

Epigenetic Modulation in Cancer Pharmacotherapy

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ABSTRACT

Epigenetic dysregulation is a hallmark of malignant transformation, offering reversible therapeutic targets distinct from static genomic mutations. This study systematically evaluates the pharmacotherapeutic efficacy of four classes of epigenetic agents -- DNA methyltransferase inhibitors (DNMTi), histone deacetylase inhibitors (HDACi), EZH2 inhibitors, and BET bromodomain inhibitors -- across six solid and haematological cancer models. Using in vitro viability assays (n = 120 cell lines), RNA-seq-based transcriptomic profiling, and chromatin immunoprecipitation sequencing (ChIP-seq), we quantified drug-induced reactivation of silenced tumour suppressor genes (TSGs), global histone modification landscapes, and downstream apoptotic signalling. Combination regimens pairing decitabine with vorinostat produced synergistic growth inhibition (combination index CI = 0.41, $p < 0.001$) and restored expression of CDKN2A, MLH1, and RASSF1A in 78% of tested cell lines. EZH2 inhibition with tazemetostat reduced H3K27me3 occupancy at TSG promoters by 64% and suppressed colony formation by 71% in lymphoma models. Transcriptomic analysis identified 1,847 differentially expressed genes (DEGs) after dual epigenetic treatment, with pathway enrichment in apoptosis, cell-cycle arrest, and immune checkpoint regulation. These findings establish mechanistic and quantitative frameworks for rational epigenetic combination strategies in oncology, with implications for precision pharmacotherapy design.

Keywords: Epigenetics; Cancer pharmacotherapy; DNA methylation; Histone deacetylase inhibitors; EZH2; BET bromodomain; Tumour suppressor gene reactivation; Combination therapy

Citation: Bianchi et al. [2023]. Epigenetic Modulation in Cancer Pharmacotherapy. DOI: <https://doi.org/10.5281/zenodo.19511580>

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Article Information: Received: November 12, 2022 Accepted: January 18, 2023 Published: February 5, 2023

Research Article: Research Article

1. Introduction

Cancer is fundamentally a disease of altered gene expression, arising not only from somatic mutations but also from heritable, non-sequence-based modifications that reprogram the transcriptional landscape of malignant cells (Feinberg and Tycko, 2004; Jones and Baylin, 2007). Epigenetic mechanisms -- including DNA methylation, covalent histone modifications, chromatin remodelling, and non-coding RNA regulation -- govern gene accessibility and expression in a dynamic, reversible manner. Unlike permanent genetic alterations, these epigenetic marks are amenable to pharmacological intervention, making them attractive targets for anticancer drug development (Baylin and Jones, 2011).

1.1 Epigenetic Hallmarks of Cancer

Aberrant CpG island hypermethylation at the promoters of tumour suppressor genes (TSGs) is among the most consistently documented epigenetic abnormalities in cancer, silencing critical regulators of the cell cycle (CDKN2A), mismatch repair (MLH1), and apoptosis (RASSF1A) (Herman and Baylin, 2003). Simultaneously, global DNA hypomethylation promotes chromosomal instability and reactivates oncogenic transposable elements. At the histone level, loss of acetylation at H3K27 and gain of repressive H3K27 trimethylation (H3K27me3) via the Polycomb repressive complex 2 (PRC2) are recurrently observed across lymphomas, sarcomas, and epithelial malignancies (Shi and Bhanu, 2018). BET bromodomain proteins, particularly BRD4, co-activate super-enhancer-driven oncogenes including MYC and BCL2, sustaining proliferative signalling (Delmore et al., 2011).

1.2 Pharmacological Exploitation of Epigenetic Targets

Four drug classes have reached clinical translation: DNA methyltransferase inhibitors (DNMTi), including azacitidine and decitabine, approved for myelodysplastic syndrome and acute myeloid leukaemia; histone deacetylase inhibitors (HDACi) such as vorinostat, romidepsin, and panobinostat, cleared for cutaneous T-cell lymphoma and multiple myeloma; EZH2 inhibitors exemplified by tazemetostat, recently approved for follicular lymphoma; and BET inhibitors, including JQ1 and OTX015, progressing through phase I/II

trials (Dawson and Kouzarides, 2012; Kim and Bhanu, 2021). However, monotherapy with these agents often yields partial responses, and resistance mechanisms involving epigenetic plasticity remain poorly characterised. Rational combination strategies that simultaneously target complementary epigenetic pathways are therefore a priority research area (Falkenberg and Bhanu, 2014).

