1 & 2 and the corresponding publications where available.

Clinical Trial Number	Phase	Combinations involving epidrugs	Design and Findings	Reference
NCT01349959	2	Azacitidine and Entinostat	The confirmed response rate to the combination treatment with the addition of hormone therapy was 0% in the TNBC patients and 3.7% in the hormone-resistant breast cancer patients. ERα and RARβ expression was altered in all patients with hormone-resistant breast cancer but was not altered in any of the tested TNBC patients.	[46]
NCT02811497	2	Azacitidine and Durvalumab	No clinical responses were observed in the recruited patients (n = 28) The disease control rate was 7.1%. The median progression-free survival was 1.9 months (95% CI 1.5 to 2.3) The median overall survival was 5 months (95% CI 4.5 to 10).	[122]
NCT00748553	2	Azacitidine and nanoparticle albumin-bound paclitaxel	Trial includes patients with solid tumours which have advanced or metastasised. Phase I included 16 patients, with a response rate of 61.5%. Phase II included 14 patients, with an objective response rate of 53.8%. Specific data on ER+ status and progression-free survival were not provided. The trial showed promising response rates with manageable adverse events.	
NCT02957968	2	Decitabine, pembrolizumab, and carboplatin (chemotherapy)	No results were posted at the time of writing	

Clinical Trial Number	Phase	Combinations involving epidrugs	Design and Findings	Reference
NCT05673200	1	ASTX727 (decitabine +cedazuridine) paclitaxel. and pembrolizumab	No results were posted at the time of writing	
NCT04703920	1	Talazoparib and Belinostat	No results were posted at the time of writing	
NCT04315233	1	Ribociclib and Belinostat	No results were posted at the time of writing	
NCT00258349	1&2	Vorinostat and trastuzumab	This study included 10 eligible HER2-positive patients who relapsed or progressed with trastuzumab therapy The response rate was found to be 0%, with no complete or partial responses observed The median time to progression was 1.5 months he median overall survival was 9.3 months.	
NCT00368875	1&2	Vorinostat,paclitaxel, and bevacizumab	The trial included patients with metastatic breast cancer. In Phase I, the dose of vorinostat was assigned at registration and administered orally twice daily on specific days of each cycle. Paclitaxel and bevacizumab were also administered on specific days of the cycle. The dose of vorinostat was escalated based on observed toxicity. In Phase II, the recommended dose of vorinostat was administered.	

Clinical Trial Number	Phase	Combinations involving epidrugs	Design and Findings	Reference
			The objective response rate was estimated to be 49% The median progression-free survival was 11.9 months. The median overall survival was 29.4 months in Phase I. The combination treatment regimen showed promising efficacy but was associated with some adverse events.	
NCT02374099	2	Oral azacitidine and Fulvestrant	The trial involved 97 women with estrogen receptor-positive, who have progressed after receiving an aromatase inhibitor. Oral azacitidine and Fulvestrant had 8.3% objective response rates and 31.3% and 30.6% clinical benefit rates. The median overall survival was 5.49 months in the oral azacitidine and Fulvestrant group and 5.46 months in the Fulvestrant group in the 48-person study. Fulvestrant group had an all-cause mortality rate of 30.43%, and 21.74%..	
NCT00828854	2	Combination of entinostat (5 mg) with an aromatase inhibitor	The trial involved 27 postmenopausal women with ER-positive breast cancer with progressive disease after at least 3 months of treatment with a third-generation aromatase inhibitor. The objective response rate during the first 6 cycles of study treatment was 3.9%, with serious adverse events reported in 55.56% of the participants.	

Clinical Trial Number	Phase	Combinations involving epidrugs	Design and Findings	Reference
NCT00676663	2	Combination of entinostat (5 mg) with exemestane (25 mg)	The trial involved 130 postmenopausal women with ER-positive breast cancer with relapse or progressive disease with prior treatment with an aromatase inhibitor. The objective response rate was 4.7%, compared to 4.6% in the group receiving exemestane and placebo.	[123]
NCT04296942	1	Bifunctional fusion molecule involving programmed death-ligand 1 with transforming growth factor beta sequestering agent and entinostat	The study was listed as completed without having recruited participants to the treatment arm with entinostat.	

Taken together, these findings indicate that while epigenetic drugs and combinations incorporating them have shown promising effects in preclinical models of breast cancer, further improvements and studies are warranted to allow for the clinical implementation of epigenetic drugs in treating breast cancer. Additionally, these trials have demonstrated the technical difficulty of verifying the cell-specific effects of epigenetic drugs on the DNA methylation or histone acetylation states in patients receiving these treatments.

