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REVIEW

DNA methyltransferase inhibitors in oncology: clinical progress, limitations and future directions

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ABSTRACT

Over the past century, cancer therapy has evolved from broadly cytotoxic approaches to mechanism-based treatments. Recognition of epigenetic dysregulation as a cancer hallmark paved the way for epigenetic therapies. The earliest to gain U.S. Food and Drug Administration (FDA) approval were DNA methyltransferase inhibitors (DNMTis), such as azacitidine and decitabine, which remain cornerstone agents in epigenetic therapy. By reversing aberrant DNA hypermethylation, DNMTis restore silenced tumor suppressor pathways, induce cellular differentiation, trigger DNA-damage-driven apoptosis, and enhance tumor immunogenicity. Although DNMTi monotherapy shows limited efficacy particularly in solid tumors, DNMTis can potentiate immunotherapy, chemotherapy or targeted agents in optimized combinatorial modalities to produce anti-tumor responses and overcome therapeutic resistance. For instance, combining DNMTis with the BCL-2 inhibitor venetoclax has produced substantial clinical benefit in hematologic malignancies and is now an FDA-approved standard-of-care regimen. Emerging dual-epigenetic strategies, including DNMTi and histone deacetylase inhibitors (HDACi), further expand therapeutic potential particularly in hormone-negative cancers. A deeper mechanistic understanding of standard DNMTis, together with further refinement of next-generation DNMTis beyond pharmacokinetic improvements, is essential to achieve more durable anti-cancer responses. Future efforts should prioritize optimized dosing and combinatorial regimens, alongside biomarker-guided patient selection and more targeted epigenetic approaches to improve efficacy, especially in solid tumors.

PLAIN LANGUAGE SUMMARY

Cancer treatments have changed over time. Early therapies mainly killed fast-growing cells which often caused strong side effects. Today, many treatments are designed to target specific changes inside cancer cells. One important type of change is called "epigenetics." Epigenetics acts like a set of switches that control whether genes are turned on or off, without changing the DNA itself. Some cancers misuse these switches to turn off genes that normally protect the body. DNA methyltransferase inhibitors (DNMTis) were the first epigenetic drugs approved for cancer treatment. The best-known examples are azacitidine and decitabine. These drugs can switch important protective genes back on, help cancer cells mature into more normal cells, trigger cell death, and make tumors more visible to the immune system. DNMTis work best in blood cancers and usually have limited effects when used alone in solid tumors, such as breast or lung cancer. However, they can make other treatments work better when used together. A major success is combining DNMTis with the drug venetoclax, which is now a standard treatment for some blood cancers. DNMTis can also improve the effects of chemotherapy, immunotherapy, and other targeted treatments. Researchers are now testing new strategies that combine different epigenetic drugs, such as DNMTis with histone deacetylase inhibitors (HDACis), especially for cancers that are difficult to treat. Future progress will depend on better understanding how these drugs work, choosing safe and effective drug combinations, and identifying which patients are most likely to benefit, especially those with solid tumors.

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1. Introduction

Cancer remains a leading cause of death worldwide, with approximately 20 million new cases diagnosed each year. Despite remarkable advances in diagnosis and treatment, resistance to conventional therapies and disease recurrence continue to pose major clinical challenges. Traditional cancer treatments, particularly radiotherapy, and chemotherapy, have historically relied on mechanisms that preferentially target rapidly dividing cells, often with limited specificity for malignant tissues. While these modalities remain first-line options for many cancers, particularly aggressive or advanced malignancies, their clinical efficacy is frequently limited due to broad off-target effects, limited specificity, substantial toxicity, and the emergence of adaptive tumor resistance [1].

As understanding of the molecular pathways driving oncogenesis has deepened, targeted therapies have emerged since the early 2000s. These agents exploit specific molecular vulnerabilities within tumors and are now used as first-line treatments in several human cancers. Examples include small-molecule inhibitors of the anti-apoptotic protein B-cell lymphoma 2 (BCL-2), monoclonal

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Article highlights

- DNMTis are a cornerstone of modern epigenetic therapy, with the ability to reprogram tumor epigenomes, reactivate silenced tumor-suppressor pathways, enhance tumor immunogenicity, induce cellular differentiation and DNA damage responses.
- In hematologic malignancies, DNMTi-based combinations especially with BCL-2 inhibitors achieve superior remission and survival outcomes, redefining frontline treatment strategies in AML and MDS.
- In solid tumors, DNMTis act as "epigenetic primers" that resensitize resistant cancers to immunotherapy, targeted agents, chemotherapy, radiotherapy and endocrine therapy by reprogramming the epigenetic landscape, activating viral-mimicry – driven immune responses, and disrupting DNA-repair pathways.
- Next-generation DNMTis, including oral formulations and prodrugs with improved pharmacokinetics, expand treatment accessibility and hold promise for enhancing solid tumor efficacy through better systemic exposure and tumor penetration.
- DNMTis remain central to the future of epigenetic oncology, with emerging opportunities in biomarker-guided precision therapy, rational combination strategies, and dual-epigenetic targeting aimed at overcoming resistance and achieving durable clinical responses across tumor types.

antibodies directed against tumor associated receptors or ligands, cancer growth blockers, angiogenesis inhibitors, and poly (ADP-ribose) polymerase (PARP) inhibitors that target defects in DNA damage repair pathways [2].

Immunotherapies have also transformed cancer treatment by harnessing the immune system to detect and eliminate cancerous cells. Immune checkpoint inhibitors, cytokine-based therapies, chimeric antigen receptor (CAR)-T-cell therapies, and cancer vaccines are examples of such treatments [3,4]. Although both targeted and immune-based therapies have greatly improved clinical outcomes, their efficacy in many solid tumors remains limited; the ongoing adaptability of cancer cells continues to generate therapeutic resistance; and treatment-associated toxicities remain a barrier [4]. Notably, many cancers are immunologically "cold" and do not respond well to immune monotherapies, highlighting the critical need for innovative and synergistic treatment strategies.

Combination therapy has emerged as the mainstay of contemporary oncology over recent decades. By integrating multiple treatment modalities, such as chemo-immunotherapy regimens, dual targeted therapies, or surgery combined with chemotherapy and/or radiotherapy, clinicians can target cancer cells through complementary and, in some cases, convergent mechanisms [5]. This strategy enables the simultaneous modulation of multiple oncogenic pathways, thereby enhancing antitumor efficacy, promoting synergistic effects and reducing the likelihood of drug resistance.

One of the major challenges in cancer treatment today is identifying the effective combination partners capable of targeting the diverse cancer-driving genes, including oncogenes and tumor suppressor genes (TSGs), as well as the signaling pathways they control, particularly those that are considered "undruggable." The complex network of transcriptional dysregulation and structural heterogeneity characteristic of cancer makes it difficult to directly target many of the molecular alterations that drive cancer progression using conventional small-molecule inhibitors, reactivators and other biologics. For example, TSGs such as *TP53* and *PTEN* which are inactivated by genetic mutations, cannot be readily restored and often lack obvious druggable features. The RAS gene family (including *KRAS*, *NRAS*, and *HRAS*) comprises some of the most frequently mutated driver oncogenes in human cancers, with *KRAS* being the most altered oncogene in solid tumors [6,7]. Although targeting RAS proteins with conventional therapies was long considered challenging due to their structural complexities, advances in structure-guided drug design have overturned this view, as demonstrated by the successful development of allele-specific inhibitors targeting $KRAS^{G12C}$ notably, sotorasib and adagrasib [8,9]. Nevertheless, therapeutic efficacy remains limited by resistance mechanisms, tumor context and the continued difficulty of directly targeting most non-G12C RAS mutants.

Epigenetic therapies have emerged to improve approaches for targeting cancer by modulating gene expression and chromatin architecture without changing the DNA sequence itself. Unlike genetic mutations, epigenetic alterations such as DNA methylation and histone modifications are reversible, offering indirect strategies to overcome "undruggability," and reveal new cancer vulnerabilities. Drugs targeting these mechanisms are often referred to as "epidrugs" or "epigenetic therapies." Epidrugs have the potential to overcome resistance mechanisms and expand the therapeutic target by reprogramming malignant cells toward less aggressive phenotypes or sensitizing them to standard treatments [10]. The clinical validation of epigenetic therapy began with the FDA approval of DNA methyltransferase inhibitors (DNMTis) [11], followed by histone deacetylase inhibitors (HDACis) [12] for hematologic malignancies, demonstrating that epidrugs could achieve meaningful clinical efficacy. These early successes paved the way for the development of the next generation epidrugs targeting diverse epigenetic modifiers, including enhancer of zeste homolog 2 (EZH2) histone methyltransferase, lysine-specific histone demethylase 1 (LSD1), bromodomain and extra-terminal (BET) proteins, and protein arginine methyltransferase (PRMT), most of which have now entered clinical trials [10,13].

DNMTis are among the most prominent and actively investigated epidrugs in current clinical development, evaluated both as monotherapies and in combination regimens against hematologic malignancies and increasingly in solid tumors. The nucleoside analog DNMTis, primarily azacitidine (AZA) and decitabine (DAC), have been widely used in the treatment of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) since their FDA approvals in 2004 and 2006 respectively [11,14]. DNMTis have emerged at the forefront of epigenetic therapy due to their proven ability to reverse aberrant DNA methylation patterns, which cancers exploit to silence TSGs. Their integration with other epidrugs and other therapeutic modalities represents a paradigm shift toward precision oncology. DNMTis are widely explored as sensitizing or priming agents in combination regimens with targeted therapies, immunotherapies, and chemotherapies, to mitigate resistance and, in some settings, improve clinical outcomes [15,16].

Despite their promise, the clinical application of DNMTis is not without challenges. Their effectiveness in solid tumors remains limited, and their use is often accompanied by

significant adverse effects, particularly for first-generation nucleoside DNMTis which incorporate into both DNA and RNA, and induce nonspecific global hypomethylation, leading to dose-dependent and context-dependent gene reactivation [17]. The development of next-generation DNMTis together with rational combination regimens aims to overcome these limitations by offering improved target selectivity, reducing off-target cytotoxicity and enhancing therapeutic synergy, thereby maximizing antitumor efficacy and establishing epigenetic therapy as a major focus of modern anticancer drug development [18,19].

In this review, we summarize the evolving clinical landscape of DNMTis, including new formulations, mechanism of action, evidence for scheduling and combination strategies with other therapeutic modalities, key limitations to efficacy particularly in solid tumors, and future directions including targeted epigenetic modulation and biomarker driven personalized medicine.

2. Epigenetic rewiring in cancer

Cancer arises as a complex disease driven by the gradual accumulation of genetic mutations and extensive dysregulation of gene expression [20]. Among these regulatory disturbances, non-mutational epigenetic reprogramming has been recognized as a central hallmark of cancer, promoting tumor initiation, progression, metastasis, and resistance to therapy [21,22]. Epigenetics broadly encompasses heritable changes to gene expression that occur without alterations to the underlying DNA sequence. The core epigenetic mechanisms include DNA methylation, histone modifications, chromatin remodeling, and regulatory non-coding RNA-mediated gene silencing [13]. Other commonly discussed additions include RNA modifications known as epitranscriptomic changes and three-dimensional (3D) genome organization [13,23].

Epigenetic changes are catalyzed or mediated by enzymes and complexes broadly classified as writers, readers, erasers, non-coding RNAs, remodelers, and structural epigenetic regulators (Supplementary Table S1) [13,20,24]. Writers introduce modifications to DNA, RNA, or histone proteins; notable examples include DNA methyltransferases (DNMTs), RNA N^6-methyladenosine (m^6 A) methyltransferase complex (METTL3/14), histone acetyltransferases (HATs), and histone methyltransferases (HMTs). Readers recognize, bind, and interpret these epigenetic marks without modifying them directly, thereby recruiting other effector proteins and translating marks into functional outcomes. Erasers remove epigenetic modifications, reversing the actions of writers, for instance, ten-eleven translocation (TET) enzymes demethylate DNA marks placed by DNMTs, histone deacetylases (HDACs) counteract HATs, and histone demethylases (HDMs) remove methylation deposited by HMTs [24] (Figure 1).

A wide array of non-coding RNAs, including long non-coding RNAs (lncRNAs), microRNAs (miRNAs), circular RNAs (circRNAs), small interfering RNAs (siRNAs), and PIWI interacting RNAs (piRNAs) also function as versatile epigenetic regulators. These molecules guide chromatin modifiers, recruit protein complexes, and influence transcriptional and post-transcriptional processes, underscoring their integral role in the epigenetic control of gene expression [23,29]. Although these ncRNAs act

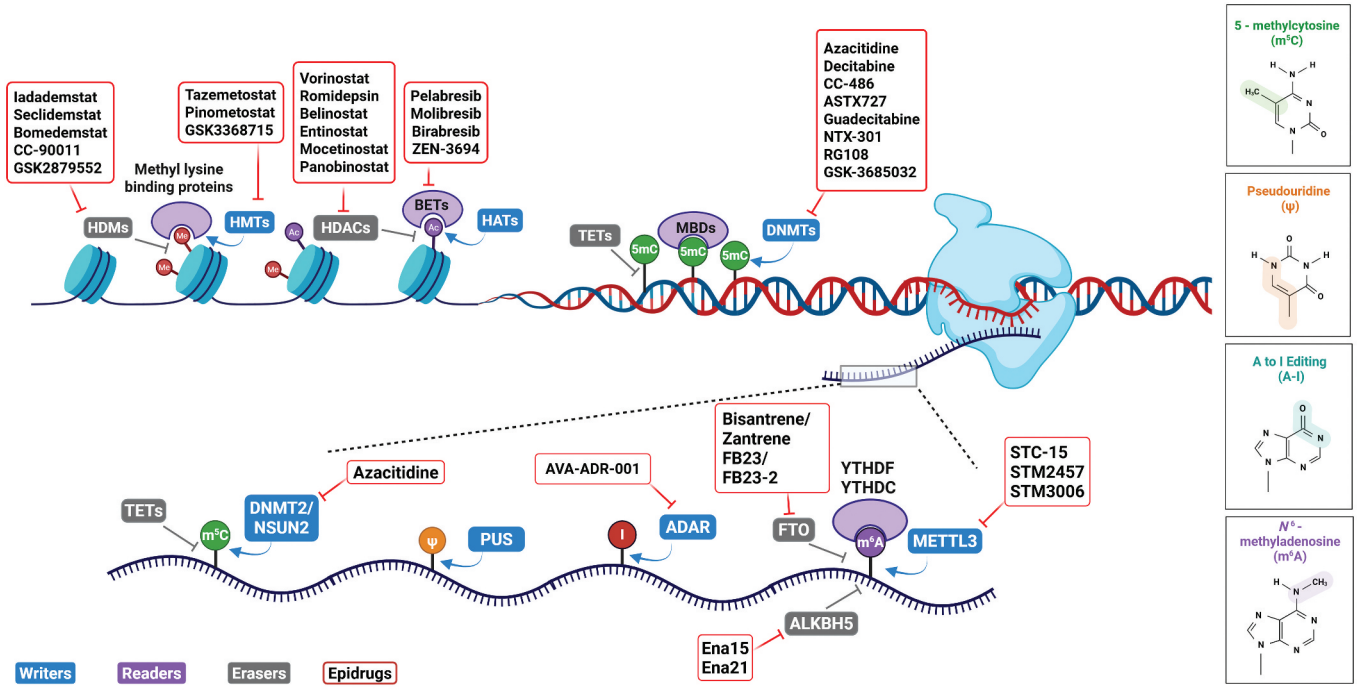

Figure 1. Epigenetic and epitranscriptomic modifiers and their corresponding epidrugs.

