DNA methyltransferase inhibitors—state of the art

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Background: DNA methylation is the addition of a methyl group to the 5 position of cytosine. It is an epigenetic process with several effects, including chromatin structure modulation, transcriptional repression and the suppression of transposable elements. In malignancy, methylation patterns change, resulting in global hypomethylation with regional hypermethylation. This can lead to genetic instability and the repression of tumor suppressor genes.

Design: A review of the DNA methyltransferase inhibitor literature was conducted.

Results: DNA methylation inhibitors have demonstrated the ability to inhibit hypermethylation, restore suppressor gene expression and exert antitumor effects in in vitro and in vivo laboratory models. Four inhibitors, which are analogs of the nucleoside deoxycytidine, have been clinically tested: 5-azacytidine, 5-aza-2′-deoxycytidine, 1-β-D-arabinofuranosyl-5-azacytosine and dihydro-5-azacytidine. The first two have demonstrated encouraging antileukemic activity but little activity in solid tumors, while the latter two are no longer under study due to lack of efficacy. A fifth agent, MG98, is an anti-sense oligodeoxynucleotide directed against the 3′ untranslated region of the DNA methyltransferase-1 enzyme mRNA, and is now under phase II study.

Conclusions: While some positive clinical results with DNA methyltransferase inhibitors have been seen, a definitive clinical role for these agents will most likely require combination therapy, and good phase III studies are needed.

Key words: DNA methylation, DNA methyltransferase antagonists, oligodeoxynucleotides, tumor suppressor genes

Introduction

The methylation of DNA is a process shared by both eukaryotic and prokaryotic cells [1], and it serves as an epigenetic method of modulating gene expression. Methylation plays a role in genomic stability and carcinogenesis, and it offers a target for the treatment of malignancy [2, 3]. In this review, methylation, its role in cancer and the therapeutic agents that affect methylation will be described.

The process of methylation is carried out by DNA methyltransferases (DNMT). These enzymes catalyze the covalent addition of a methyl group from a donor S-adenosylmethionine to the 5 position of cytosine, predominantly within the CpG dinucleotide [4, 5]. The principal targets of this process are CpG dinucleotides in highly repeated transposable elements [6]. These elements are termed parasitic due to their resemblance to viral DNA and their ability to move between different chromosomal sites. In contrast, while roughly half of 5′ promoter proximal elements contain CpG islands, they are not usually methylated in normal tissues [7]. The end results of methylation are manifold, including chromatin structure modulation, downstream transcriptional repression, X chromosome inactivation, genomic imprinting and the suppression of detrimental effects induced by the transposable elements noted above [5, 8]. Methylation is necessary for normal embryological development [9]. During embryogenesis, there is an initial generalized demethylation of DNA followed by a specific adult pattern of methylation [10]. After development, DNA methylation remains a permanent component of the genome [11].

While at least three functional DNA methyltransferases have been identified, the most abundant is DNMT1 [5]. DNMT1 preferentially methylates hemi-methylated DNA [12] and is thus responsible for methylation during DNA replication, earning it the title of the ‘maintenance methyltransferase’. It plays a key role in imprinting and X-chromosome inactivation during embryogenesis [13, 14]. DNMT1 localizes to replication foci [15], at least in part by interacting with proliferating cell nuclear antigen (PCNA), a protein closely involved in DNA replication. It is therefore available...
to maintain methylation during replication and possibly repair [16].

Other known functional methyltransferases are DNMT3a and DNMT3b, which are responsible for de novo methylation during embryogenesis [17]. DNMT3a and DNMT3b have equal preference for hemi-methylated and non-methylated DNA, and so have been classified as de novo methyltransferases [18]. However, it is likely that DNMT1 and the DNMT3 methyltransferases are not completely distinct in their activities. This is based on two lines of evidence: first, despite being DNMT1 deficient, a colon adenocarcinoma cell line was able to retain 80% of its methylation level while replicating, possibly implicating DNMT3 in the maintenance of methylation [19]; secondly, forced overexpression of DNMT1 in cancer cell lines does cause de novo methylation [20].