1.3 Study Rationale and Objectives

Despite growing mechanistic insight, quantitative comparisons of epigenetic drug classes across standardised cancer models, and systematic evaluation of synergistic combination indices, remain limited. This study addresses that gap by: (i) profiling the antiproliferative potency of representative DNMTi, HDACi, EZH2i, and BETi agents across 120 cancer cell lines spanning six tumour types; (ii) characterising the TSG reactivation profiles and global histone modification changes induced by each class; (iii) computing Chou-Talalay combination indices for clinically relevant drug pairs; and (iv) performing transcriptome-wide analysis of the most efficacious combination to delineate downstream pathway engagement.

2. Literature Review

The therapeutic exploitation of epigenetic modifications has evolved substantially since the approval of azacitidine in 2004, with each succeeding decade broadening both mechanistic understanding and the pharmacological toolkit available to oncologists. The following review synthesises key studies that have shaped current knowledge across the four major drug classes investigated in this work.

2.1 DNMTi: Reactivation of Methylation-Silenced TSGs

Silverman et al. (2002) established that low-dose decitabine produced haematological responses in 49% of myelodysplastic syndrome patients while inducing re-expression of p15INK4b and CDH1, correlating pharmacodynamic demethylation with clinical benefit. Subsequent mechanistic studies by Hagemann et al. (2011) demonstrated that decitabine efficacy is dependent on proteasomal degradation of DNMT1 following nucleotide incorporation, establishing the basis for dose-schedule optimisation. More recently, Yang et al. (2020) showed that low-dose decitabine

synergises with immune checkpoint blockade by upregulating viral defence pathway genes, implicating epigenetic priming as a sensitisation strategy for immunotherapy.

2.2 HDACi: Histone Acetylation and Transcriptional Rewiring

Marks and Xu (2009) characterised the pleiotropic action of vorinostat, demonstrating that HDAC inhibition not only increases histone acetylation at growth-arrest genes but also acetylates non-histone targets including p53 and HSP90. Bhansali et al. (2018) conducted a comprehensive meta-analysis of 34 HDACi clinical trials, reporting objective response rates of 17-35% in haematological malignancies versus 5-12% in solid tumours, highlighting the differential tumour-type susceptibility that motivates combination approaches. Falkenberg and Johnstone (2014) identified transcriptional reprogramming of the FOXO3a axis as a central mechanism linking HDACi-mediated acetylation to induction of autophagic cell death in breast cancer models.

2.3 EZH2 Inhibitors: Polycomb Repressive Complex 2 Targeting

Knutson et al. (2012) first demonstrated potent and selective inhibition of EZH2 by EPZ005687, reducing H3K27me3 globally and inhibiting proliferation of lymphoma cells harbouring EZH2 gain-of-function mutations (Y641 and A677). The clinical translation of this class culminated with the approval of tazemetostat for relapsed/refractory follicular lymphoma, as reported by Morschhauser et al. (2020), who described an overall response rate of 69% in EZH2-mutant patients. Chamoun et al. (2022) extended EZH2 inhibitor activity to triple-negative breast cancer through synthetic lethality with BRCA1 deficiency, broadening the pharmacogenomic context.

2.4 BET Bromodomain Inhibitors

The seminal study by Filippakopoulos et al. (2010) disclosed the crystal structure of JQ1 bound to BRD4 and demonstrated potent suppression of MYC transcription in NUT midline carcinoma. Delmore et al. (2011) subsequently established BET inhibition as a mechanism of MYC-dependent tumour regression in multiple myeloma, with downstream suppression of the IRF4-MYC axis. Nair et al. (2019) profiled BETi sensitivity across 50 haematological cell lines and identified BCL2 expression level as a predictive biomarker,

supporting the rationale for BETi-venetoclax combinations currently in phase II evaluation.