Schematic overview of key epigenetic and epitranscriptomic modifiers; writers, readers, and erasers, acting on DNA, histones, and RNA in cancer. The figure highlights major therapeutic targets and recently developed epidrugs, including DNA methyltransferase (DNMT) inhibitors, histone deacetylase (HDAC) inhibitors, histone demethylase (HDM) inhibitors (notably lysine-specific demethylase 1 (LSD1) inhibitors), histone methyltransferase (HMT) inhibitors, the ADAR1p150 inhibitor AVA-ADR-001 [25], emerging m^6A RNA methylation inhibitors including (STC-15, STM2457 and STM3006) [26], as well as ALKBH5 inhibitors [27] and FTO inhibitors [28]. Together, these agents underscore the expanding landscape of epigenetic and epitranscriptomic therapies in oncology. *Created in BioRender. Mehdipour, P. (2026) https://BioRender.com/zeprki6.*

through diverse mechanisms, they do not directly modify nucleic acids or histones; rather, they serve as guides, scaffolds, and regulators that orchestrate DNA methylation, histone modifications, chromatin structure [30].

Remodelers comprise ATP-dependent chromatin-modifying complexes, such as switch/sucrose non-fermentable (SWI/SNF), imitation switch (ISWI), and chromodomain-helicase DNA-binding (CHD) that reposition, eject, or restructure nucleosomes to regulate chromatin accessibility [31]. Structural epigenetic regulators in contrast, influence higher order chromatin or genome architecture through persistent, non-enzymatic or scaffolding functions distinct from catalytic modification of histones or DNA [32]. These regulators modulate genome topology and long-range interactions without directly writing, erasing, or reading epigenetic marks. Examples include histone variants, oncohistones, architectural proteins such as CTCF (CCCTC-binding factor), cohesin and condensin complexes and nuclear lamina components and periphery organizers such as lamins and nucleoporins [33–37].

In parallel, epitranscriptomic modifications, modulate gene expression through chemical modifications at the RNA level rather than through DNA or histone marks. Examples of these chemical modifications include m^6 A, 5-methylcytidine (m^5 C), pseudouridine (Ψ), and adenosine-to-inosine (A-to-I) RNA editing catalyzed by ADAR enzymes [38]. These modifications influence RNA splicing, stability, localization, translation, and innate immune recognition, thereby shaping gene expression programs without changing the underlying DNA sequence [38]. Although epitranscriptomic changes are conceptually distinct from classical epigenetics, they are discussed here briefly because of their emerging role in post-transcriptional gene regulation and cancer biology (Figure 1).

These distinct modifications provide a framework for understanding how diverse protein and RNA families coordinate to establish, maintain, and dynamically remodel epigenetic states in both physiological and oncogenic contexts. While multiple epigenetic mechanisms contribute to cancer pathogenesis, the primary focus of this review is DNA methylation and its therapeutic targeting through DNMTis. Other epigenetic and epitranscriptomic alterations have been briefly compiled in Supplementary Table S1. This section therefore provides insight into the role of DNA methylation in cancers and the mechanistic rationale for DNMT inhibition.

2.1. DNA methylation

DNA methylation is one of the most extensively studied epigenetic marks in cancer [39] and plays critical roles in regulating gene expression, maintaining genomic integrity, directing cell fate during development, maintaining cell identity, and controlling cellular differentiation [39–45]. DNA methylation involves the covalent addition of a methyl group to the 5′ carbon position of cytosine, typically within cytosine-phosphate-guanine (CpG) dinucleotides, forming 5-methylcytosine (5mC). When present in gene regulatory regions such as promoters, 5mC establishes transcriptionally repressive chromatin states, whereas 5mC within transcribed regions of genes (gene bodies) is generally associated with active gene expression [46–48].

Methylation at promoter CpG islands silences genes by blocking transcription factor binding either directly or through the recruitment of methyl-CpG – binding domain (MBD) proteins [49,50]. These MBD proteins, in turn, recruit repressive complexes that reinforce transcriptional inhibition and maintain silenced chromatin [51]. The enzymes responsible for catalyzing the formation of 5mC are collectively known as DNA methyltransferases (DNMTs), while ten-eleven translocation (TET) enzymes (TET1/2/3) catalyze the removal of 5mC, thereby facilitating active DNA demethylation [52,53]. In cancer, both aberrant hypermethylation and hypomethylation are observed, disrupting normal gene regulation and contributing to tumorigenesis [39].

Under normal physiological conditions, most CpG sites in the mammalian genome are methylated, particularly those in repetitive elements, gene bodies, and intergenic regions, whereas CpG islands – which are regions of high CpG density typically found in gene promoters, remain largely unmethylated in normal somatic tissues which are permissive for transcriptional activation of associated genes; however, a subset of CpG islands show tissue-specific or context-dependent methylation [54,55]. In cancer cells, however, a distinctive duality in the methylation landscape is frequently observed: a global decrease in methylation (global hypomethylation), especially at repetitive DNA sequences known as transposable elements (TEs), accompanied by gene-specific hypermethylation at CpG islands within promoters of key genes, particularly TSGs [20,21,56–58]. Large-scale cancer genome consortia have confirmed that virtually all cancers harbor widespread methylation abnormalities, and several frequently mutated genes in cancer are directly involved in methylation regulation [39,59]. These alterations are not random, they affect genes controlling cell cycle progression, DNA repair, apoptosis, and immune evasion, collectively promoting oncogenic transformation and tumor progression.

While aberrant DNA methylation is a universal hallmark of cancer, the specific methylation patterns vary across cancer types. Methylation-mediated silencing of genes involved in DNA damage repair, cell cycle regulation, and apoptosis facilitates the accumulation of genetic mutations, thereby promoting tumor initiation and progression [60]. For example, hypermethylation of TSGs leads to transcriptional silencing of critical regulators, including *BRCA1* in breast and ovarian cancer [61], *MLH1/MSH2* in colorectal cancer [62], *VHL* in clear cell renal carcinoma [63], *MGMT* in glioblastoma [64], and *CDKN2A*/$p16^{INK4A}$ in various other malignancies [21,65,66]. In colorectal cancer and certain gliomas, a distinct CpG Island Methylator Phenotype (CIMP) is observed, characterized by coordinated hypermethylation of hundreds of promoter CpG islands, defining a unique molecular subtype of tumors [67,68]. In cervical cancer, HOTAIR-driven *PTEN* promoter hypermethylation represses *PTEN* and sustains PI3K/AKT and Wnt/β-catenin signaling, supporting an oncogenic phenotype [69]. DNA hypermethylation also contributes to the epithelial-to-mesenchymal transition (EMT), a key process in metastasis, by repressing epithelial markers such as E-cadherin and modulating microRNA expression in human carcinomas [70,71]. Hematologic malignancies such as MDS and AML

habor mutation of genes regulating cellular differentiation, signaling pathways and methylation-related genes including mutations in *DNMT3A*, *TET2* and *IDH1/2* thereby perturbing methylation homeostasis [59,72–74]. Collectively, these findings underscore the therapeutic potential of targeting aberrant DNA methylation in cancer.

Conversely, global DNA hypomethylation predominantly affects repetitive DNA sequences including TEs such as long interspersed nuclear elements (LINEs) and long terminal repeats (LTRs) [56]. Hypomethylation of TEs contributes to genomic instability by increasing susceptibility to aberrant recombination and chromosomal instability, including rearrangements and aneuploidy [75,76]. In addition, hypomethylation-mediated reactivation of TEs leads to the accumulation of TE-derived double-stranded RNAs (dsRNAs) from normally silenced genomic regions. These dsRNAs activate antiviral defense pathways, inducing type I and type III interferon (IFN) responses within cancer cells, a phenomenon referred to as viral mimicry [77]. Furthermore, hypomethylation can activate normally silent oncogenes such as *MYC* [78] and the *RAS* gene family (*HRAS, KRAS*) [79], as well as cancer-testis antigens (CTAs), contributing to cellular dedifferentiation and tumor aggressiveness [80].

Taken together, the dual phenomena of promoter hypermethylation and global hypomethylation play complementary roles in driving cancer development by silencing tumor-suppressive functions and creating an environment conducive to genomic instability [21,41].

2.1.1. Classification of DNA methyltransferase enzymes

DNA methylation is mediated by DNMTs, which transfer a methyl group from S-adenosyl-L-methionine (SAM or AdoMet) to the 5′-position of cytosine residues in DNA. The DNMT family comprises DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L, which differ in their structural features and biological functions [81,82] (Figure 2). The canonical DNA methyltransferases, DNMT1, DNMT3A, and DNMT3B are responsible for establishing and maintaining cytosine-5 methylation in the genome. In contrast, DNMT2 and DNMT3L are considered non-canonical members: DNMT2 primarily methylates RNA rather than DNA, whereas DNMT3L lacks catalytic activity but serves as a regulatory cofactor that stimulates the activity of DNMT3A and DNMT3B [43,82–84].

2.1.1.1. DNMT1. DNMT1 is the most abundant DNA methyltransferase in somatic cells and functions primarily as a maintenance methyltransferase, reestablishing cell-specific methylation patterns during cell divisions. During S phase, DNMT1 recognizes hemimethylated CpG sites on the newly synthesized strand and restores the full methylation pattern by copying the parental methylation marks onto the daughter strand, thereby faithfully preserving the cell's epigenetic state [85–88]. The catalytic mechanism of DNMT1 involves base flipping of the target

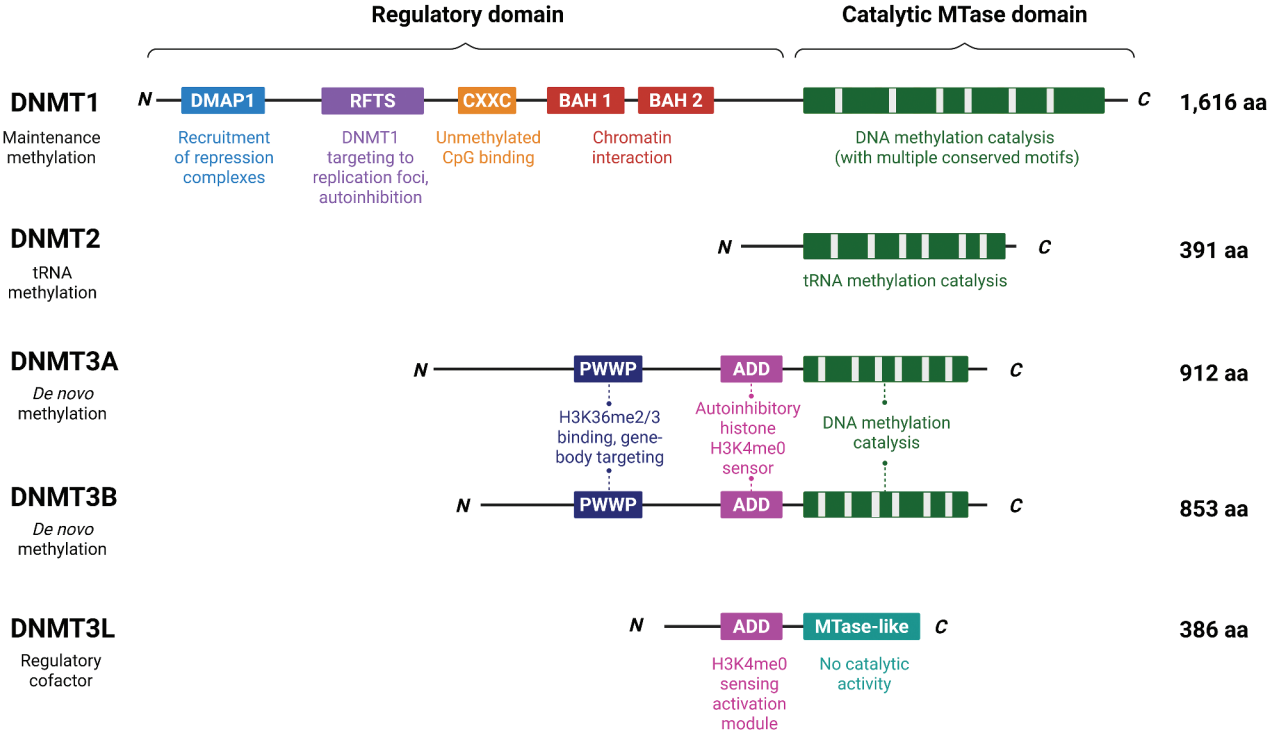

Figure 2. Structural domains of human DNMT Enzymes.