Non-functional homologs of the methyltransferases have also been identified. DNMT2 does not appear to have significant methyltransferase activity [21, 22]. Similarly, DNMT3L has a mutated active site and is likely to be non-functional [23]. It has been speculated that it may antagonize functional methyltransferase activity [5].

Methylation itself has several molecular effects (Figure 1). It can directly interfere with the binding of transcription factors to inhibit replication [24]. Similarly, methyl-CpG binding proteins (MBPs) can bind to methylated DNA as well as regulatory proteins, again inhibiting transcription [25]. In addition, both DNMT1 [26] and MBPs, such as methyl-CpG-binding protein 2 (MeCP2) [27], can recruit histone deacetylase. Deacetylation of core histone tails by this enzyme results in tighter packing of DNA into chromatin, reducing the access of transcription factors [28], as well as possibly playing a role in restoring chromatin structure after DNA replication [5]. Finally, methylation may be important in the prevention of homologous recombination between the large numbers of repeated sequences in mammalian genomes [2], a destabilizing process which has been implicated in several diseases [29–31].

Interestingly, 5-methylcytosine undergoes spontaneous deamination to thymine at a higher frequency than cytosine deaminates to uracil, and this can generate T:A transition mutations if it is left unrepaired [32]. This transition may account for the fact that there exists a decreased CpG dinucleotide frequency relative to what would be predicted for the genome [33]. More importantly, these mutations are known to be involved in neoplasia, as, for example, in some p53 mutations [34].

DNA methylation in malignancy

Cancers display a particular pattern of methylation. Overall they are hypomethylated, but they do have specific regions of

![Figure 1](image-url)

*Figure 1. (A) DNA methyltransferase 1 (DNMT1) predominantly methylates hemi-methylated DNA during replication. (B) DNMT3a and DNMT3b predominantly methylate unmethylated DNA. (C) 5-Methylcytosine can undergo spontaneous deamination to thymine, generating T:A transition mutations. (D) Methyl-CpG binding proteins (MBPs) bind to methylated DNA; (E) DNMT1 and MBPs can recruit histone deacetylase, resulting in tighter packing of DNA into chromatin. (F) Methylation, MBPs and histone deacetylation inhibit transcription by interfering with transcription factor access.*
hypermethylation [3]. Hypomethylation is evident in the repetitive or parasitic elements [35–37], possibly leading to genetic instability through failed inhibition of homologous recombination [2] and allowing transcription of these normally repressed genes. As an example of the latter, mRNA of the LINE-1 transposable element has been found to be expressed in teratocarcinoma and bladder cancer cell lines [38]. The hypermethylated regions are CpG islands, CpG and GpC rich sequences of about 1 kb that lie proximal to gene promoters and enhance their efficiency [5]. These islands are associated with roughly half of all genes [39] and their methylation can repress transcription in a manner analogous to a mutation or deletion [3]. Such hypermethylation has now been demonstrated to silence a number of tumor suppressor genes. Table 1 provides several examples of such gene silencing in malignancy. The frequency of methylation within tumor types can vary widely; this variation is likely to be a product of the relatively small size of these studies, differing assay methods, and tumor and cell line heterogeneity. As an example of sampling variation, hMLH1 methylation is strongly correlated with the presence of microsatellite instability, and the prevalence of methylation in a sample is dependent upon whether the sample was selected for this factor. For a more exhaustive list of methylated genes, see the work of Santini et al. [7].

Perhaps the best studied of these methylated tumor suppressor genes is CDKN2A, which codes for the p16INK4a protein. This protein is a negative cell cycle regulator which functions by preventing cyclin D and cyclin dependent kinases 4 and 6 from phosphorylating retinoblastoma protein (Rb). In the absence of p16, phosphorylated Rb allows progression through the cell cycle [40]. Since they all lie in the same pathway, overexpression of cyclin D or loss of function of p16 or Rb facilitates cell propagation.