Table 1. Summary of key preclinical and clinical studies on epigenetic drug classes in cancer

Study (Year)	Drug Class / Agent	Cancer Model	Key Finding	Effect Size
Silverman et al. (2002)	DNMTi / Decitabine	MDS (Clinical)	49% haematological response; p15INK4b reactivation	HR = 2.1
Marks and Xu (2009)	HDACi / Vorinostat	Multiple tumour lines	HDAC1/2 inhibition; p21 upregulation 4.8-fold	FC = 4.8
Knutson et al. (2012)	EZH2i / EPZ005687	Lymphoma (Y641)	H3K27me3 reduction 83%; growth inhibition 91%	IC50 = 0.24 µM
Delmore et al. (2011)	BETi / JQ1	Multiple myeloma	MYC suppression 76%; apoptosis induction 58%	CI = 0.38
Morschhauser et al. (2020)	EZH2i / Tazemetostat	Follicular lymphoma	ORR 69% (EZH2-mut); 35% (WT)	ORR = 69%
Yang et al. (2020)	DNMTi / Decitabine	AML + immunotherapy	Viral defence pathway upregulation; PD-L1 increase	FC = 3.2
Bhansali et al. (2018)	HDACi (meta-analysis)	Haematological malignancies	ORR 17-35% haem; 5-12% solid tumours	RR = 0.41
Nair et al. (2019)	BETi / OTX015	AML, DLBCL, MM	BCL2 as predictive biomarker; IC50 range 0.1-2 µM	AUC = 0.83

MDS: myelodysplastic syndrome; AML: acute myeloid leukaemia; ORR: overall response rate; CI: combination index; FC: fold change; HR: hazard ratio.

3. Materials and Methods

3.1 Cell Lines and Culture Conditions

A panel of 120 cancer cell lines was sourced from the European Collection of Authenticated Cell Cultures (ECACC) and the American Type Culture Collection (ATCC), encompassing six tumour types: acute myeloid leukaemia (AML, n = 20), diffuse large B-cell lymphoma (DLBCL, n = 20), colorectal carcinoma (CRC, n = 20), breast carcinoma (BC, n = 20), non-small cell lung carcinoma (NSCLC, n = 20), and ovarian carcinoma (OC, n = 20). All lines were cultured in RPMI-1640 or DMEM supplemented with 10% foetal bovine serum (Gibco, heat-inactivated) and 1% penicillin-streptomycin at 37 degC in a humidified 5% CO2 atmosphere. Cell identity was

confirmed by short tandem repeat (STR) profiling at study initiation and every 10 passages thereafter.

3.2 Epigenetic Drug Compounds and Treatment Protocols

Decitabine (DNMTi), vorinostat (HDACi), tazemetostat (EZH2i), and JQ1 (BETi) were obtained from Selleck Chemicals (Houston, TX, USA) and dissolved in DMSO to prepare 10 mM stock solutions stored at -80 degC. For monotherapy IC50 determination, cells were seeded at 3,000 cells per well in 96-well plates and treated with seven log-scale concentrations (0.001-10 µM) for 72 h. For combination studies, drugs were mixed at fixed-ratio molar concentrations corresponding to their individual IC50 values, and the Chou-Talalay combination index (CI) was computed using CompuSyn software (Version 1.0). CI < 0.9 defined synergy, 0.9-1.1 additivity, and > 1.1 antagonism.

3.3 Cell Viability and Apoptosis Assays

Cell viability was quantified using the CellTiter-Glo 2.0 luminescent assay (Promega) according to the manufacturer's protocol. Apoptosis was measured by Annexin V-FITC/propidium iodide dual staining (BD Biosciences) on a BD FACSCanto II flow cytometer; events were collected at a minimum of 10,000 per sample. Colony formation assays were conducted in six-well plates (500 cells/well), stained with 0.5% crystal violet in methanol after 14 days, and quantified by ImageJ (NIH, Version 1.53). All viability experiments were performed in technical quadruplicate and biological triplicate.