Each DNMT family member is depicted with its characteristic regulatory and catalytic domains. Domain abbreviations include DNA methyltransferase 1-associated protein 1 (DMAP1), replication foci targeting sequence (RFTS), CXXC zinc finger domain (CXXC), bromo-adjacent homology domains (BAH1 and BAH2) domains, Pro-Trp-Pro-Trp (PWWP) domain, ATRXDNMT3-DNMT3L (ADD) zinc finger domain, and the conserved C-terminal DNA methyltransferase catalytic (MTase) domain. DNMT3L lacks intrinsic catalytic activity but is included for its regulatory ADD domain, which modulates DNMT3A and DNMT3B function. The domain organization highlights the functional specialization of individual DNMTs in DNA methylation maintenance, *de novo* methylation, and epigenetic regulation. Created in BioRender. Mehdipour, P. (2026) https://BioRender.com/iatd45m.

cytosine out of the DNA helix and positioned within the catalytic pocket, where the active-site cysteine forms a transient covalent enzyme – substrate, enabling transfer of the methyl group from SAM to the C5 position of cytosine [85,86].

Structurally, DNMT1 contains an N-terminal regulatory region comprising the DNA methyltransferase 1-associated protein 1 (DMAP1)-binding domain, a replication foci targeting sequence (RFTS) domain, which facilitates DNMT1 recruitment to replication foci through interactions with *UHRF1* and proliferating cell nuclear antigen (PCNA), a CXXC zinc finger domain that binds unmethylated CpG sites, and two bromo-adjacent homology (BAH) domains that contribute to chromatin association and protein-protein interactions (Figure 2) [85,89]. The C-terminal catalytic methyltransferase (MTase) domain binds the cofactor SAM and the target cytosine. In its unbound state, the RFTS domain interacts directly with the MTase domain, serving as an autoinhibitory module that regulates substrate access [85]. Conversely, when DNMT1 binds to unmethylated DNA, the CXXC – BAH1 linker suppresses enzymatic activity to prevent *de novo* methylation [85]. These structural features collectively coordinate DNMT1's recruitment and activity during DNA replication and methylation maintenance.

DNMT1, along with DNMT3A and DNMT3B, is frequently overexpressed in various cancers, where elevated expression correlates with poor prognosis and EMT signatures [90]. Moreover, DNMT1-driven methylation cooperates with polycomb group proteins particularly the histone methyltransferase EZH2 to enforce stable epigenetic silencing of differentiation-associated genes, thereby promoting tumor progression and maintenance of an undifferentiated, proliferative cancer cell phenotype [44,91,92].

2.1.1.2. DNMT3 family. DNMT3 has three members: DNMT3A, DNMT3B, and DNMT3L (Figure 2). The catalytically active isoforms DNMT3A and DNMT3B are primarily responsible for establishing new DNA methylation patterns on previously unmethylated DNA during embryonic development and cellular differentiation, a process known as *de novo* DNA methylation [90,93]. Early in development, DNMT3A and DNMT3B establish tissue-specific methylation marks, while DNMT3A in cooperation with DNMT3L is essential for genomic imprinting; these marks are subsequently maintained by DNMT1 [93,94]. These enzymes are highly expressed in germ cells, embryonic tissues, and undifferentiated embryonic stem cells (including embryonal carcinoma cells), but are downregulated in differentiated adult somatic cells [94,95]. Functionally, DNMT3A tends to methylate specific gene regions later in development (and even postnatally), whereas DNMT3B acts earlier, targeting broader repetitive sequences in the early embryo [95].

Unlike the catalytically active DNMT1, DNMT3A and DNMT3B, DNMT3L lacks intrinsic methyltransferase activity but functions as an essential regulatory cofactor that interacts with DNMT3A and DNMT3B to enhance their *de novo* methylation activity, particularly during gametogenesis and early embryogenesis [96,97]. DNMT3A and DNMT3B have a similar protein structure with three key conserved domains: an N-terminal proline – tryptophan – tryptophan – proline (PWWP) domain, an ATRX – DNMT3 – DNMT3L (ADD) zinc finger domain, and a C-terminal MTase catalytic domain [97,98] (Figure 2). The PWWP domain binds to both DNA and histone tails, particularly H3K36me2/3, directing DNMT3A and DNMT3B to specific chromatin regions such as gene bodies and heterochromatin [99–101]. The ADD domain in DNMT3A functions as an autoinhibitory histone sensor, switching the enzyme on or off in response to H3K4 methylation status [102]. In DNMT3A, the ADD domain forms an autoinhibitory interface with the catalytic MTase domain, binding to the unmethylated H3K4 tail releases this autoinhibition and activates the enzyme [102]. In contrast, the ADD domain of DNMT3B does not establish a comparable DNMT3A-like autoinhibitory interaction and exhibits reduced dependence on H3K4 methylation status, indicating a distinct mode of catalytic regulation [103]. The MTase domains of both DNMT3A and DNMT3B catalyze the transfer of a methyl group to C5 position of cytosine predominantly within CpG dinucleotides, enabling *de novo* DNA methylation [97,98].

Unlike DNMT1, DNMT3A is frequently mutated in cancers. DNMT3A is one of the most commonly mutated genes in AML, occurring in approximately 20–25% of adult AML cases, often involving a hotspot mutation R882 that impairs its enzymatic function [104]. DNMT3A mutations are early events in clonal hematopoiesis and leukemogenesis, suggesting they confer a pre-leukemic state [104,105]. These mutations generally lead to a loss-of-function or dominant-negative effect, resulting in a global loss of DNA methylation fidelity [104–106]. Beyond blood cancers, DNMT3A abnormalities have been noted in other malignancies; for instance, DNMT3A heterozygous loss or mutation has been implicated in some lymphomas and solid tumors, and decreased DNMT3A expression is observed in a subset of cancers [90]. However, overexpression of DNMT3A is also seen in certain tumors (such as breast cancer), where it can drive hypermethylation of gene promoters such as ADAMTS8 and promote invasion and tumor progression [107–109]. Overall, DNMT3A can act as an oncogene or tumor suppressor depending on context; mutations usually impair normal methylation programming (contributing to malignant transformation in hematopoietic cells), whereas overexpression can contribute to aberrant methylation of TSGs in solid tumors.

DNMT3B is essential for early development (Dnmt3b KO mice are embryonic lethal) and plays a distinct role in methylating centromeric repeats, linking its dysfunction to human ICF syndrome (immunodeficiency, centromeric instability, facial anomalies) [95]. Functionally, DNMT3B may cooperate with DNMT1 in cancer cells to maintain aberrant methylation. Even though DNMT3B is a *de novo* enzyme, cancer cells sometimes repurpose it to reinforce methylation at promoters of genes that were not methylated in the cell of origin [52]. Like DNMT1 and DNMT3A, higher DNMT3B levels generally result in worse prognosis [110].

DNMT3L is a catalytically inactive regulatory protein that lacks the conserved catalytic motifs required for methyl transfer, instead, it partners with DNMT3A (and to some extent DNMT3B) to stimulate their activity. DNMT3L is crucial in the germline; it is predominantly expressed in embryonic germ cells (but expressed at very low levels or absent in somatic

tissues), and it is required for establishing maternal imprints and methylating transposons during spermatogenesis [90]. DNMT3L helps DNMT3A and DNMT3B recognize unmethylated histone H3K4 as a cue for DNA methylation and facilitates their chromatin targeting and complex formation. In most cancers, DNMT3L expression is downregulated or absent (likely reflecting its germline-restricted function) [90]. Another *de novo* DNA methyltransferase, DNMT3C, has been identified in mice, and it specializes in methylating the promoters of young retrotransposons in the male germline, thereby contributing to transposon repression and male fertility [111].

2.1.1.3. DNMT2. DNMT2 (also known as TRDMT1) is not a bona fide DNA methyltransferase, although it was initially misclassified as one. It primarily functions as an RNA methyltransferase, specifically introducing 5mC at cytosine 38 (C38) in the anticodon loop of tRNAs [112]. Consequently, DNMT2 has little impact on genomic DNA methylation, and its biological role is distinct from that of other DNMT family members. However, DNMT2 utilizes SAM as the methyl donor and retains the SAM-dependent catalytic mechanism characteristic of the DNMT family [113].

Although the precise role of DNMT2 in tumor biology remains unclear, recent evidence associates high DNMT2 expression with poor prognosis in liver cancer [114]. Deletion of DNMT2 increases expression of the pro-apoptotic gene ligand TNFSF10 (TRAIL), through upregulation of the m^6A demethylase FTO and reduced global m^6A levels, and is associated with decreased tumor cell proliferation and metastasis [114]. Moreover, DNMT2 loss markedly increases expression of the chemokines CXCL9, CXCL10, and CXCL11, enhancing immune cell recruitment and promoting a more immunogenic "hot" tumor microenvironment (TME) [114]. These findings highlight DNMT2 as a potential therapeutic target, and inhibitors of DNMT2 are currently under preclinical investigation as anticancer agents [115].

2.2. DNA demethylation

2.2.1. TET enzymes

The TET family of dioxygenases catalyzes the sequential oxidation of 5mC, a key step in the active DNA demethylation pathway. DNA demethylation can occur through either a passive or active mechanism.

Passive DNA demethylation refers to the replication-dependent depletion of 5mC marks over successive cell divisions [53]. This process occurs when DNMT1, the maintenance DNA methyltransferase, fails to remethylate hemimethylated CpG sites during DNA replication, leading to a gradual loss of methylation across daughter strands. Passive demethylation is therefore replication-coupled and enzyme-independent process, prominent in cells with reduced DNMT1 expression or activity [53,116].

In contrast, active DNA demethylation is an enzyme-mediated process that removes 5mC independently of DNA replication. This pathway is catalyzed by the TET family enzymes: TET1, TET2, and TET3, which oxidize 5mC to 5-hydroxymethylcytosine (5hmC), then to 5-formylcytosine (5fC), and finally to 5-carboxylcytosine (5caC) [116–118]. These oxidized bases are subsequently recognized and excised by thymine DNA glycosylase (TDG), after which base excision repair replaces them with unmodified cytosine, thereby completing the demethylation cycle [53,116].

TET enzymes primarily act on DNA methylated by canonical DNMTs and have also been reported, in certain contexts, to oxidize RNA 5mC deposited by DNMT2, suggesting potential substrate versatility [119,120]. The entire TET-mediated reaction sequence requires α-ketoglutarate (α-KG) as an essential cofactor. Under normal conditions, isocitrate dehydrogenase 1 and 2 (IDH1/2) enzymes generate α-KG; however, neomorphic mutations in IDH1 or IDH2 lead to the aberrant production of the oncometabolite D-2-hydroxyglutarate (2-HG) [121]. 2-HG acts as a competitive inhibitor of α-KG – dependent dioxygenases, including TET enzymes, thereby blocking the generation of 5hmC and subsequent oxidation products. This inhibition results in hypermethylated, undifferentiated cellular phenotype, contributing to epigenetic silencing of TSGs and facilitating malignant transformation, as observed in IDH-mutant AML and other cancers [72].

In recent years, 5hmC has been recognized not only as an intermediate in the active DNA demethylation pathway but also as a stable and functional epigenetic modification with distinct regulatory roles [122]. 5mC and 5hmC often co-occur in a disproportionate manner at the same genomic loci, either on the same cytosine residues or on complementary DNA strands, creating a dynamic and tunable epigenetic landscape [123,124]. While 5mC generally represses gene expression and decreases chromatin accessibility to transcriptional regulators, 5hmC can facilitate the gene reactivation or enable context-dependent transcriptional regulation in response to developmental or environmental cues [124,125].

In many cancers, global loss of 5hmC occurs due to downregulation or mutation of TET enzymes, and this reduction does not necessarily correlate with global DNA hypomethylation [126–128]. Changes in 5hmC and 5mC profiles may serve as promising biomarkers for early cancer detection, including through their detection in circulating cell-free DNA [129]. 5hmC supports normal gene regulation by limiting excessive DNA methylation. Its loss is frequently observed across malignancies, and this disrupts the protective epigenetic balance, promoting aberrant gene silencing, uncontrolled proliferation and tumorigenesis [130].

Overall, abnormal DNA methylation is central to cancer biology, which is why therapies targeting epigenetic changes are being actively developed and tested.

3. Overview of clinically approved epigenetic therapies

Reversible epigenetic alterations cooperate with genetic mutations to drive cancer progression, positioning epigenetic therapies that reprogram the cancer epigenome, as promising therapeutic tools [131].

Epidrugs can be classified as broad or narrow reprogrammers depending on their molecular targets. Broad reprogrammers, such as DNMTis and HDACis (classes I – IV), induce widespread changes in chromatin structure and gene expression across the genome. In contrast, narrow or targeted

reprogrammers selectively modulate genes within specific epigenetic regulatory pathways, including inhibitors of isocitrate dehydrogenase 1/2 (IDH1/2), LSD1, EZH2, PRMTs, disruptor of telomeric silencing 1-like (DOT1L) histone lysine methyltransferase, and BET proteins [132,133].

DNMTis, notably AZA and DAC, were the first and remain the most extensively used epidrugs in clinical oncology. They have longstanding approval and broad adoption in hematologic malignancies, including AML and MDS [11,134]. DNMTis remain the backbone of many combination therapies, including those with BCL-2 inhibitors (e.g., venetoclax), IDH inhibitors (e.g., ivosidenib, enasidenib), and immune checkpoint inhibitors [17]. Alongside HDACis, DNMTis are the most widely employed epidrugs in combination regimens for hematologic malignancies, with their use increasingly extending to solid tumors [133]. Their proven clinical efficacy is reflected in the comparatively high number of regulatory approvals granted by both the European Medicines Agency (EMA) and the FDA. Notably, FDA-approved HDACis, such as romidepsin [135–137] and vorinostat [12] demonstrate clinically meaningful monotherapy activity primarily in cutaneous and peripheral T-cell lymphomas, whereas first-generation DNMTis, including AZA and DAC, have established frontline efficacy in higher-risk MDS and AML, underscoring their complementary roles across distinct hematologic malignancies. Several epidrugs are currently under preclinical and clinical investigation, however, all the FDA and EMA approved epidrugs for cancer treatment to date are compiled in Table 1 to briefly highlight their epigenetic mechanism of action and their expanding potential in targeting the cancer epigenome. Further details on DNMT enzymes, their inhibitors, mechanisms of antitumor activity, and clinical applications are discussed in the subsequent sections.

4. Emerging strategies in DNMT-directed cancer therapy

Targeting DNMTs represents an important therapeutic approach for cancer treatment, as these enzymes are key mediators of aberrant DNA methylation patterns that drive tumorigenesis. Because DNA methylation is reversible, DNMTis can reprogram the cancer epigenome and potentiate the efficacy of other therapies, thereby enhancing tumor sensitivity and overcoming treatment – related resistance [88].