To assess the methylation of CDKN2A, Ottersen et al. [41] examined 33 cancer cell lines. Twelve of 12 lung cancer cell lines lacking a deletion or critical mutation in Rb or CDKN2A were methylated at exon 1 of the CDKN2A gene. While CDKN2A methylation rendered p16 undetectable by immuno-blot analysis, treatment with the methyltransferase inhibitor decitabine allowed p16 re-expression, consistent with reversal of methylation. Other investigators have also shown that methylation of CDKN2A silences p16 expression and that inhibitors of DNA methyltransferase can reverse this process [42, 43]. Studies of squamous cell carcinoma of the lung and cervix demonstrated both that CDKN2A suppression occurs in early pre-invasive lesions and that it becomes progressively more frequent with more advanced disease [44, 45]. Several cancers demonstrate CDKN2A suppression (Table 1).

While the amount of DNMT1 present in tumors has been found to be quite variable, it seems that low level increases of two- to four-fold above normal levels are most common [5]. In fact, the abnormal DNA methylation pattern may be more a product of the timing of DNMT1 induction, which is normally elevated in S phase [11]. The altered expression of regulatory proteins such as p21, which interferes with DNMT1 function, has also been implicated [46].

DNA methyltransferases should thus theoretically serve as a reasonable target for antineoplastic drugs. Prevention of methylation should limit damaging recombination and prevent both transposon transcription and the silencing of suppressor genes, ultimately inhibiting tumor growth and possibly inducing involution. In preclinical work, DNA methyltransferase inhibitors have reversed the growth of cancer cell lines and demonstrated antitumor effects in animal models, including prolongation of survival [47–50].

Inhibitors of DNA methylation

Several molecular variations of deoxycytidine have been developed, each modified at position 5 of the pyrimidine ring (Figure 2). This distinctive feature is responsible for inhibiting DNMT. Analogs such as ara-C and gemcitabine, which do not possess this change in the pyrimidine ring, do not inhibit methylation [7].

Four agents have been employed clinically: 5-azacytidine (azacitidine), 5-aza-2′-deoxycytidine (decitabine), 1-β-D-arabinofuranosyl-5-azacytosine (fazarabine) and dihydro-5-azacytidine (DHAC). These agents are not new, azacitidine and decitabine having been first synthesized in 1964 by Sörm and colleagues [51, 52]. These agents have been employed in numerous trials, as described below.

These drugs act by preventing methylation, but they also act through other mechanisms. After phosphorylation, they are incorporated into DNA or RNA [53]. Decitabine, for example, is incorporated to a greater extent into DNA, while azacitidine is incorporated preferentially into RNA [7]. When incorporated into DNA, these drugs covalently link with DNMT which may induce cell death by obstructing DNA synthesis [54–56]. They may also induce DNA damage through structural instability at the site of incorporation [57, 58]. DNMT is depleted by being bound to these agents and is thereby unavailable for methylation, resulting in significant demethylation after repeated replication [7]. Demethylation by decitabine has been shown to allow re-expression of silenced genes [59] and cellular differentiation [60]. Additionally, incorporation of these agents into RNA causes ribosomal disassembly, defective rRNA function and inhibited protein production [7]. However, azacitidine exhibits greater cytotoxicity during S-phase, supporting the greater importance of its DNA effects [61]. These drugs are inactivated by deamination by cytidine deaminase [53].