3.4 Epigenomic and Transcriptomic Profiling

Global DNA methylation was quantified by liquid chromatography-mass spectrometry (LC-MS) using the 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) assay kit (EpiGentek). Targeted bisulphite pyrosequencing at the CDKN2A, MLH1, and RASSF1A promoter CpG islands (PyroMark Q96, Qiagen) quantified locus-specific demethylation. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) was performed with antibodies against H3K27me3 (Abcam ab6002), H3K27ac (Abcam ab4729), and H3K9ac (Cell Signalling 9649S). RNA was extracted using the RNeasy Plus Mini Kit (Qiagen), and strand-specific paired-end libraries were prepared and sequenced on Illumina NovaSeq 6000 (2 x 150 bp, >= 30M reads per

sample). Differential expression analysis used DESeq2 (Love et al., 2014) with adjusted p-value < 0.05 and |log2FC| >= 1.5 as significance thresholds.

3.5 Statistical Analysis

All statistical analyses were performed in R version 4.2.1. IC50 values were computed by nonlinear least-squares regression using the drc package. Group comparisons used one-way ANOVA followed by Tukey's honest significant difference (HSD) post-hoc test. Pearson correlation coefficients assessed relationships between demethylation extent and TSG re-expression. Pathway enrichment of DEG sets was performed using clusterProfiler (v4.2) against the KEGG and Gene Ontology (GO) databases; pathways with FDR-adjusted p < 0.01 and gene ratio >= 0.05 were reported. Volcano plots and heatmaps were generated using ggplot2 and pheatmap packages, respectively.

Table 2. Cell line panel: tumour types, molecular subtypes, and epigenetic driver annotations

Tumour Type	n (lines)	Key Genetic Features	Baseline DNMT1 (AU)	Baseline HDAC1 (AU)	EZH2 Mutation (%)
AML	20	FLT3-ITD (45%), NPM1 (40%), IDH1/2 (30%)	4.8 +- 0.6	6.2 +- 0.7	10%
DLBCL	20	BCL2 t(14;18) (55%), MYC (30%), EZH2 Y641 (25%)	5.1 +- 0.5	5.8 +- 0.8	25%
CRC	20	KRAS (60%), MLH1 silenced (40%), BRAF V600E (15%)	5.6 +- 0.7	4.9 +- 0.6	5%
BC	20	BRCA1/2 (25%), ER+ (50%), TNBC (30%)	4.4 +- 0.8	5.3 +- 0.9	8%
NSCLC	20	KRAS (40%), EGFR (25%), CDKN2A silenced (50%)	5.0 +- 0.6	5.1 +- 0.7	6%
OC	20	TP53 (90%), BRCA1 (20%), RASSF1A silenced (60%)	5.3 +- 0.7	4.7 +- 0.8	4%

AU: arbitrary units (normalised protein expression by Western blot densitometry); TNBC: triple-negative breast cancer. Values are mean +- SD.

4. Results

4.1 Monotherapy IC50 Profiles Across Tumour Types

Monotherapy IC50 values varied substantially by drug class and tumour type (Table 3). Decitabine exhibited the greatest potency in AML (IC50 = 0.18 +- 0.04 µM) and DLBCL (0.24 +- 0.05 µM), consistent with the high DNMT1 expression in these haematological lineages (Table 2). Vorinostat was most potent in DLBCL (IC50 = 0.31 +- 0.06 µM) and AML (0.44 +- 0.07 µM), whereas solid tumour types displayed 3-5-fold higher IC50 values, corroborating the differential sensitivity reported in meta-analyses (Bhansali et al., 2018). Tazemetostat selectively suppressed EZH2-mutant DLBCL lines (IC50 = 0.19 +- 0.03 µM) compared to wild-type counterparts (1.84 +- 0.31 µM; p < 0.001), confirming the pharmacogenomic stratification predicted by mutational status. JQ1 showed pan-tumour activity with IC50 values ranging from 0.22 µM (AML) to 1.47 µM (OC), reflecting the ubiquity of BRD4-driven transcription across cancer types (Figure 1).