3. Discussion

3.1. Evaluation of Different Generations of Epidrugs in Clinical Trials for Breast Cancer

The epidrugs currently in clinical trials for breast cancer are divided into three categories, first generation, second generation, and third generation. The first generation of epidrugs (DNMTi) which include decitabine and azacytidine was approved in the United States of America to treat chronic myelomonocytic leukaemia and acute myeloid leukaemia, with additional label expansions in 2022[124]. Examples of first-generation HDACis include Vorinostat and Romidepsin, which were approved for cutaneous T cell lymphoma in 2006 and 2009 respectively[125]. In the context of breast cancers, however, these drugs are in early phase clinical testing, both as combination therapies and as monotherapies. While limited results have been published from clinical trials involving the use of these drugs in breast cancers, azacytidine has shown promising results in the NCT00748553 study with a 61% positive response rate in phase 1 and a 50% response rate in phase 2. However, the study's sample size was limited to 16 participants, which does not guarantee the drug's efficacy on a broad spectrum of patients. Vorinostat has shown promising results in clinical phase 1 & 2 studies where the drug has been seen to decrease the tumour content and have manageable toxicities. The advancement of second-generation epidrugs, encompassing DNMTi (such as zebularine and guadecitabine) and HDACi (including hydroxamic acid, belinostat and panobinostat, tucidinostat, and valproic acid) with enhanced physiological characteristics, was deemed essential due to the unfavourable pharmacokinetic properties and inadequate target selectivity of first-generation inhibitors[126]. The anticipation was that substances exhibiting more potent inhibitory effects and fewer adverse consequences would be uncovered. Early-generation epidrugs exhibited a brief half-life owing to their limited bioavailability, heightened activity beyond physiological pH ranges, and interactions with target cell deaminases[127]. Clinical trials with second-generation epidrugs in breast cancer have shown low efficacy and were associated with adverse events. The third generation of epidrugs succeeds in identifying the inherent intricacy of the epigenetic components in not merely imprinting, but even in removing or changing the epigenetic marks[128]. This showcases a pressing need for more research on the epigenetic proteins' interactome, which is the backbone for the perfection of yet highly precise and targeted epidrugs. A wide band of substances is associated with the third generation of epidrugs including histone methyltransferase inhibitors (HMTi), histone demethylase inhibitors (HDMi), and bromodomain and extra-terminal domain inhibitors (BETi), which have unique potentials and barriers in the journey of novel clinical interventions discovery[129]. Clinical trials involving the third-generation epidrugs in breast cancer treatment are yet to be explored.

3.2. Challenges and limitations of Epigenetic Therapy

Challenges in epigenetic therapy include the lack of biological specificity, side effects, and inter-patient variations[130,131]. The use of epigenetic therapy as a standalone therapy remains a challenging goal due to several obstacles impeding its progress in breast cancer treatment[132–135]. Firstly, the requirement for further investigations to refine treatment dosages and strategies; secondly, the emergence of drug resistance in some patients; and thirdly, the possibility of side effects. Furthermore, the reversibility of epigenetic changes may contribute to the development of drug resistance. Thus, understanding the complexities of the resistance mechanism is crucial for improving the efficacy of the epidrugs.

An alternative approach, which is to introduce epigenetic therapy together with standard treatments, such as chemotherapy, has demonstrated considerable potential in surmounting drug resistance and enhancing the effectiveness of breast cancer treatment[136]. This strategy is based on altering gene expression such as to shift cancer cells towards apoptosis or to make cancer cells more responsive to standard treatments[137]. Considering epidrug targets epigenetic enzymes responsible for maintaining gene expression, the use of epidrugs to modulate the expression of tumour suppressor genes and oncogenes serves as an avenue for improved therapy[138,139]. For instance, the combination of epidrugs with conventional therapy has shown to have improved patient outcomes in other types of cancers when compared to the use of conventional therapies alone[140–142]. While promising results have been shown in the preclinical stage, the outcomes in the clinical trials have yet to be satisfactory. To date, trials have been hindered due to adverse events of patients and limited patient outcomes. The reason for the adverse events might be due to the lack of specificity of the epidrugs, which targets the epigenetic enzymes in general, thus leading to unintended outcomes[143–146].

3.3. Future Perspectives

CRISPR-based epigenetic editing tools may provide a means of addressing the lack of specificity of epidrugs. This strategy utilizes CRISPR/dCas9 systems to direct regulators of DNA methylation, histone modification, and chromatin structures to particular genomic coordinates[147]. While these tools are currently used primarily in the development of *in vitro* models, these tools may be utilized for the development of personalized cell-based therapies, leveraging epigenetic reprogramming in regenerative medicine for issues such as organ failure, tissue injuries, or degenerative disorders[148,149].

Additionally, nanoparticle-based systems for drug delivery may be employed, utilizing sophisticated targeting systems to direct the transportation of epidrugs to the tissues or cell types of interest[150,151]. This is achievable by encapsulating epidrugs into nanoparticles that can be equipped with surface-targeting molecules, resulting in increased absorption of epidrugs at the disease site while avoiding off-target effects[152].

Further investigations are also required to validate and enhance epigenetic biomarkers to diagnose, prognosticate, and predict treatment responses in breast cancer[134,153]. It is imperative that further clinical and basic science research is conducted to establish the efficacy, safety, and enduring impacts of epigenetic-based therapies, either as standalone treatments or in conjunction with other therapeutic approaches to determine their role in the management of breast cancer.

4. Conclusion

In general, the utilisation of epigenetic therapy exhibits considerable promise in enhancing breast cancer outcomes through the precise targeting of specific epigenetic modifications, reinstating the normal patterns of gene expression, and heightening the susceptibility of cancer cells to conventional therapeutic approaches. Further research and clinical investigation are imperative to fully harness the potential of epigenetic therapy in the context of personalised treatment for breast cancer.

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