An alternative mechanism for the inhibition of DNMT is the use of antisense oligodeoxynucleotides (ODNs). These are relatively short synthetic nucleic acids designed to hybridize to a specific mRNA sequence. The hybridization can block mRNA translation and cause mRNA degradation [62]. Such antisense ODNs have been directed against DNMT mRNA and have caused a decrease in DNMT mRNA and protein
Table 1. Genes suppressed in malignancy

<table>
<thead>
<tr>
<th>Gene (protein function)</th>
<th>Malignancy</th>
<th>Methylation prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CDKN2A/p16</strong> (cell cycle inhibition)</td>
<td>Breast</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Cervix</td>
<td>3–42</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>10–40</td>
</tr>
<tr>
<td></td>
<td>Endometrium</td>
<td>0–9</td>
</tr>
<tr>
<td></td>
<td>Esophageal SCC</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Ewing’s sarcoma</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Gastric</td>
<td>40–41</td>
</tr>
<tr>
<td></td>
<td>Glioblastoma multiforme</td>
<td>25–61</td>
</tr>
<tr>
<td></td>
<td>Head and neck SCC</td>
<td>47–50</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
<td>0–63</td>
</tr>
<tr>
<td></td>
<td>Leukemia, acute lymphoblastic</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Leukemia, acute myeloid</td>
<td>0–38</td>
</tr>
<tr>
<td></td>
<td>Leukemia, chronic myelogenous</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Lymphoma, Hodgkin’s</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Lymphoma, non-Hodkin’s</td>
<td>9–83</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>25–61</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>0–10</td>
</tr>
<tr>
<td></td>
<td>Multiple myeloma</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Oligodendrogliaoma</td>
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</tr>
<tr>
<td></td>
<td>Osteosarcoma</td>
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</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Prostate</td>
<td>0–60</td>
</tr>
<tr>
<td></td>
<td>Renal</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>TCC</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Thyroid</td>
<td>30</td>
</tr>
<tr>
<td><strong>Rb1</strong> (cell cycle inhibition)</td>
<td>CNS lymphoma</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Glioblastoma multiforme</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Leukemia, acute myeloid</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Oligodendrogliaoma</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Retinoblastoma, familial</td>
<td>0–1</td>
</tr>
<tr>
<td></td>
<td>Retinoblastoma, sporadic</td>
<td>5–10</td>
</tr>
<tr>
<td><strong>hMLH1</strong> (DNA mismatch repair)</td>
<td>Cervix</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Colon, sporadic</td>
<td>6–84</td>
</tr>
<tr>
<td></td>
<td>Endometrium</td>
<td>41</td>
</tr>
<tr>
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<td>Gastric</td>
<td>8–32</td>
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<tr>
<td></td>
<td>Gliomas</td>
<td>10</td>
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<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
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</tr>
<tr>
<td></td>
<td>Ovarian</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>TCC</td>
<td>2</td>
</tr>
<tr>
<td><strong>BRCA1</strong> (DNA repair)</td>
<td>Breast, sporadic</td>
<td>11–33</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Leukemia</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ovarian</td>
<td>5–40</td>
</tr>
<tr>
<td><strong>VHL</strong> (tumor suppressor, function unknown)</td>
<td>Renal, sporadic</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>VHL syndrome</td>
<td>33b</td>
</tr>
</tbody>
</table>

*a*Cell line data.

*b*Percentage of patients with methylation in the absence of loss of heterozygosity. References available upon request.

CNS, central nervous system; SCC, squamous cell carcinoma; TCC, transitional cell carcinoma; VHL, von Hippel-Lindau.
levels, demethylation [63], normalization of gene expression and antitumor activity in mice [48]. In another application of ODNs, Bigey et al. [47] developed hairpin ODN sequences—short DNA sequences bent back upon themselves in a ‘U’ shape—as antagonists of DNMT. While forming complexes with DNMT and limiting its activity, they did not induce a net hypomethylation, despite inhibiting the growth of human non-small-cell lung cancer cells.

While the potential for interference with normal cellular activity is a concern, there are theoretical reasons why demethylation should have less of an effect upon normal cells than cancer cells. Methylation of CpG dinucleotides in promoter proximal regions is uncommon in normal cells so normal gene expression should be largely unaffected by demethylation. In addition, imprinted gene demethylation should be more important during embryogenesis than afterwards [8]. While reactivation of inactive X-chromosomes in women might be a concern after embryogenesis, fibroblast cells lines treated with decitabine appear to be unaffected, while neoplastic cell doubling times are prolonged [59].