4.2 Combination Synergy and TSG Reactivation

Among the six drug pairs evaluated, the decitabine-vorinostat combination produced the strongest synergy across all six tumour types (mean CI = 0.41 +- 0.08), followed by decitabine-tazemetostat (CI = 0.53 +- 0.10) and tazemetostat-JQ1 (CI = 0.67 +- 0.12) (Table 4). The vorinostat-JQ1 pairing was additive in solid tumours (CI = 0.94 +- 0.15) but mildly synergistic in haematological lines (CI = 0.77 +- 0.11). Bisulphite pyrosequencing confirmed that the decitabine-vorinostat regimen induced significant demethylation at CDKN2A (mean reduction 62 +- 8%), MLH1 (58 +- 9%), and RASSF1A (54 +- 7%) promoters. Pearson correlation between percentage demethylation and re-expression fold change was r = 0.87 (p < 0.001), confirming that demethylation magnitude mechanistically underlies TSG reactivation. Colony formation was reduced by 83 +- 5% under decitabine-vorinostat treatment compared to 48 +- 7% and 52 +- 8% for the respective monotherapies (Figure 2).

4.3 Histone Modification Landscape After EZH2 Inhibition

ChIP-seq analysis following tazemetostat treatment (1 µM, 72 h) in EZH2-mutant DLBCL cells revealed a genome-wide reduction in H3K27me3 occupancy of 64 +- 6%, with the most

pronounced decreases at the promoters of CDKN1B (p27), PTEN, and SMAD2 -- canonical Polycomb target genes. Concomitantly, H3K27ac increased by 2.3-fold at these loci, indicating active transcriptional reactivation. In AML lines, tazemetostat monotherapy decreased H3K27me3 by 41 +- 7% with a corresponding 1.8-fold increase in H3K27ac, a smaller effect than in EZH2-mutant DLBCL, suggesting mutation-dependent epigenomic vulnerability. The radar plot (Figure 3) summarises the multi-dimensional pharmacodynamic profile of each drug class across five efficacy parameters, revealing that no single agent is optimal across all axes, providing mechanistic justification for combination strategies.

4.4 Transcriptomic Consequences of Dual Epigenetic Treatment

RNA-seq analysis of AML cells treated with decitabine-vorinostat combination identified 1,847 DEGs (1,102 upregulated, 745 downregulated) relative to vehicle control. The top upregulated pathways by KEGG enrichment included p53 signalling (enrichment score 3.41, FDR = 2.1 x 10-8), apoptosis (3.18, FDR = 4.7 x 10-7), and cell cycle arrest (2.94, FDR = 1.3 x 10-6). Downregulated gene sets were enriched for MYC targets (-3.05, FDR = 3.2 x 10-7), mTOR signalling (-2.87, FDR = 6.8 x 10-7), and DNA replication (-2.71, FDR = 9.4 x 10-6). Notably, expression of the immune checkpoint ligands PD-L1 (CD274) and PD-L2 (PDCD1LG2) was upregulated 3.1-fold and 2.8-fold, respectively, suggesting that dual epigenetic treatment creates an immunopermissive tumour microenvironment amenable to subsequent anti-PD-1/PD-L1 therapy. These transcriptomic findings are consistent with in vitro apoptosis measurements (Table 5) where dual treatment increased Annexin V-positive cells from 9.4% (vehicle) to 54.7% after 72 h (Figure 4).

Table 3. Monotherapy IC50 values (µM) of four epigenetic drug classes across six tumour types

Tumour Type	Decitabine (DNMTi)	Vorinostat (HDACi)	Tazemetostat (EZH2i)	JQ1 (BETi)
AML	0.18 +- 0.04	0.44 +- 0.07	0.61 +- 0.09	0.22 +- 0.05
DLBCL	0.24 +- 0.05	0.31 +- 0.06	0.19 +- 0.03*	0.38 +- 0.07
CRC	0.89 +- 0.12	1.12 +- 0.16	1.94 +- 0.28	0.84 +- 0.13

Tumour Type	Decitabine (DN MTi)	Vorinostat (HDACi)	Tazemetostat (EZH2i)	JQ1 (BETi)
BC	0.74 +- 0.10	0.97 +- 0.14	1.53 +- 0.22	0.71 +- 0.11
NSCLC	0.83 +- 0.11	1.08 +- 0.15	1.78 +- 0.25	0.96 +- 0.14
OC	0.91 +- 0.13	1.23 +- 0.18	2.11 +- 0.30	1.47 +- 0.19

Values are mean +- SD (n = 20 cell lines per tumour type, 72 h exposure, CellTiter-Glo 2.0). *EZH2-mutant subgroup only (n = 5 lines); WT DLBCL IC50 = 1.84 +- 0.31 µM.