4.1. Classes and generations of DNMTis

DNMTis are commonly classified according to their chemical class and generation. Two broad chemical classes are recognized: nucleoside analogs (nDNMTis), which incorporate into nucleic acids and covalently trap DNMTs, and non-nucleoside DNMTis (nnDNMTis), which reversibly inhibit DNMT enzymatic activity without DNA incorporation (Figure 3). Several nDNMTis have received FDA approvals including: (i) azacitidine (AZA/vidaza/5-azaC), administered intravenously or subcutaneously [11], (ii) oral azacitidine tablets (onureg/CC-486) [140,141], (iii) decitabine (DAC/dacogen/5-aza-dC), administered intravenously [134,156], and (iv) oral combination of decitabine and cedazuridine (inqovi/ASTX727) [143]. Several other nDNMTis including guadecitabine [157–160] and zebularine [161,162] remain under active development, and together with nnDNMTis are broadly classified as next-generation DNMTis (Table 2). In addition, DNMTis are commonly stratified into three generations according to their chemical structures, mechanism of action and pharmacokinetic properties.

4.1.1. First-generation DNMTis (nucleoside analogs)

First-generation agents comprise the FDA-approved cytidine analogs azacitidine and decitabine. AZA is a ribonucleoside analog that incorporates predominantly into RNA (~80–90%) and to a lesser extent into DNA (~10%), affecting RNA metabolism as well as DNMT function; DAC is a deoxyribonucleoside analog that incorporates exclusively into DNA and is a more direct DNMT1 inhibitor [198,199]. Both AZA and DAC are prodrugs that undergo phosphorylation to active triphosphates (5-aza-CTP and 5-aza-dCTP, respectively) and when incorporated into nascent DNA during S-phase, act as "suicide" substrates that covalently trap DNMT1, leading to passive DNA demethylation upon replication and eventual enzyme degradation (Figure 3).

Some of the limitations of first-generation agents are toxicity at high doses, drug related resistance and short half-life due to cytidine deaminase (CDA) activity. One of the kinases involved in the activatory phosphorylation cascade for DAC is deoxycytidine kinase (DCK) and some patients produce DCK at reduced levels, making them unable to respond to DAC treatment over time despite continued drug administration. This DCK-associated DAC resistance can be overcome by switching to AZA, which does not depend on DCK for activation [200,201].

CDA can rapidly metabolize both AZA and DAC, leading to low oral bioavailability, and it is overexpressed in many cancers where it has been associated with resistance to DNMTis [200]. Short plasma half-lives due to CDA-mediated degradation motivated the development of oral formulations, including CC-486 (onureg), an oral azacitidine formulation, and ASTX727 (inqovi), which combines decitabine with the CDA inhibitor cedazuridine to improve bioavailability, while retaining first-generation cytidine analog backbones [140,143]. Although clinically validated, especially in hematologic malignancies, first-generation agents are limited by dose-dependent cytotoxicity and pharmacokinetic instability [17].

4.1.2. Second-generation DNMTis (improved nucleoside analogs)

Second-generation DNMTis include AZA derivatives such as zebularine [161] and CP-4200 [179], as well as DAC derivatives such as guadecitabine (SGI-110) [157,158], 4′-Thio-2′-deoxycytidine (TdCyd) [163], NTX-301 (Aza-TdCyd) [163], and 5-fluoro-2′-deoxycytidine (FdCyd) administered in combination with tetrahydrouridine (THU) [165]. These agents were developed to improve metabolic stability, resist CDA-mediated degradation, and sustain DNA demethylation. Guadecitabine, a DAC prodrug designed to resist CDA-mediated degradation, showed on-target DNA hypomethylation and clinical activity in early-phase trials across hematologic malignancies (e.g., NCT01261312; NCT02131597), however, phase III studies in AML (NCT02920008, NCT02348489) [160] and in

Table 1. FDA and EMA approved epidrugs for cancer treatment.

Class/Target	Mechanism	Rationale	Epidrug (Brand)	Cancer type (Indication)	Approval status	Ref
DNA methyl-transferase inhibitor (DNMTi)	Cytidine analog that incorporates into DNA (and RNA for azacitidine) and irreversibly traps and depletes DNMT1, leading to passive DNA demethylation	Reactivation of silenced tumor suppressor genes (TSGs)	**Azacitidine**: 5-azaC (Vidaza) – injectable (subcutaneous or intravenous)	AML, MDS, CMML, JMML	FDA: 2004, EMA: 2008	[11,118,138]
			Azacitidine (CC-486 or Onureg) – oral formulation	Maintenance therapy in AML (post-remission)	FDA: 2020	[139–141]
			Decitabine: 5-aza-dC (Dacogen)	AML, MDS, CMML	FDA: 2006, EMA: 2012	[14,142]
DNMTi + Cytidine Deaminase Inhibitor	Oral bioavailable prodrug of decitabine; preventing cytidine deaminase from degrading decitabine before reaching systemic circulation	Cedazuridine prevents decitabine degradation, enabling oral administration	**Decitabine + Cedazuridine** (ASTX727 or Inqovi) – oral fixed dose combination	MDS, CMML	FDA: 2020	[143]
DNMTi + BCL-2 inhibitor	DNMT inhibition (hypomethylation) + BCL-2 blockade (apoptosis)	Synergistic combination therapy that sensitizes cancers to apoptosis via BCL-2 blockade	**Azacitidine** [or **Decitabine**] + **Venetoclax** (Venclexta)	AML (especially in elderly or unfit for intensive combo)	FDA: 2018 EMA: 2021	[16,144,145]
Histone deacetylase inhibitor (HDACi)	Inhibition of class I/II HDAC, increases histone acetylation	Relieves transcriptional repression of TSGs, induces cell cycle arrest and apoptosis, maintains a more relaxed chromatin structure.	**Vorinostat**: SAHA (Zolinza)	CTCL	FDA: 2006, EMA: 2008	[12]
			Romidepsin: FK228 (Istodax)	CTCL, PTCL	FDA: 2009 (CTCL), FDA: 2011 (PTCL), EMA: 2012	[135,136]
			Belinostat: P×D101 (Beleodaq)	PTCL	FDA: 2014	[146–148]
EZH2 (HMT) inhibitor	Inhibits histone methyltransferase EZH2, reducing H3K27me3 levels thereby reducing gene silencing	Targets histone methylation in EZH2-driven cancers, reactivates TSGs	**Tazemetostat**: EPZ6438 (Tazverik)	Epithelioid sarcoma; Follicular lymphoma	FDA: 2020	[149]
Isocitrate dehydrogenase 1 (IDH1) inhibitor	Inhibits mutant IDH1, decreases the oncometabolite 2-hydroxyglutarate (2-HG) which blocks TET and histone demethylases	Reverses epigenetic block by restoring TET/histone demethylase function which in turn restores normal gene expression	**Ivosidenib**: AG120 (Tibsovo)	AML and cholangiocarcinoma with IDH1 mutation	FDA: 2018 EMA: 2023	[150]
			Olutasidenib (Rezlidhia)	Relapsed or refractory AML with IDH1 mutation	FDA: 2022	[151]
IDH2 inhibitor	Inhibits mutant IDH2, reduces 2-HG and restores normal TET and histone demethylases activity	Same as above	**Enasidenib**: AG221 (Idhifa)	Relapsed or refractory AML with IDH2 mutation	FDA: 2017	[152]
Dual mutant IDH1/2 inhibitor	Selectively inhibits mutant IDH1 (R132) and IDH2 (R172), reducing 2-HG accumulation, restoring α-KG – dependent dioxygenase and TET activity.	First systemic therapy targeting IDH mutations as a brain-penetrant small molecule.	**Vorasidenib**: AG-881 (Voranigo)	Adults and adolescents > 12 yrs with grade 2 astrocytoma or oligodendroglioma post-surgery with susceptible IDH1/2 mutation	FDA: 2024 EMA: 2024	[153,154]
DNMTi + IDH1 inhibitor	Inhibits mutant IDH1, lowers (2-HG) and restoring normal gene expression	More robust inhibition of DNMTs and mutant IDH1	**Azacitidine + Ivosidenib**	newly diagnosed AML with IDH1 mutation	FDA: 2022 EMA: 2023	[72,155]

Abbreviations: AML, Acute myeloid leukemia; MDS, Myelodysplastic syndromes; CMML, Chronic myelomonocytic leukemia; JMML, Juvenile myelomonocytic leukemia; CTCL, Cutaneous Tcell lymphoma; PTCL, Peripheral Tcell lymphoma.

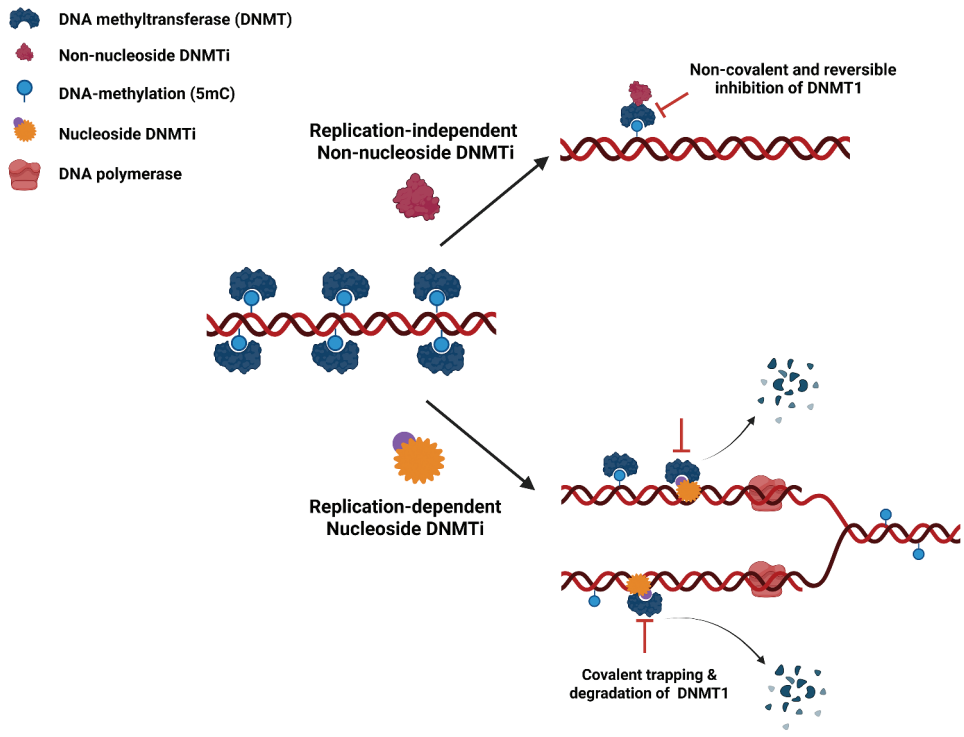

Figure 3. Nucleoside vs Non-nucleoside DNMT Inhibitors.

Replication-independent non-nucleoside DNMT inhibitors suppress DNMT activity through reversible, non-covalent binding to catalytic or allosteric sites. In contrast, replication-dependent nucleoside DNMT inhibitors are incorporated into DNA during S-phase and act as "suicide" substrates, covalently trapping DNMTs, primarily DNMT1, leading to irreversible enzyme inactivation and degradation. *Created in BioRender. Mehdipour, P. (2026) https://BioRender.com/4ts2yu9.*

refractory MDS/CMML (NCT02907359) failed to improve overall survival compared with physician's-choice therapy. Other candidates, such as zebularine and CP-4200, have shown promising preclinical immunomodulatory activity and therapeutic efficacy, however, both compounds have faced significant barriers to clinical translation, including limited or unproven clinical efficacy and challenges in establishing safe and effective dosing regimens [179,202].

Selected second and third-generation DNMTis (also known as next generation DNMTis) are summarized in Table 2 to highlight their key features and utility in preclinical and clinical studies.

4.1.3. Third-generation DNMTis (non-nucleoside analogs)

Third-generation approaches encompass non-nucleoside inhibitors, oligonucleotide strategies, and multi-target epigenetic agents that inhibit DNMT activity without DNA incorporation. They are chemically distinct molecules that can reversibly bind to the catalytic or allosteric sites of DNMTs independent of DNA replication phase (Figure 3). These compounds such as RG108 [189] and GSK3685032 [193] aim for greater specificity, activity in non-dividing cells, lower cytotoxicity, and oral dosing. Dual-target compounds, such as CM-272 [196], which inhibit both DNMTs and histone methyltransferases (e.g., G9a), have demonstrated potent preclinical antitumor and immune-activating effects through concurrent modulation of DNA and histone methylation [19,196].

To date, however, no nnDNMTi has received regulatory approval, and most remain in preclinical or early clinical development (see Table 2 for more details). Next-generation DNMTi agents have primarily achieved improvements in pharmacokinetics and safety profiles (e.g oral bioavailability, reduced dosing frequency, better tolerability) rather than demonstrating consistent monotherapy superiority over AZA or DAC in randomized clinical trials. This has reinforced the field's shift toward rational combination therapies and biomarker-guided strategies [203–205].

4.2. Mechanisms of anti-tumor activity

Both nucleoside and non-nucleoside DNMTis influence gene regulation through multiple, interrelated mechanisms. Originally developed to reactivate silenced TSGs via promoter demethylation, their therapeutic effects also include activation of DNA damage response [206], induction of CTAs [207], and stimulation of viral mimicry and other immune-modulatory pathways that enhance tumor immunogenicity [77] (Figure 4).

4.2.1. Reactivation of epigenetically silenced TSGs

The promoters of TSGs such as *CDKN2A*/p16INK4a [208], *BRCA1* [61] and *CDH1* [209] are often aberrantly hypermethylated, leading to their transcriptional silencing in cancers. By inhibiting DNMT-mediated CpG methylation, DNMTis induce promoter hypomethylation and re-expression of these TSGs, thereby reducing tumor cell fitness through multiple mechanisms, including cell cycle arrest, proliferation control, differentiation, and apoptosis [206]. Low-dose DNMTis have been shown to erode stem-like and self-renewal states in hematologic malignancies and breast cancer by epigenetically reactivating lineage or differentiation-associated transcriptional programs and

Table 2. Next Generation DNMT Inhibitors in Cancer Therapy.