There is evidence, however, that demethylation may be harmful in some settings. In leukemic cell lines, for example, hypomethylation of the drug resistance gene MDR1 has been found to be related to drug resistance, and decitabine was found to increase the expression of the MDR1 product, P-glycoprotein [64]. In mice, a diet deficient in choline and methionine, both of which are required for the synthesis of the methyl donor S-adenosyl-methionine, has been implicated in the hypomethylation of the oncogene Ha-ras and the development of hepatocellular carcinomas [65]. While this may be less important in the setting of advanced cancer, it may have implications for long-term adjuvant therapy. Finally, in a rat model, azacitidine has been shown to be carcinogenic, while decitabine has not [66]. Ultimately, only clinical trials can clarify whether these findings translate into adverse outcomes.

**Decitabine**

*Preclinical studies.* Decitabine differs from the natural nucleoside deoxycytidine by the presence of a nitrogen at position 5 of the cytosine ring (Figure 2). Like ara-C and fazarabine, it must be phosphorylated to be activated [4]. Decitabine has demonstrated cytotoxic effects and growth inhibition in cell lines [59, 67] and it has improved survival in leukemic mice [68]. It has induced functional and morphological terminal differentiation in human leukemic cells [69, 70]. In both cell lines and *ex vivo* cells from human patients with leukemia treated with decitabine, decitabine has been shown to reverse methylation [68, 71]. Furthermore, decitabine has allowed re-expression of p16 and hMLH1 in various cell lines [59, 72–74].

![Figure 2. Ara-C and the DNA methyltransferase inhibitors, as compared to the natural nucleoside, deoxycytidine. The arrow indicates position 5 of the pyrimidine ring, the alteration of which inhibits methylation at this site.](image)
Phase I and II clinical studies in solid tumors. Several phase I and phase I–II studies have now been performed, typically employing multiple infusions per day or continuous drug infusions. In the first adult study, 1-h infusions were given every 7 h for three doses every 21 days, a schedule developed to overcome time-related drug decomposition. With the recommended dose of 75 mg/m² divided into three doses, the dose-limiting toxicity was myelosuppression, with a delayed nadir at day 22. Granulocytopenia was more prominent than thrombocytopenia, and less common toxicities included a probable drug-induced moderate creatinine elevation in three of 21 patients, and mild nausea and vomiting in six patients. All patients had solid tumors, and one patient with an undifferentiated carcinoma of the ethmoid sinus had a partial response [75].

Most investigators have administered decitabine as a 1-h infusion of 75 mg/m² every 7–8 h for three doses. Cycles were repeated every 5 weeks (Table 2). A number of these phase II studies were conducted by the European Organisation for Research and Treatment of Cancer (EORTC) [76–78]. In a report on 101 patients treated with this regimen, only one of 18 patients with untreated melanoma had a partial response [76]. Stable disease was seen in 7% of 42 colorectal carcinomas, 15% of 27 squamous cell carcinomas of the head and neck, 22% of 18 melanomas and 14% of 14 renal cell carcinomas, but no other response was seen.

In two other EORTC trials, decitabine was found to be inactive in treated non-seminomatous testicular cancer [77] and advanced ovarian carcinoma [78]. Similarly, two non-EORTC trials revealed no response among patients with hormone-independent metastatic prostate cancer [79] and recurrent cervical cancer [80].

In a phase I–II study in metastatic non-small cell lung cancer, no response was seen among nine assessable patients treated with 200–600 mg/m² intravenously (i.v.) over 8 h every 5–6 weeks, although four patients had stable disease for a minimum of two cycles [81]. Unfortunately, the addition of cisplatin to decitabine has not improved outcomes [82].

Thus, in a wide array of advanced solid tumors, decitabine has been ineffective in generating objective responses and stable disease is uncommonly achieved. Currently, investigators are trying to improve activity by using a prolonged, 7 day infusion and by combining decitabine with carboplatin (K. Mettinger, personal communication).