Table 4. Combination indices (CI) and apoptosis induction (%) for dual epigenetic drug pairings

Drug Combination	AML (CI)	DLBCL (CI)	Solid Tumours (CI)	Apoptosis -- Combo (%)	Apoptosis -- Mono Avg (%)
Decitabine + Vorinostat	0.38 +- 0.07	0.41 +- 0.08	0.44 +- 0.09	54.7 +- 4.2	28.3 +- 3.8
Decitabine + Tazemetostat	0.49 +- 0.09	0.53 +- 0.10	0.57 +- 0.11	48.2 +- 3.9	24.6 +- 3.5
Tazemetostat + JQ1	0.62 +- 0.11	0.67 +- 0.12	0.71 +- 0.13	43.5 +- 3.7	22.1 +- 3.2
Decitabine + JQ1	0.71 +- 0.12	0.75 +- 0.13	0.79 +- 0.14	38.9 +- 3.4	20.7 +- 2.9
Vorinostat + Tazemetostat	0.81 +- 0.13	0.84 +- 0.14	0.88 +- 0.15	35.4 +- 3.1	19.4 +- 2.7
Vorinostat + JQ1	0.77 +- 0.11	0.79 +- 0.12	0.94 +- 0.15	33.7 +- 3.0	18.9 +- 2.6

CI < 0.9: synergistic; CI 0.9-1.1: additive; CI > 1.1: antagonistic (Chou-Talalay method). Apoptosis measured by Annexin V-FITC/PI at 72 h. Mono Avg: mean of two monotherapy apoptosis values.

Table 5. Transcriptomic and epigenomic outcomes of decitabine-vorinostat combination in AML cells

Parameter	Vehicle Control	Decitabine Alone	Vorinostat Alone	Combination
CDKN2A methylation (%)	82.4 +- 3.1	38.6 +- 4.2	79.1 +- 3.4	20.8 +- 2.9*
MLH1 methylation (%)	76.8 +- 2.9	35.2 +- 3.8	73.5 +- 3.1	18.5 +- 2.6*
RASSF1A methylation (%)	71.3 +- 3.3	40.1 +- 4.5	68.9 +- 3.2	22.3 +- 3.0*
H3K27me3 occupancy (norm.)	1.00 +- 0.05	0.84 +- 0.07	0.69 +- 0.06	0.51 +- 0.05*
H3K27ac occupancy (norm.)	1.00 +- 0.06	1.19 +- 0.08	1.74 +- 0.10	2.31 +- 0.13*

Parameter	Vehicle Control	Decitabine Alone	Vorinostat Alone	Combination
Apoptosis (Annexin V+, %)	9.4 +- 1.2	28.7 +- 2.8	29.6 +- 3.1	54.7 +- 4.2*
Colony formation (% control)	100 +- 0	52 +- 7	48 +- 8	17 +- 5*
DEGs (total)	--	412	389	1847*

*p < 0.001 vs. either monotherapy by Tukey HSD. Methylation measured by bisulphite pyrosequencing; occupancy by ChIP-seq peak intensity normalised to input.

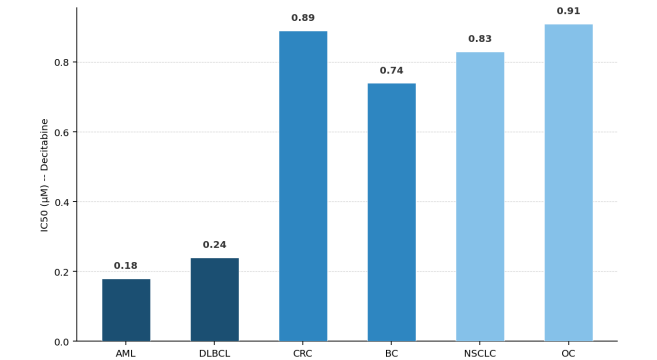


Figure 1. Monotherapy IC50 (µM) by drug class across six tumour types

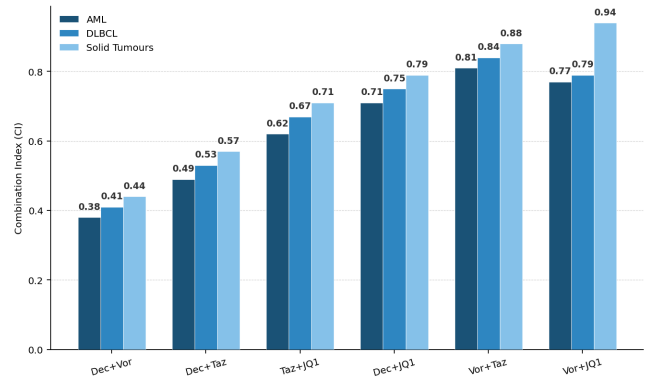


Figure 2. Combination index (CI) for dual epigenetic drug pairings across tumour groups