Compound	Class	Status/Phase	Condition	Key Features	References
Guadecitabine (SGI-110)	Nucleoside analog (decitabine guanosine dinucleotide)	**Phase I/II** (ongoing) **Phase II/III** (completed)	Hematologic and Advanced solid tumors	Second-generation cytidine deaminase (CDA)-resistant decitabine prodrug with a prolonged half-life.	[157–159] [NCT03220477, NCT03257761, NCT02131597, NCT01752933, NCT02920008, NCT02907359, NCT01261312, NCT02920008]
4′-Thio-2′-deoxycytidine (TdCyd)	Nucleoside analog (thio-substituted deoxycytidine)	**Phase I** (completed)	Advanced solid tumors	Structurally modified deoxycytidine that incorporates into DNA and traps DNMT1. More chemically stable than decitabine	[163] [NCT02423057]
NTX-301 (5-Aza-4′-thio-2′-deoxycytidine) (Aza-TdCyd)	Nucleoside analog (aza + thio modification)	**Phase I** (ongoing) **Phase I/II** (completed)	Hematologic (phase I trial) and advanced solid tumors (phase I/II trial)	Orally bioavailable DNMT1-depleting agent with reduced off-target toxicity in preclinical models.	[163,164] [NCT03366116, NCT04167917, NCT04851834]
5-Fluoro-2′-deoxycytidine (FdCyd) + Tetrahydrouridine (THU)	Nucleoside analog (fluorinated cytidine) + THU CDA inhibitor	**Phase I/II** (completed)	Advanced solid tumors (breast, NSCLC, head & neck, urothelial)	FdCyd traps DNMTs in DNA and co-administration with THU increases FdCyd exposure and oral dosing. In Phase I/II trials, FdCyd + THU induced TSG demethylation and occasional partial responses, with modest disease stabilization (notably in urothelial carcinoma).	[165] [NCT01534598, NCT00978250]
MG98	Non-nucleoside (antisense phosphorothioate oligodeoxynucleotide)	**Phase I** (completed)	Advanced solid tumors	MG98 targets DNMT1 mRNA 3′ UTR inducing DNA demethylation and TSG reactivation in preclinical models, but clinical development stalled due to dose-limiting transaminitis and fatigue.	[166,167] [NCT00003890]
Hydralazine	Non-nucleoside small molecule (repurposed antihypertensive)	**Phase I/II** (completed)	Solid tumors (including cervical cancer)	Hydralazine is an orally available DNMTi evaluated in combination with HDACi valproic acid. Phase II trial in cervical cancer evaluated hydralazine/valproate as an epigenetic adjunct to cisplatin-based chemoradiation, achieving high clinical response with good tolerability.	[168,169] [NCT00996060, NCT00404326]
EGCG (Epigallocatechin-3-gallate)	Non-nucleoside (natural polyphenol – green-tea catechin))	**Phase I/II**	CLL, Prostate cancer and CRC (ongoing)	Green tea extract that *in vitro* inhibits DNMT activity. Generally safe; and advanced to phase 2 trials in CLL and prostate cancer.	[170,171] [NCT00676780, NCT02891538]
Caffeic Acid	Non-nucleoside (natural polyphenol)	**Phase III** (esophageal cancer studies)	Mostly solid tumors (esophageal, breast and prostate cancer)	Caffeic acid inhibit human DNMT1 predominantly through a noncompetitive mechanism and shows strong preclinical promise in cancer, inhibiting proliferation, inducing apoptosis, modulating signaling and immune responses in various cancers (prostate, breast, esophageal). Shows promise for use as adjuvant with chemotherapy and radiotherapy and have so far advanced to phase III in esophageal cancer trials.	[172–175] [NCT04648917, NCT03070262]
Zebularine	Nucleoside analog (2-pyrimidone)	**Preclinical** (research)	Investigational (solid and hematological cancers)	Orally bioavailable and stable cytidine analog with low toxicity and capacity to enhance anti-tumor immunity by inducing immunogenic cell death.	[161,176–178]
CP-4200 (Azacitidine-5′-elaidic acid)	Nucleoside analog (lipophilic prodrug of AZA)	**Preclinical**	Investigational (solid and hematological cancers)	Fatty acid ester of AZA designed to improve cellular uptake independent of nucleoside transporters, resulting in enhanced half-life and bioavailability	[179,180]
F-aza-T-dCyd (NSC801845)	Nucleoside analog (fluoro-aza-thio cytidine)	**Preclinical**	Investigational (solid and hematological cancers)	Multi-modified cytidine analog derived from TdCyd, Aza-TdCyd, and FdCyd. Designed for stronger DNMT1 inhibition and improved stability. Preclinical data show potent DNMT1 depletion, but it remains under laboratory evaluation.	[181]
SGI-1027	Non-nucleoside (quinoline-based small molecule)	**Preclinical**	Investigational (solid and hematological cancers)	Inhibits DNMTs, reactivates silenced TSGs, inducing cell death in various cancer cells like liver (HCC) and renal cancers by upregulating pro-apoptotic proteins and sensitizing cells to other treatments. The SGI-1027 analog, **MC3353** show greater demethylating activity than AZA and DAC, exhibiting broad anticancer activity.	[182–184]
Procainamide/Procaine	Non-nucleoside (DNA-binding drugs)	**Preclinical** (repurposing)	Investigational (mostly solid tumors)	Procaine, a local anesthetic and DNMTi, inducing cell cycle arrest and apoptosis, modulating oncogenic signaling, anti-proliferative and pro-apoptotic effects on human tongue squamous cell carcinoma cells, gastric and colon cancers.	[185–188]

(*Continued*)

Table 2. (Continued).

Compound	Class	Status/Phase	Condition	Key Features	References
RG108 (N-Phthaloyl-l-tryptophan)	Non-nucleoside (indole small molecule)	**Preclinical**	Investigational (solid and hematological cancers)	Directly suppresses DNMT catalytic activity and radioresistance in esophageal cancers, inducing antitumor effect and apoptosis in cancer cell lines and often with lower cytotoxicity than traditional nucleoside DNMTis.	[189–191]
GSK-3484862	Non-nucleoside (dicyanopyridine)	**Preclinical**	Investigational (solid and hematological malignancy)	DNMT1-selective inhibitor/degrader that promotes proteasomal degradation via UHRF1-dependent ubiquitination, causing rapid DNMT1 loss and global DNA demethylation in cancer cells. Effects appear within hours and reverse after drug withdrawal, indicating a non-covalent, degradative mechanism.	[192]
GSK-3685032	Non-nucleoside	**Preclinical**	Investigational (AML models)	First-in-class reversible DNMT1 with high selectivity for maintenance DNMT1. Competes at the DNMT1-DNA interface, driving genome-wide demethylation and gene reactivation *in vitro*. Shows stronger tumor regression than DAC in AML mouse models with better tolerability.	[18,193]
Nanaomycin A	Non-nucleoside (Streptomyces-derived quinone antibiotic)	**Preclinical**	Investigational (*in vitro* models)	DNMT3B-selective inhibitor that directly inhibits DNMT3B catalytic activity, causing global DNA demethylation, reactivation of TSGs like RASSF1A and anti-tumor activity in cancer cell lines.	[194]
DC-517	Non-nucleoside (carbazole analog)	**Preclinical**	Solid tumors (lung and CRC models)	Structure-based specific and highly potent DNMT1 inhibitor. Inhibits cancer cell proliferation at low μM concentrations, and moderate oral bioavailability (~37%) *in vivo* rat model.	[195]
CM-272	Non-nucleoside (quinol-based dual DNMT/HMT inhibitor)	**Preclinical**	Investigational (solid and hematological malignancy)	Reversibly inhibits DNMTs (sub-μM) and G9a (nM, H3K9 methyltransferase), a novel strategy combining DNA and histone methylation blockade. Inhibits cell proliferation and induce apoptosis and immune response in xenogeneic models, with anti-tumor effect and reduction in cancer survival in other 2D and 3D model system.	[196,197]

Abbreviations: AZA, Azacitidine; DAC, Decitabine; CDA, Cytidine deaminase; AML, Acute myeloid leukemia; MDS, Myelodysplastic syndromes; NSCLC, Non-small cell lung cancer; CMML, Chronic myelomonocytic leukemia; RCC, Renal Clear Carcinoma; CLL, Chronic lymphocytic leukemia; CRC, Colorectal cancer; HCC, Hepatocellular carcinoma.

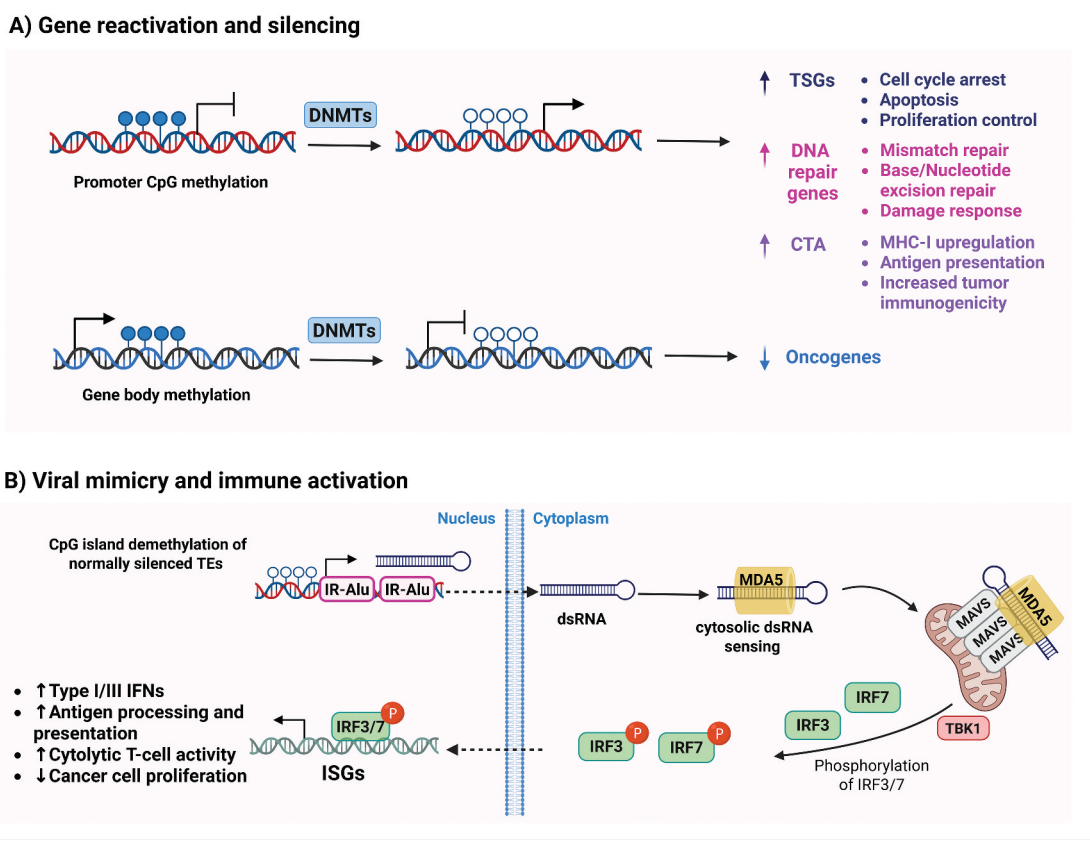

Figure 4. Underlying Mechanism of DNMT Inhibitors in Cancer Treatment.

(A) Gene reactivation and silencing: DNMT inhibitors (DNMTis) exert context-dependent effects on gene expression. By reducing promoter CpG methylation, DNMTis reactivate silenced genes such as tumor suppressor genes (TSGs), cancer-testis antigens (CTAs), and DNA repair genes. In contrast, demethylation within gene bodies, particularly in oncogenes, can lead to transcriptional suppression. (B) Viral mimicry and immune activation: DNMTis induce a viral mimicry response by demethylating promoters of SINE retroelements, leading to accumulation of double-stranded RNAs (dsRNAs) from inverted Alu (IR-Alu) repeats. These dsRNAs are sensed by the pattern-recognition receptor MDA5, which signals through MAVS and TBK1 to activate IRF3/IRF7, thereby driving type I/III interferon production and interferon-stimulated gene (ISG) expression. The resulting immune activation enhances antigen presentation, promotes cytotoxic T-cell responses, and suppresses tumor growth. *Created in BioRender. Mehdipour, P. (2026)* https://BioRender.com/h20czxl.

tumor suppressive cell-cycle checkpoints while dampening proliferative and EMT-linked networks [210]. Beyond promoter reactivation, DNMTis can also indirectly and context-dependently modulate oncogenic transcriptional programs, including c-MYC–driven networks, through inducing gene-body demethylation and broader effects on chromatin organization and transcriptional regulation [48].

4.2.2. Induction of DNA damage response/DNA repair and apoptosis

Nucleoside analog DNMTi are incorporated into DNA, where they form irreversible DNA-DNMT protein adducts which cause replication stress, induce DNA double-strand breaks, and trigger the DNA damage response, ultimately leading to cell death [206]. DNMTi can also demethylate and reactivate silenced DNA repair genes such as *MLH1*, which is a key mediator of DNA mismatch repair, thereby limiting mutational accumulation and shifting cancer cells from mutation-prone survival toward damage-induced death [211–213]. Although DNMTis restore the expression of DNA repair genes, they simultaneously induce replication-coupled DNA damage, the resulting acute and excessive DNA damage overwhelms repair capacity and restores checkpoint-mediated apoptotic signaling, favoring cell death over survival [214].

Paradoxically, DNMTis can induce a "BRCAness" phenotype through transcriptional suppression of homologous recombination repair (HRR) genes, rendering HRR-proficient tumors selectively sensitive to PARP inhibitors and other DNA-damaging agents [215]. This HRR-deficient state can arise in cancers with intact BRCA1/2 through DNMTi-induced activation of innate immune signaling pathways that impair HRR and/or through replication stress that amplifies DNA repair defects and enforces dependence on HRR pathways [216]. Collectively, these effects enable DNMTis to induce synthetic lethality and sensitize HRR-proficient cancers to PARP inhibition and apoptosis, providing a major emerging rationale for their use beyond classical DNA demethylation in both HRR-proficient and HRR-deficient tumors [215,217].