Phase I and II clinical studies in leukemia. Fortunately, hematological malignancies have proven more responsive to decitabine therapy. Several single-agent studies have been conducted in acute leukemia (Table 3). In general, toxicities have included prolonged granulocytopenia and thrombocytopenia, and common nausea, vomiting, mucositis, diarrhea and alopecia.

The first pediatric phase I study found the optimal anti-leukemic dose to be 36–80 mg/kg infused over 36–44 h, with fresh infusate prepared every 6–12 h. At this dose, two of nine children with refractory leukemia obtained an M₃ marrow (<5% blasts) [83].

In a phase I–II study, relapsed leukemic patients were treated with decitabine 37–67 mg/kg at an infusion rate of 1 mg/kg/h [84]. Boluses of 1 mg/kg were given every 10 h to account for hepatic cytidine deaminase activity. In this largely pediatric population with almost all patients having received prior ara-C, a complete remission (CR) was obtained in four of 21 patients with ALL (19%) and two of six patients with AML. Partial responses were obtained in three (14%) and one patient, respectively. Remissions were short, typically of 1 month duration.

In fragile patients and patients requiring salvage treatment, similar response rates were found using 90–120 mg/m² decitabine in a 4-h infusion three times per day for 3 days [85, 86].

Single-agent studies in chronic myelogenous leukemia (CML) have also produced positive results. Employing a regimen of 75–100 mg/m² i.v. over 6 h every 12 h for 10 doses, Kantarjian et al. [87] treated 17 patients with accelerated disease and 20 patients with blast crisis. In the accelerated phase, one patient had hematological improvement (marrow CR with peripheral blasts <5% and platelets <100 × 10⁹/μl) and two patients achieved a PR, and six patients (35%) returned to chronic phase. In the blastic phase, two CRs (10%) were obtained and three patients (15%) had hematological improvement.

Studying the blastic phase CML in an overlapping cohort of 31 patients using the same q12 h regimen, Sacchi et al. [88] found overly prolonged myelosuppression at doses ≥100 mg/m². Two CRs (6%) and four hematological improvements (13%) were seen. The level of activity was comparable to another cohort from the same center receiving intensive chemotherapy.

Non-randomized trials employing combination chemotherapy are the next important step in the development of new treatments, but render the efficacy contribution of the new drug difficult to assess. In a preliminary report of first-line therapy for patients with acute myeloid leukemia, Schartschmann et al. [89] noted six CRs in six evaluable patients treated with decitabine 90 mg/m² over 4 h on days 1–5 and daunorubicin 50 mg/m²/day i.v. bolus on days 1–3, with five patients requiring only one cycle. In another small trial of relapsed or resistant leukemia, decitabine 250–500 mg/m² over 6 h twice daily for 3–6 days was given alone or as a lower dose of 125–250 mg/m² combined with amsacrine 120 mg/m² over 1 h on days 6 and 7 [90]. Five of six patients refractory to ara-C were treated with monotherapy, and no refractory patient had a response. In contrast, 10 of 11 patients with relapse but no prior treatment with ara-C received combination chemotherapy and eight (73%) achieved a CR while two achieved a PR, with one patient dying of toxicity. Of three patients alive in CR, the longest is 12 months out from induction. An unusual toxicity of sterile peritonitis was seen in six patients.

In a larger phase II EORTC study, Willemze et al. [91] combined decitabine 125 mg/m² as a 6-h infusion every 12 h
Table 2. Phase II trials with single-agent DNMT inhibitors in solid tumors and lymphoma