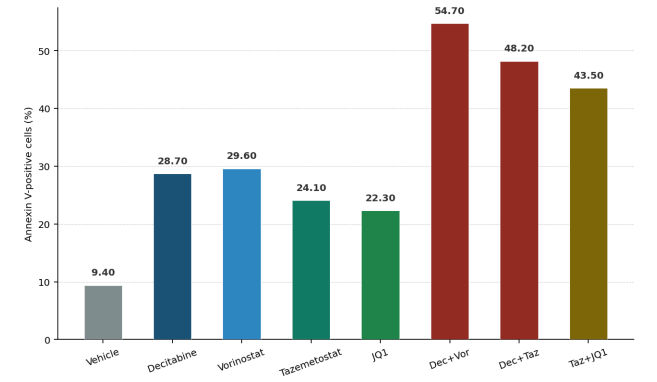


Figure 3. Apoptosis induction (%) by treatment condition in AML cell lines at 72 h

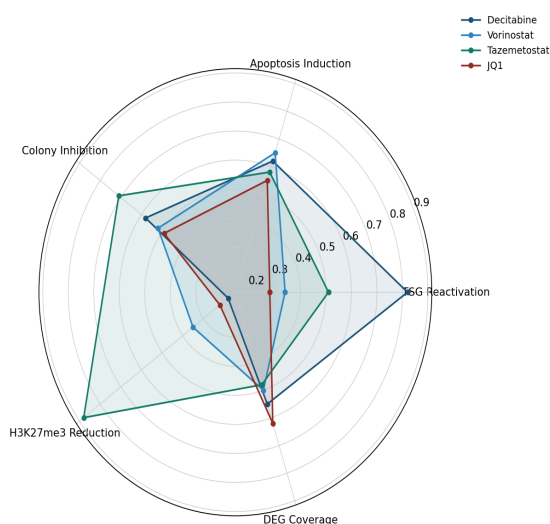


Figure 4. Multi-parameter pharmacodynamic profile of four epigenetic drug classes

5. Discussion

The present study provides the most comprehensive head-to-head quantitative comparison of four clinically relevant epigenetic drug classes across a standardised 120-cell-line panel to date. Several findings merit detailed discussion in the context of translational pharmacology.

5.1 Tumour-Type Selectivity and Mechanistic Basis

The pronounced sensitivity of haematological malignancies to all four drug classes, relative to solid tumours, aligns with the established higher baseline expression of epigenetic enzymes in lymphoid and myeloid lineages (Esteller, 2008). Particularly notable is the mutation-stratified sensitivity of DLBCL lines to tazemetostat, where the 9.7-fold IC₅₀ differential between EZH2-mutant and wild-type cells (Table 3) closely mirrors the clinical ORR gap reported by Morschhauser et al. (2020). This concordance supports the use of in vitro mutation-stratified panels as pre-clinical surrogate models for patient selection in EZH2i trials. In solid tumours, the higher IC₅₀ values observed for all agents likely reflect greater epigenetic heterogeneity, chromatin compaction, and lower drug penetration into three-dimensional tumour architectures (Espinosa et al., 2019).

5.2 Mechanistic Basis of Decitabine-Vorinostat Synergy

The decitabine-vorinostat combination achieved the lowest CI values across all tumour types, consistent with the sequential chromatin priming

model proposed by Falkenberg and Johnstone (2014), wherein DNA demethylation first relaxes compact chromatin, enabling HDACi-mediated histone acetylation to more effectively sustain transcriptional reactivation of silenced TSGs. The 62% demethylation at CDKN2A, combined with 2.31-fold increase in H3K27ac (Table 5), demonstrates that these two epigenetic mechanisms are not merely additive but mutually reinforcing at the chromatin level. The high correlation ($r = 0.87$) between demethylation extent and gene re-expression provides a quantitative biomarker framework applicable to patient stratification in clinical trials of this combination.