4.2.3. Modulation of anti-tumor immune response

CTAs are a class of antigens normally expressed in germ cells but epigenetically silenced in somatic tissues. In cancer, their expression can be reactivated by DNMTi treatment [207]. DNMTis demethylate the promoters of CTA-encoding genes, leading to transcriptional reactivation and translation of

proteins not ordinarily expressed in adult tissues [207]. This reactivation produces novel antigenic peptides that are processed and presented on the tumor cell surface via MHC class I molecules. Enhanced antigen presentation facilitates recognition by cytotoxic T lymphocytes (CTLs), boosting T-cell – mediated tumor cell killing [207,218,219]. The ability of DNMTis to induce CTA expression, prime antitumor immune responses, and overcome immune evasion mechanisms makes them effective combination partners for immunotherapies, including adoptive T-cell therapy, cancer vaccines targeting CTAs, and immune-checkpoint blockade in refractory solid tumors and hematologic malignancies [218,220].

Another key immunomodulatory mechanism of DNMTis is the reactivation of epigenetically silenced TEs, which triggers a viral mimicry response. Viral mimicry is a cellular state where endogenous stimuli, such as dsRNAs from TEs, trigger an active antiviral response like that caused by an actual viral infection [18,77]. Derepressed Alu retrotransposons, belonging to the SINE family, are transcribed into panhandle-like double-stranded RNA (dsRNA) structures that activate innate immune signaling [221]. This, in turn, induces the production of type I and III IFNs and pro-inflammatory cytokines [219,222]. These cytokines promote dendritic-cell maturation, enhance $CD8^+$ T-cell infiltration, and upregulate MHC class I expression on tumor cells, collectively fostering an immune-reactive TME [223]. The viral mimicry response may also generate neoantigen-like peptides, broadening the tumor-antigen repertoire and further sensitizing tumors to immune attack [207,223].

Collectively, these findings highlight how DNMTis serve as a bridge between epigenetic reprogramming and immune activation, linking DNA demethylation to the induction of innate and adaptive antitumor immunity.

It is also important to note that other epigenetic therapies, as well as non-epigenetic mechanisms, can induce viral mimicry and immunogenic reprogramming. As a result, tumor cells become more visible to the immune system, and the TME is further primed for enhanced immune-cell infiltration and activation. Overall, viral mimicry represents a unifying mechanism through which epigenetic and non-epigenetic factors can enhance tumor immunogenicity and improve cancer treatment outcomes [224].

These mechanisms provide the mechanistic rationale for DNMTi combination regimens.

4.3. DNMTi combination strategies: preclinical and clinical studies

Combination therapy has emerged as a pivotal strategy to target cancer cells through complementary mechanisms. While DNMTis reverse gene silencing at low doses, higher doses cause cytotoxicity that limits clinical use. Using low-dose DNMTis with agents that induce DNA damage, regulate proliferation, or stimulate immune responses enhances epigenetic reprogramming while minimizing toxicity and drug-resistance [217]. A meta-analysis confirmed that combination regimens achieve better outcomes than monotherapy by acting on multiple oncogenic pathways and broadening the therapeutic window [5,13]. Moreover, of the 386 ongoing DNMTi trials analyzed in this study, only 37 are monotherapies, while 349 involve combination therapies, underscoring the strong shift toward DNMTi-based combination strategies (Supplementary Table S2).

Combination therapies dominate the clinical landscape for DNMTis, with key combination partners and their mechanism of action summarized in Figure 5.

4.3.1. DNMTi and targeted therapy

Targeted therapies directed against specific molecular drivers represent the most common and rapidly expanding class of combination partners with DNMTi epidrug, especially for hematological malignancies. It currently dominates ongoing clinical trials, with about 48% of 386 trials involving one or more targeted therapy with DNMTis across hematological and solid tumors (Figure 6). An additional 15% of these trials involve multiple combination regimens (comprising three or more therapeutic combinations) of which targeted therapy is one of the combination partners (Supplementary Table S2). Some of the top candidates in this category are briefly discussed in this section.

4.3.1.1. BCL-2 inhibitors (venetoclax). The B-cell lymphoma 2 (BCL-2) protein family regulates apoptosis by balancing pro-apoptotic and anti-apoptotic signals. In many cancers, anti-apoptotic BCL-2 protein is overexpressed, suppressing apoptosis and allowing cancer cells to survive and proliferate unchecked [225]. BCL-2 inhibitors such as venetoclax inhibit the BCL-2 protein leading to the cancer cell death. AZA/DAC combined with venetoclax is FDA (2018) and EMA (2021) approved for newly diagnosed AML in patients unfit for intensive chemotherapy and represents the clearest clinical success of DNMTi combinations [16,226]. AZA primes cells for caspase-mediated apoptosis by inducing pro-apoptotic BH3-only protein NOXA via the integrated stress response (ISR) pathway. NOXA subsequently binds and neutralizes the anti-apoptotic protein MCL-1, thereby removing a crucial survival mechanism and making cells more dependent on BCL-2. Venetoclax capitalizes on this dependency by displacing pro-apoptotic effectors (e.g., BAX/BAK or BIM) from BCL-2, thereby triggering caspase-mediated apoptosis providing a mechanistic basis for synergy [227,228] (Figure 5). The combination of BCL-2 inhibitors and DNMTis is the leading targeted therapy strategy in ongoing clinical trials especially for hematologic malignancies with over 180 of 386 (46%) trial investigations. (Supplementary Table S2).

4.3.1.2. IDH inhibitors. IDH1/2 mutations occur in ~15 – 20% of AML patients and in subsets of gliomas and cholangiocarcinomas, producing 2-HG which inhibits the enzymatic activity of α-KG-dependent dioxygenases, including lysine histone demethylases and TET enzymes [72]. This results in the persistence of cancer cells in an undifferentiated, progenitor-like state characterized by aberrant histone and DNA methylation programs that support long-term self-renewal and tumorigenesis [72,229]. This mechanistic rationale underpins the FDA approval of IDH1 inhibitors (ivosidenib [150] and olutasidenib [151]), IDH2 inhibitor (enasidenib [152]) and dual mutant IDH1/2 inhibitor (vorasidenib [154]) for the treatment of IDH-mutant AML. In this context, the combination of

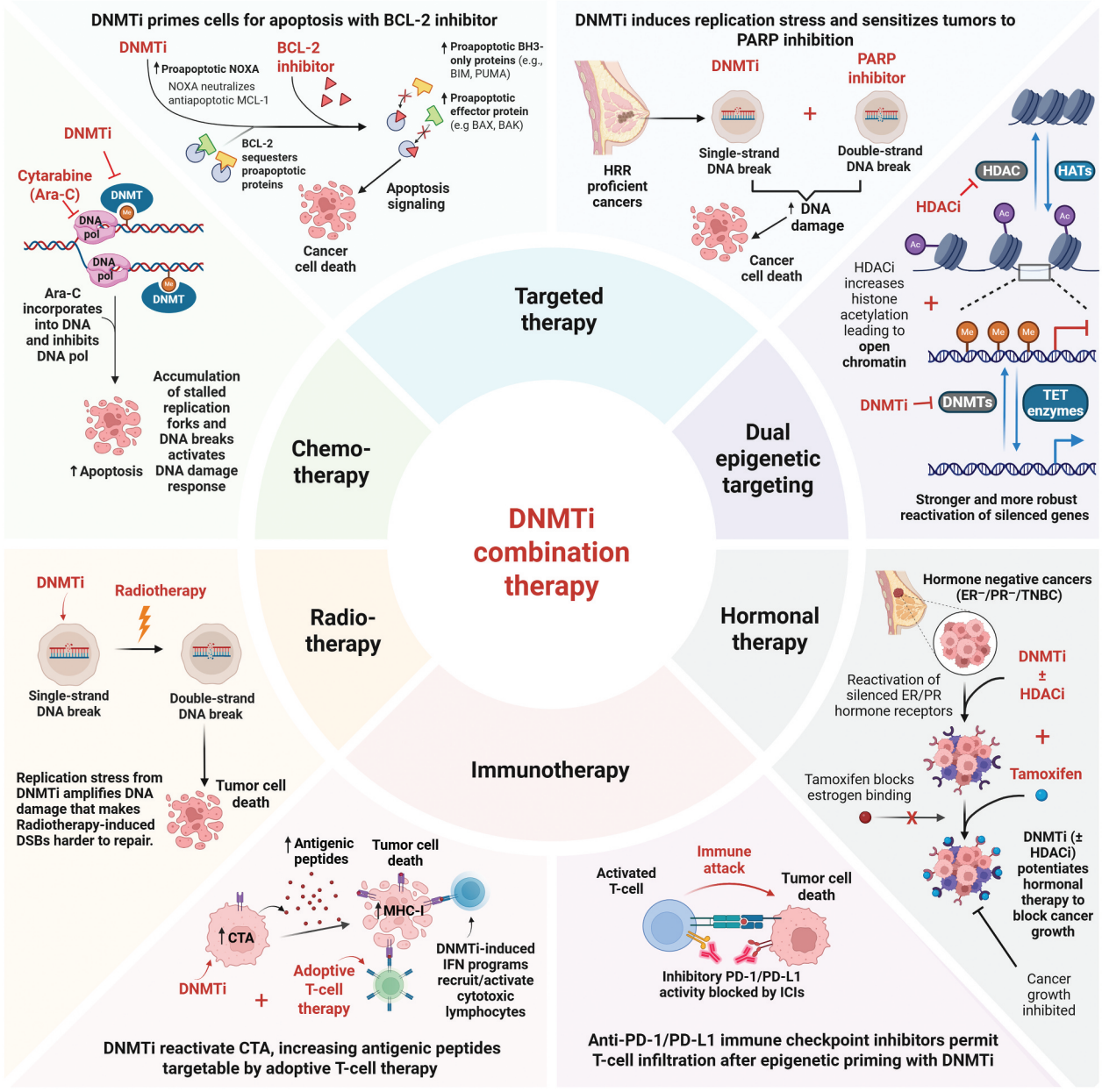

Figure 5. Mechanisms of DNMTi-based Combination Therapy.

DNA methyltransferase inhibitors (DNMTis) serve as a therapeutic backbone in combination with agents targeting complementary pathways. DNMTi-induced upregulation of pro-apoptotic proteins (e.g., NOXA) sensitizes tumor cells to BCL-2 inhibition, while induction of homologous recombination repair (HRR) deficiency enhances sensitivity to PARP inhibitors. Dual epigenetic targeting such as the combination of DNMTis and HDAC inhibitors (HDACis), achieves stronger gene reactivation or repression and is often used to potentiate hormonal therapies in hormone-negative cancers. DNMTis also enhance tumor immunogenicity through cancer-testis antigen expression and viral mimicry, thereby potentiating immunotherapies such as adoptive T-cell therapy (e.g CAR T-cell therapy) and immune checkpoint inhibitors (ICIs). Additionally, DNMTi-induced replication stress augments cytarabine efficacy and impairs the repair of radiotherapy-induced double-strand breaks, amplifying antitumor effects. *Created in BioRender. Mehdipour, P. (2026)* https://BioRender.com/8dfoxy8.

IDH inhibitors with DNMTis has emerged as a clinically meaningful strategy in this regard, with a recent meta-analysis of 11 prospective cohorts [230], including randomized trials such as NCT03173248 and NCT02677922, demonstrating that combining IDH inhibitors with AZA significantly improves remission rates and survival outcomes while maintaining a manageable safety profile compared with IDH inhibitor monotherapy in newly diagnosed IDH-mutant AML [155,230].

4.3.1.3. PARP inhibitors (PARPi). Combining DNMTi with PARP inhibitors (such as olaparib, talazoparib, rucaparib and niraparib) has found application in both HRR-proficient and HRR-deficient tumors as described earlier in section 4.2.2. DNMTis induce a "BRCAness" phenotype that sensitizes HRR-proficient cancer cells to PARP inhibition leading to a synthetic lethal interaction [215]. Mechanistically, Orta et al. showed that DAC-induced DNMT1–DNA trapping engages base-excision repair (BER) pathways, generating single-strand

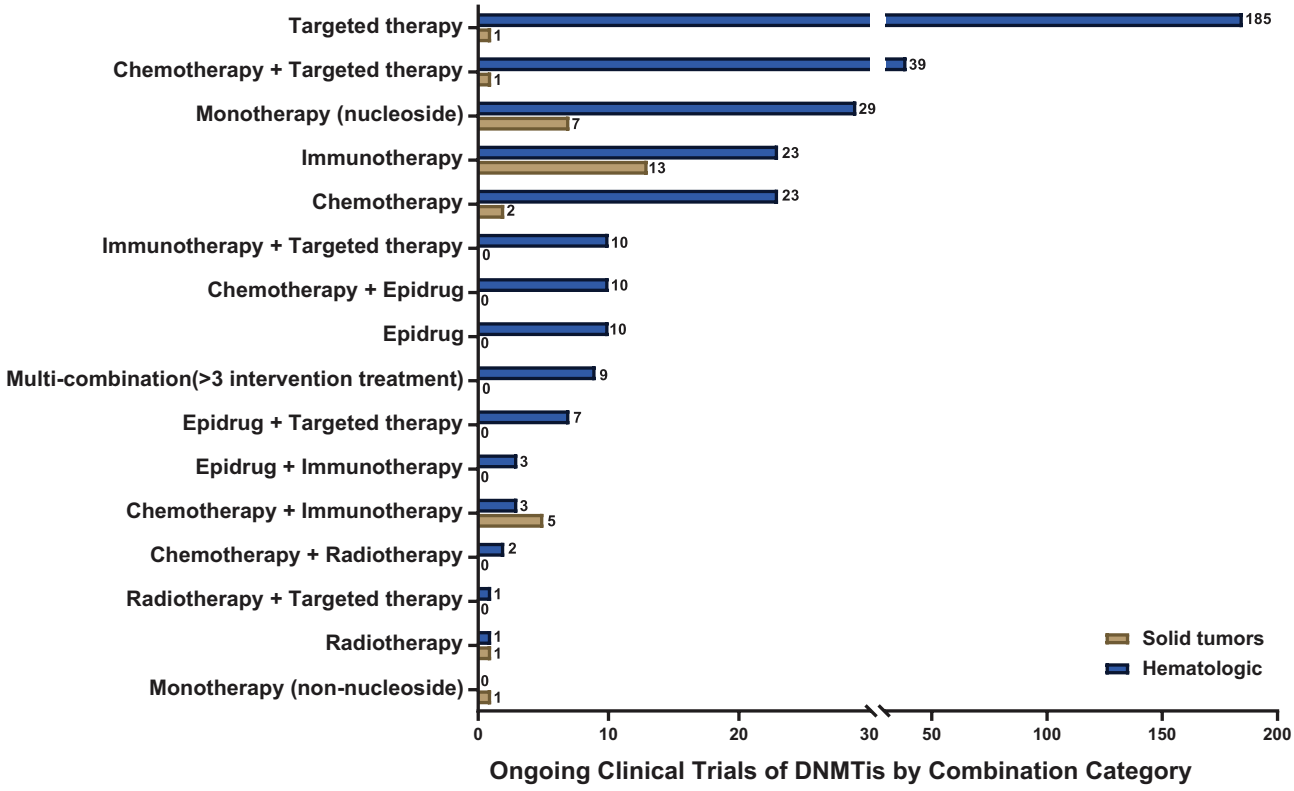

Figure 6. Ongoing Clinical Trials of DNMT Inhibitors by Combination Category.