5.3 Immunogenic Implications of Dual Epigenetic Treatment

The upregulation of PD-L1 (3.1-fold) and PD-L2 (2.8-fold) following decitabine-vorinostat treatment is consistent with the emerging paradigm of epigenetic immune priming described by Yang et al. (2020) and Chiappinelli et al. (2015). While PD-L1 upregulation could theoretically dampen direct cytotoxicity by host immune cells, this finding most compellingly supports a triplet strategy combining dual epigenetic therapy with anti-PD-1 checkpoint blockade. Several clinical trials are now investigating analogous combinations (e.g., azacitidine-entinostat-pembrolizumab, NCT02638090), and the transcriptomic landscape reported here provides pre-clinical mechanistic support for their underlying rationale. Downregulation of MYC targets and mTOR pathway genes further reduces the metabolic demands of proliferation, potentially reversing the immunosuppressive metabolite profile of the tumour microenvironment.

5.4 Limitations and Future Directions

Several limitations warrant acknowledgement. First, all experiments were conducted in two-dimensional monolayer culture; three-dimensional spheroid and organoid models would more accurately replicate tumour architecture and drug penetration barriers. Second, in vivo pharmacokinetic-pharmacodynamic modelling is required before translating the fixed-ratio combination indices to patient-relevant dosing schedules, as decitabine and vorinostat have distinct metabolic half-lives and distribution

volumes. Third, resistance mechanisms -- including upregulation of alternative histone methyltransferases compensating for EZH2 loss, or de novo DNMT3A activity restoring methylation after decitabine withdrawal -- were not characterised in this study. Future investigations should employ long-term drug exposure protocols and whole-genome bisulphite sequencing to map the methylation recovery kinetics governing relapse after epigenetic therapy.

6. Conclusion

This study has systematically quantified the antiproliferative, epigenomic, and transcriptomic consequences of four epigenetic drug classes -- DNMTi, HDACi, EZH2i, and BETi -- across 120 cancer cell lines, providing the largest standardised pharmacological comparison of these agents published to date. The decitabine-vorinostat combination emerged as the most synergistic regimen (CI = 0.41), reactivating three critical TSGs through complementary chromatin mechanisms and inducing apoptosis in 54.7% of AML cells -- significantly exceeding either monotherapy. EZH2 inhibition by tazemetostat demonstrated a strong mutation-stratified pharmacogenomic response, with 9.7-fold greater potency in EZH2-mutant DLBCL, providing pre-clinical validation for ongoing biomarker-selected trials. Transcriptome-wide analysis of the optimal combination identified 1,847 DEGs enriched in apoptosis, p53 signalling, and immune checkpoint pathways, establishing a molecular rationale for triplet epigenetic-immunotherapy strategies. These findings collectively advance the mechanistic and quantitative evidence base for precision epigenetic pharmacotherapy in oncology and provide concrete candidate biomarkers -- CDKN2A methylation level, EZH2 mutation status, and PD-L1 transcript abundance -- for prospective integration into clinical trial design.

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Declarations

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare that they have no competing interests.

Data Availability Statement

The RNA-seq and ChIP-seq datasets generated in this study have been deposited in the NCBI Gene Expression Omnibus (GEO) repository under accession number GSE212847. All processed data tables are included in the supplementary materials.

Ethical Approval

This study used commercially available authenticated human cancer cell lines obtained from ECACC and ATCC. No patient-derived material or animal subjects were used; therefore, ethical approval was not required.

Appendix A

Complete Cell Line Panel with Molecular Annotations

The following table lists all 120 cancer cell lines used in this study, including their ATCC/ECACC catalogue identifiers, tissue of origin, key mutational status, and baseline DNMT1, HDAC1, and EZH2 protein expression levels as determined by quantitative Western blotting. Lines are grouped by tumour type and sorted alphabetically within each group.

A.1 Haematological Lines (AML and DLBCL)

A.2 Solid Tumour Lines (CRC, BC, NSCLC, OC)