This analysis classifies ongoing clinical trials involving DNA methyltransferase inhibitors (DNMTis) according to their combination partners in cancer therapy. Data were retrieved from ClinicalTrials.gov for all ongoing interventional studies (excluding completed, terminated, or withdrawn trials) as of September 2025 (see Supplementary Table S2). Among the 386 trials identified, 355 were conducted in hematologic malignancies and 31 in solid tumors. Targeted therapies, particularly BCL-2 inhibitors, dominate hematologic studies, whereas immune checkpoint inhibitors are the most common combination partners in solid tumor trials.

break (SSB) intermediates that are processed in an XRCC1-dependent and PARP-facilitated manner [231]. PARP inhibition disrupts this XRCC1-dependent processing, promoting the persistence of SSB intermediates that are converted into double strand breaks (DSBs), with consequent HRR signaling and enhanced cytotoxicity, consistent with DNMTi-mediated sensitization to PARP inhibition in AML cell models [231] (Figure 5). In BRCA1/2-defective (HRR-deficient) tumor xenograft and patient-derived xenograft (PDX) mouse models, low-dose DAC synergized with the PARP inhibitor talazoparib to induce G2/M cell-cycle arrest, cumulative DNA damage, and significantly increased tumor cell death compared with talazoparib monotherapy, whereas in HRR-proficient models only limited growth suppression and cell death were observed [217]. Although Pacaud et al. [217] observed minimal apoptosis in HRR-proficient triple negative breast cancer (TNBC) models at the tested low-dose regimen, other studies suggest that low-dose DNMT inhibition can also sensitize selected HRR-proficient models, including breast cancer, ovarian cancer, non-small cell lung cancer (NSCLC), and pancreatic ductal adenocarcinoma (PDAC) to PARP inhibition, indicating that response in HRR-proficient disease may be context and regimen-dependent [215,232,233].

Clinical translation of combined DNMT inhibition with PARP inhibition is underway. A phase I trial (NCT02878785) in AML provides clinical evidence that DNMT inhibition can induce a functional HRR-deficient state, thereby amplifying PARP inhibitor associated DNA damage [234]. More recently, solid-tumor trials such as ASTX727 in combination with olaparib (NCT06177171) have been initiated in advanced HRR-deficient solid tumors to evaluate the safety and tolerability of this combination in genetically DNA repair compromised cancers.

4.3.2. DNMTi and immunotherapy

Immunotherapy works by enabling the immune system to recognize cancer cells as "non-self" and/or eliminate them more effectively. DNMTis can prime cancer cells toward a more immunogenic state by inducing CTA expression, increasing tumor antigen presentation and MHC class I expression and activating viral mimicry and type I interferon signaling (as described in section 4.2.3). Together, these effects provide a strong rationale for combining DNMTis with immunotherapies such as immune-checkpoint inhibitors (ICIs), adoptive cell therapies (e.g CAR T-cells) and cancer vaccines [207,219,224] (Figure 5).

Multiple clinical trials are currently evaluating combination of DNMTis with ICIs, including anti-PD-1/PD-L1 and anti-CTLA-4 antibodies. A phase II, open-label study in relapsed or refractory classical Hodgkin lymphoma reported a 95% objective response rate (ORR) and a 71% complete response (CR) with low-dose DAC priming plus camrelizumab in PD-1–naïve patients, compared with an ORR 90% and a CR rate of 32% with PD-1 monotherapy. In PD-1–pretreated or resistant patients treated with the combination, ORR was 52% with a 28% CR, suggesting partial activity in some resistant cases [235]. This combination approach is also emerging theme in solid tumors, supported by growing preclinical and early clinical evidence of efficacy [236,237].

4.3.3. DNMTi and chemotherapy

DNMTis sensitize tumors to chemotherapy by inducing replication stress and DNA lesions, particularly at nucleoside analog doses, that overwhelm DNA repair machinery [214], as described in section 4.2.2. This pre-existing replication stress and replication fork instability greatly increase the lethal impact of S-phase – active chemotherapeutics (e.g., cytarabine, platinum, topoisomerase poisons), which add more lesions on an already compromised replication machinery [214,238,239] (Figure 5).

While traditional chemotherapies primarily target rapidly dividing cancer cells to induce cell death, some cancer cells can undergo epigenetic reprogramming as an adaptive response, reducing their sensitivity to treatment and promoting the emergence of chemoresistance and tumor regrowth [240]. DNMTis can help reprogram tumor cells into a more therapy-sensitive state and reverse epigenetic drug-resistance programs through the re-expression of pro-apoptotic and cell-cycle checkpoint genes, thereby enabling chemotherapy-induced damage to culminate in cell death rather than survival or tolerance [214,241]. Clinically, this strategy has been explored using DNMTi priming prior to full-dose chemotherapy, with tumor hypomethylation correlating with major histopathologic responses reported in gastroesophageal cancer, directly linking epigenetic priming to clinical benefit [242]. A phase II study also shows that epigenetic priming with DAC followed by cytarabine is a feasible and well-tolerated induction strategy in AML patients, producing high remission rates, low early mortality, and meaningful clinical benefit even in adverse-risk and TP53-mutated disease [243].

4.3.4. DNMTi and radiotherapy

Radiotherapy exerts its cytotoxic effects largely by inducing extensive DNA damage particularly DNA DSBs and oxidative stress, leading to defective DNA repair, and consequently, proliferative death or mitotic catastrophe during subsequent cell divisions [244]. DNMTi act as radiosensitizers, primarily by impairing DNA repair pathways which makes cancer cells more vulnerable to the DNA-damaging effects of ionizing radiation [245,246] (Figure 5). In preclinical studies, pre-treatment of lung and glioblastoma cell lines with DNMTis such as DAC and zebularine significantly reduced survival after irradiation relative to radiotherapy alone, accompanied by increased apoptotic markers, indicating compromised DNA damage resolution after radiotherapy when DNA methylation is inhibited [245]. More recent mechanistic work in nasopharyngeal carcinoma models shows that combining DAC with radiation can partially overcome radioresistance linked to gene methylation, restoring expression of radiosensitivity-associated gene and increasing radiotherapy-induced apoptosis *in vitro* and *in vivo* [247]. Although preclinical data strongly support DNMTis as radiosensitizers, clinical evidence remains limited, as most trials include radiotherapy only as part of standard-of-care regimens alongside chemotherapy or surgery rather than as a defined combination partner (NCT06997094).

4.3.5. Dual epigenetic targeting

Tumors frequently employ multiple epigenetic mechanisms in parallel to silence genes. Combining DNMTis with other epigenetic modulators, such as HDAC, EZH2, LSD1, BET inhibitors, aims to overcome compensatory epigenetic reprogramming in cancer cells. Among these strategies, combination regimens involving DNMTis and HDACis represent an appealing strategy for achieving more robust reactivation of silenced genes. While DNMTis demethylate promoters to reactivate gene expression, HDACis increase histone acetylation, resulting in a more relaxed chromatin conformation that facilitates transcriptional activation. Preclinical studies have indicated that sequential or simultaneous administration of these agents can produce synergistic effects, leading to enhanced reactivation of silenced TSGs, apoptotic pathways and differentiation programs [248,249] (Figure 5).

In ovarian cancer models, AZA combined with the HDAC6 inhibitor nexturastat A, amplified type I interferon signaling and increased MHC-I antigen presentation relative to either agent alone [250]. Clinically, AZA in combination with the class I HDACi entinostat was tested in patients with advanced non-small cell lung cancer (NSCLC) and showed epigenetic immune priming, including upregulation of interferon-response and antigen-presentation genes, with occasional objective responses observed in PD-1–refractory patients (NCT01928576).

4.3.6. DNMTi and hormonal (endocrine) therapy

Hormonal therapies (e.g tamoxifen and aromatase inhibitors) are generally ineffective in hormone-receptor-negative tumors because these cancers lack functional estrogen and/or progesterone receptors required for hormone-driven growth, in contrast to hormone-receptor-positive tumors. In hormone-negative breast cancers, receptor expression is frequently silenced through hypermethylation of promoter regions [251]. DNMTis can potentiate hormonal therapy in this context by reversing aberrant DNA methylation and restoring hormone receptor expression, thereby enabling hormonal therapies to block receptor signaling or inhibit hormone production and ultimately curbing cancer growth. DNMTi treatment has been shown to restore PR expression through promoter demethylation of the epigenetically silenced *PGR* gene [252] in leukemia cancer cells. In addition, DNMTis in combination with HDACis, can restore estrogen receptor-α (ERα) expression in ER^- breast cancer models thereby re-sensitizing tumor cells to tamoxifen more effectively than tamoxifen alone [253].

Dietary phytochemicals such as resveratrol and pterostilbene have been shown to epigenetically reactivate ERα and restore hormonal responsiveness in ER^- breast cancer cells by inhibiting DNMT and HDAC activities [254]. Similarly, combined DNMT and HDAC inhibition has been shown to re-express ERα and ERβ in TNBC cells, and the addition of an ERβ-selective agonist further enhanced anti-tumor markers, supporting the potential for endocrine re-sensitization [255]. The rationale for combining DNMTis and HDACis to potentiate hormonal therapy instead of using DNMTis alone, was demonstrated in a study by Yang et al in which dual epigenetic targeting resulted in a more robust induction of ER expression (up to 10-fold) compared with single-agent treatment [251].

Despite compelling preclinical evidence, the clinical benefit of combining epigenetic therapies with endocrine treatment

has remained modest. In a randomized phase II trial of ER^+, human epidermal growth factor receptor 2 negative ($HER2^-$) metastatic breast cancer that had progressed on aromatase inhibitors, the addition of oral azacitidine (CC-486) to fulvestrant did not improve progression-free or overall survival compared with fulvestrant monotherapy, resulting in early trial termination due to lack of efficacy (NCT02374099). Similarly, a phase II study of DNMTi (AZA) combined with the HDACi (entinostat) in advanced $HER2^-$ breast cancer demonstrated minimal clinical benefit with only a single partial response observed in the hormone-resistant ER^+ cohort and none in TNBC [256]. Notably, a small subset of patients showed prolonged disease stabilization only after endocrine therapy was reintroduced at progression, suggesting limited, context-dependent sensitization rather than robust therapeutic efficacy [256].

Overall, clinical studies evaluating DNMTi – endocrine therapy combinations with or without HDACi remain limited to early-phase trials and have demonstrated modest outcomes, including occasional partial responses and evidence of hormone receptors re-expression. These findings underscore the need for further investigation to better define patient selection, optimal treatment scheduling, and clinical contexts in which epigenetic reprogramming may meaningfully enhance endocrine therapy responsiveness.

5. Emerging clinical insights and trends

DNMTis continue to lead epigenetic therapy, especially in hematologic malignancies, and function increasingly as the backbone for combination regimens across cancer types. Oral DNMTi formulations (e.g., ASTX727, CC-486) and triplet combination strategies are gaining momentum. The most clinically impactful combinations to date pair DNMTis with targeted agents (notably BCL-2 inhibitors); the AZA (or DAC) + venetoclax regimen has markedly influenced the standard of care for older or unfit AML patients [16]. In solid tumors, combination approaches particularly those combining DNMTis with immunotherapies, particularly ICIs, represent the principal strategy to overcome the limited single-agent efficacy of DNMTis [15,257]. Current trial patterns indicate a strong tilt toward combination studies versus monotherapy (Figure 6). The estimated distribution based on current clinical trial patterns is approximately 90% combination trials versus approximately 10% monotherapy in hematologic malignancies, whereas in solid tumors approximately 70–80% of trials evaluate combination regimens and approximately 20–30% assess monotherapy (Supplementary Table S2).

5.1. Combination approaches in hematologic malignancies

In hematologic cancers such as AML and MDS, DNMTis, particularly AZA and DAC are established first-line, low-intensity treatments that provide substantial clinical benefit [257,258]. The DNMTi – venetoclax combination disrupts epigenetic silencing of pro-apoptotic genes while directly priming cells for apoptosis, demonstrating strong synergy and achieving regulatory approval for hematologic cancers [16]. Regimens of ASTX727 with venetoclax in higher risk MDS/MPN, as well as the combination of DAC with tagraxofusp, an IL-3 receptor-targeting agent (NCT05038592), have produced encouraging safety and efficacy signals thereby demonstrating promising synergistic activity [259,260]. Nearly half of the ongoing clinical trials involving the use of DNMTi in cancers include BCL-2 inhibitor as a combination partner, predominantly in hematologic malignancies (Supplementary Table S2, Figure 6), demonstrating its widespread application in cancer treatment.

Following ASTX727 approval in 2020, maintenance trials in the post – stem-cell transplantation setting (e.g., NCT04980404) are exploring DNMTis as consolidation therapy. Combinations with immune checkpoint inhibitors (e.g., atezolizumab, pembrolizumab) are also under investigation to counter immune evasion (NCT02935361). Overall, these multifaceted regimens target epigenetic dysregulation, apoptosis, and immune suppression concurrently, yielding superior outcomes compared to monotherapy.

5.2. Combination approaches in solid tumors

Solid tumors have shown modest responses to DNMTi monotherapy, likely due to complex TMEs, rapid drug deamination, and limited intratumoral exposure [15]. Consequently, most studies now integrate DNMTis into combination regimens, particularly immunotherapy [261]. Trials combining guadecitabine with ipilimumab or pembrolizumab (NCT02608437, NCT04250246, NCT02998567) aim to harness DNMTi-induced epigenetic priming to improve immune checkpoint blockade. DNMTis enhance tumor antigenicity by demethylating immune-related genes and reactivating endogenous retroviruses (ERVs), thereby inducing viral mimicry and converting immune-cold tumors to immune-hot phenotypes. This dual modulation of gene expression and the TME positions DNMTis as ideal partners for ICIs [257,261].

Beyond immunotherapy, combinations with HDAC inhibitors (e.g., belinostat) and PARP inhibitors (e.g., olaparib) are being evaluated for solid tumors such as chondrosarcoma (NCT04340843) and HRR-deficient carcinomas (NCT06177171). These multi-agent epigenetic and targeted strategies show improved efficacy by addressing multiple resistance mechanisms simultaneously.

Recent efforts have also extended DNMTi evaluation into pediatric oncology, where epigenetic dysregulation is common in tumors such as neuroblastoma, medulloblastoma, and osteosarcoma [257]. Early-phase pediatric studies have explored DNMTi-based combinations with chemotherapy and other epigenetic agents, demonstrating biological feasibility and strong mechanistic rationale; however, clinical benefit has been limited by toxicity or insufficient efficacy signals (e.g., DNMTi + HDACi + chemotherapy in relapsed ALL, NCT01483690; and DNMTi sequential with cytarabine in relapsed/refractory AML, NCT01853228).

5.3. DNA methylation-based biomarkers

DNA methylation alterations arise early during carcinogenesis, are often cancer type-specific, and can be robustly detected in both tissue and liquid biopsies, making them attractive

biomarkers for early detection and therapy response prediction [262,263]. Aberrant promoter hypermethylation of TSGs and characteristic genome-wide methylation signatures form the basis of several stool and blood-based screening assays for colorectal cancer, most notably the FDA-approved Epi proColon [264] which detects methylated *SEPT9* in plasma, while Guardant Shield [265], an FDA-approved (2024) multi--target stool DNA test, incorporates DNA methylation markers alongside other analytes. These assays exemplify how methylation-based readouts are increasingly being integrated into routine screening pathways for non-invasive early cancer detection. In addition, multiple other registered and emerging liquid biopsy tests involving DNA methylation biomarkers are under clinical evaluation or entering clinical use across various cancer types [263].

In parallel, baseline and on-treatment DNA methylation patterns are increasingly being investigated as predictors of response or resistance to DNMTis, chemotherapy, targeted therapies, and immunotherapy, supporting DNA methylation as a dynamic biomarker of treatment sensitivity. For example, *MGMT* promoter methylation guides temozolomide treatment in glioblastoma [64,266], while *MLH1* methylation predicts immunotherapy response in microsatellite-unstable colorectal cancers [267,268]. Similarly, *BRCA1* promoter methylation identifies breast and ovarian cancers that are sensitive to PARPis [269], enabling personalized treatment selection. In addition, the expression levels of DNMT enzymes have been proposed as predictors of sensitivity to DNMTi therapy in TNBC [270]. Taken together, these developments support the view that DNA methylation-based assays for early detection and therapy response prediction represents as an emerging, yet rapidly maturing, clinical trend in oncology.

Emerging preclinical and early clinical biomarkers include multi-cancer early detection (MCED) assays such as Galleri [271] and PanSeer [272], which leverage genome-wide methylation patterns to enable detection of multiple cancer types, in some cases prior to clinical diagnosis. In addition, tissue-specific markers, such as *GSTP1* for prostate [273], *SHOX2* for lung [274], *NDRG4* for colorectal [275]) and multi-gene panels (e.g., ColoScape [276]) have demonstrated improved sensitivity and specificity in ongoing clinical studies [268]. The integration of DNA methylation biomarkers with AI-driven pattern recognition approaches, together with their relative stability in circulating cell-free DNA, positions DNA methylation as a cornerstone of precision oncology strategies for early detection, risk stratification, and therapeutic decision-making [277].

6. Challenges in patient response to DNMTi-based therapies

Despite significant advances in DNMTi development and combination strategies, several obstacles remain, including limitations in pharmacokinetics, tumor heterogeneity, biomarker identification, and toxicity management. A major limitation of DNMTi therapy is its unfavorable pharmacokinetic profile, characterized by poor bioavailability and rapid enzymatic degradation, primarily by CDA, which results in a short plasma half-life, rapid systemic clearance, low cellular uptake and variable clinical efficacy [88]. Although numerous next-generation DNMTi agents have been developed with improved pharmacokinetic properties, they have generally not yet demonstrated consistent survival benefits or superior efficacy compared with standard nucleoside analogs such as AZA and DAC in randomized trials. Novel drug delivery approaches, including nanocarrier-based system, have been proposed to improve the stability of nucleosides DNMTis, however, challenges such as lysosomal degradation, incomplete tumor targeting, and immune recognition remain significant barriers to clinical translation [278].

A second major challenge associated with DNMTi therapy is the genome-wide, nonselective mechanism of action. While therapeutic benefit is frequently attributed to the reactivation of epigenetically silenced TSGs and immune pathways, global hypomethylation can also derepress pro-tumorigenic programs, including oncogenic and cancer-germline (cancer-testis) genes, as well as pathways associated with invasion, immune evasion, or altered TME signaling [279,280].

In addition, DNMT inhibition can produce context-dependent immune effects. For example, DNMTi can activate tumor-intrinsic inflammatory programs, including interleukin-1 signaling, which remodel chemokine networks and promote the recruitment of immunosuppressive myeloid cells [281]. In certain solid tumors, these effects may counteract antitumor immunity, highlighting that DNMTi-mediated immune modulation is highly context dependent and does not uniformly result in immune stimulation [281].

Another key barrier to effective DNMTi therapy in solid tumors such as TNBC and glioblastoma is intrinsic tumor heterogeneity and epigenetic plasticity. Extensive genetic and epigenetic heterogeneity drives variable responses to DNMTis through differences in DNMT dependency, baseline methylation states and sub-clonal composition, while compensatory epigenetic programs further limit effective reprogramming, such that only a subset of tumor cells becomes epigenetically primed, ultimately undermining durable therapeutic benefit [282,283]. Moreover, DNMTi-induced state changes can be transient or rerouted into alternative resistant phenotypes rather than durable differentiation or sensitization. Resistance, both primary and acquired, can result from redundant repressive mechanisms, altered drug metabolism or uptake, or activation of alternative signaling pathways that bypass DNMTi-mediated demethylation over time [282]. In addition, the lack of reliable predictive biomarkers further complicates treatment optimization, and despite extensive research, no consistent correlation has been established between baseline methylation patterns, extent of demethylation, and clinical outcomes [258]. Collectively, these considerations highlight the need for future strategies to move beyond indiscriminate demethylation toward biomarker-selected patient selection and more targeted, gene or locus-specific approaches to methylation modulation.

Finally, optimizing dosing schedules for DNMTis whether as monotherapy or within combination regimens, remains a scientific challenge. Treatment duration and scheduling are critical determinants of DNMTi activity. Because nucleoside DNMTis require incorporation during S-phase and their epigenetic effects accumulate over successive cell divisions, durable DNA hypomethylation and transcriptional reprogramming

typically require repeated or prolonged low-dose exposure rather than brief high-dose pulses. In preclinical AML models, extended low-dose exposure to AZA results in sustained DNMT1 depletion, durable hypomethylation and long-lasting gene-expression and phenotypic changes, whereas transient high-dose exposure regimens can produce markedly different and sometimes discordant outcomes depending on the cell state (i.e stem-like vs non-stem-like leukemia cells) and the biological endpoints assessed [284]. Moreover, although both AZA and DAC induce DNA hypomethylation, they have been demonstrated to exhibit fundamentally different effects on gene expression, cell cycle regulation, DNA damage and apoptosis in NSCLC models [285], and they exhibit varying potencies depending on dosage context and exposure duration in colorectal cancer models [286]. Collectively, this evidence challenges the assumption that AZA and DAC are therapeutically equivalent and interchangeable. Combination therapies also introduce additional challenges in balancing dosing, managing overlapping toxicities, and identifying responsive patient subgroups [159] (NCT02998567, NCT03308396). Addressing these challenges will require improved pharmacodynamic monitoring, rational schedule optimization and combination regimens.

Collectively, these considerations highlight that DNMTis operate within a highly dynamic and context-dependent epigenetic landscape. Their effective clinical deployment must account for schedule-dependent effects, non-selective genome-wide methylation changes with potential oncogenic consequences, the context-specific roles of TET enzymes across tumor types, and the profound heterogeneity and epigenetic plasticity characteristic of solid tumors

7. Conclusion

DNMTis are central to modern epigenetic therapy. By reversing aberrant DNA methylation, they reactivate TSGs, induce DNA damage responses, trigger apoptotic signaling and remodel the TME. DNMTis also upregulate CTAs and TEs, inducing viral-mimicry – driven type I IFN signaling and enhanced MHC-I expression, thereby promoting antitumor immunity.

First-generation DNMTis, AZA and DAC, established proof of concept in hematologic malignancies by trapping DNMTs within DNA during DNA replication but are limited by chemical instability, off-target toxicity and modest efficacy in solid tumors. Next-generation agents such as guadecitabine and non-nucleoside inhibitors like GSK3685032 represent advances in DNMTi design with improved pharmacokinetic properties; however, they have not yet consistently demonstrated superior overall survival in solid tumors or reliably overcome resistance in hematologic malignancies, and challenges including off-target effects and complex dosing regimens remain. The need to improve the efficacy of DNMTis parallels a shift toward combination therapy. Integrating epigenetic modulation with immunotherapy, targeted agents, and chemotherapy represents a critical frontier in cancer treatment.

Clinical data confirm that DNMTi-based combinations achieve deeper remissions and more durable responses than monotherapy [15,258]. DNMTis, particularly AZA, DAC, and their oral formulations (CC-486, ASTX727), remain front-line for myeloid neoplasms and serve as the backbone for combinations with BCL-2 inhibitors (e.g., venetoclax), PARPis, and immune-checkpoint inhibitors. DNMTi + venetoclax has become the standard of care for elderly AML [16,145], while DNMTi + immunotherapy is emerging as a dominant strategy for solid tumors [257,261]. Trials combining DNMTis with immune checkpoint blockers such as ipilimumab, pembrolizumab, or durvalumab show improved survival and durable responses in melanoma, NSCLC, and renal cell carcinoma (NCT02608437, NCT03308396, NCT04250246). DNMTis also sensitize tumors to chemotherapy and radiotherapy by impairing repair of DNMTi-induced DNA damage [287]. Dual-epigenetic strategies, pairing DNMTis with other epidrugs such as HDACis, achieve synergistic reactivation of silenced genes and stronger immune stimulation. Preclinical studies have also shown that DNMTi can potentiate hormonal therapies by restoring the expression of hormone receptors in hormone-negative cancers.

Overall, preclinical and clinical evidence supports continued development of DNMTi combination regimens, which in selected contexts have demonstrated improved response rates and more durable remissions compared with DNMTi monotherapy.

8. Future perspectives

Future progress in DNMTi therapy could be driven by next-generation agents, precision delivery, and biomarker-guided personalization. New non-nucleoside DNMTis seek to overcome the poor pharmacokinetics and solid-tumor limitations of nucleoside analogs. A more comprehensive understanding of the underlying biological mechanisms will be critical to achieving earlier and more durable responses, overcoming resistance, and improving cancer treatment outcomes. CDA-resistant analogs such as zebularine and guadecitabine, and non-nucleoside compounds including RG108 and MG98, are still under active investigation for improved stability, reduced toxicity and improved survival outcomes.

The landscape of DNMTis is expanding with creative therapeutic approaches. One emerging idea is proteolysis-targeting chimeras (PROTACs) directed at DNMT1, which could induce selective enzyme degradation, achieving demethylation without DNA incorporation [288]. Likewise, DNMT3A reactivators may be explored to correct hypomethylation in DNMT3A-mutant leukemias: the idea is to restore some methylation to a chaotic hypomethylated cancer [289]. In solid tumors, advances in nanocarrier delivery and oncolytic-virus combinations can exploit DNMTi-induced viral mimicry to enhance tumor targeting.

DNA methylation alterations are highly cancer-specific, stable, and detectable in circulating cell-free DNA, making DNA methylation particularly well-suited for noninvasive, blood-based cancer screening approaches [262]. Beyond diagnosis, DNA methylation patterns also function as predictive and pharmacodynamic biomarkers. Baseline methylation states can stratify patients according to their likelihood of responding to epigenetic therapies. For example, ongoing translational studies are identifying methylation-based biomarkers that help predict patient sensitivity or resistance to DNMTis, thereby guiding future clinical use

of these agents in personalized medicine [277,290]. Moreover, large-scale genomic and epigenomic profiling, efforts are increasingly uncovering tumor subsets that show a heightened dependence (addiction) on abnormal DNA methylation patterns, enabling the identification of patients most likely to benefit from DNMTi-based therapies [291,292]. These insights will be critical in enabling the development of personalized combination regimens that target both the epigenome and the specific oncogenic drivers of individual tumors.

DNMTis affect genome-wide methylation which may inadvertently reactivate silenced oncogenes or other critical proteins which may reduce their efficacy. Novel platforms that employ engineered proteins such as zinc-finger arrays, TALEs, or CRISPR/dCas9 fused with DNMT inhibitory domains offer the potential to achieve locus-specific demethylation and reactivation of target genes with unprecedented precision [88,293]. A complementary, more targeted route is locus-directed recruitment of TET enzyme demethylating activity (e.g., dCas9–TET catalytic domain) to modulate methylation at selected regulatory elements, thereby improving specificity and mitigating risks of global hypomethylation [294].

Future work should therefore prioritize optimized dosing schedules, rational co-targeting of DNMT and TET pathways with locus-selective epigenetic tools, combination strategies that rely on personalized, biomarker-driven regimens that combine DNMTis with complementary treatment modalities to maximize efficacy, minimize toxicities, restrict phenotypic plasticity and prevent epigenetically mediated resistance. Integration of artificial intelligence (AI) and multi-omics analytics is accelerating discovery of predictive signatures, rational combination modalities, and novel DNMTi chemotypes [295], and collectively, these efforts are steering DNMTi research toward more effective, durable, and personalized cancer treatments.

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Author contributions

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