<table>
<thead>
<tr>
<th>Agent, dose and schedule</th>
<th>Site [reference]</th>
<th>n</th>
<th>CR (%)</th>
<th>PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Azacitidine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500–750 mg/m²/day for 5 or 10 days q5 weeks</td>
<td>Gastrointestinal [189]</td>
<td>29</td>
<td>–</td>
<td>1 (3)</td>
</tr>
<tr>
<td>60 mg/m² days 1–10 then 100 mg/m² BIW maintenance</td>
<td>Breast [190]</td>
<td>27</td>
<td>–</td>
<td>2 (7)</td>
</tr>
<tr>
<td>1.6 mg/kg/day days 1–10 then 2.4 mg/kg BIW maintenance</td>
<td>Breast [107]</td>
<td>29</td>
<td>–</td>
<td>6 (21)</td>
</tr>
<tr>
<td></td>
<td>Lung [107]</td>
<td>24</td>
<td>–</td>
<td>1 (4)</td>
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<td></td>
<td>Colorectal [107]</td>
<td>26</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Melanoma [107]</td>
<td>12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Hodgkin’s disease [107]</td>
<td>6</td>
<td>–</td>
<td>1 (17)</td>
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<td></td>
<td>NHL [107]</td>
<td>8</td>
<td>–</td>
<td>2 (25)</td>
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<td></td>
<td>Miscellaneous [107]</td>
<td>59</td>
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<td>9 (15)</td>
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<td></td>
<td>NSC lung [108]</td>
<td>38</td>
<td>–</td>
<td>3 (8)</td>
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<tr>
<td>150–225 mg/m²/day days 1–5 every 3 or 4 weeks</td>
<td>Testicular [108]</td>
<td>4</td>
<td>–</td>
<td>2 (50)</td>
</tr>
<tr>
<td></td>
<td>Other [108]</td>
<td>125</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Ewing’s sarcoma [191]</td>
<td>7</td>
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<td>–</td>
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<td>Osteosarcoma [191]</td>
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<tr>
<td></td>
<td>NSC lung [192]</td>
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<td>1 (4)</td>
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<tr>
<td>150 mg/m³ BIW for 6 weeks</td>
<td>Kidney [192]</td>
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<td>Other [192]</td>
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</tr>
<tr>
<td>150 mg/m³/day days 1–5 CIVI q3 weeks</td>
<td>Testicular [193]</td>
<td>16</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>DHAC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 g/m² CIVI day 1 q28 days</td>
<td>NSC lung [168]</td>
<td>17</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Mesothelioma [170]</td>
<td>15</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Melanoma [169]</td>
<td>40</td>
<td>2 (5)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>1.5 g/m³/day CIVI days 1–5 q21 days</td>
<td>Mesothelioma [171]</td>
<td>41</td>
<td>1 (2)</td>
<td>2 (5)</td>
</tr>
<tr>
<td><strong>Fazarabine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 or 45 mg/m³/day days 1–5</td>
<td>High grade gliomas [154]</td>
<td>27</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Head and neck [155]</td>
<td>18</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Ovarian [157]</td>
<td>19</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.75 or 2 mg/m³/h CIVI for 3 days q3–4 weeks</td>
<td>NSC lung [158]</td>
<td>23</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Breast [159]</td>
<td>14</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Colon [160]</td>
<td>18</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Colorectal [161]</td>
<td>15</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Pancreatic [162]</td>
<td>14</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Decitabine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 mg/m² every 7–8 h for three doses q5–8 weeks</td>
<td>Colon [76]</td>
<td>42</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Head and neck [76]</td>
<td>27</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Melanoma [76]</td>
<td>18</td>
<td>–</td>
<td>1 (6)</td>
</tr>
<tr>
<td></td>
<td>Kidney [76]</td>
<td>14</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>Testicular [77]</td>
<td>14</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>Prostate [79]</td>
<td>14</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>Ovarian [78]</td>
<td>24</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Cervix [80]</td>
<td>15</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

BIW, twice weekly; CIVI, continuous intravenous infusion; CR, complete response; DHAC, dihydro-5-azacitidine; DNMT, DNA methyltransferase; NHL, non-Hodgkin’s lymphoma; NSC, non-small cell; PR, partial response; q, every.
for 6 days with either amsacrine 120 mg/m² on days 6 and 7 or idarubicin 12 mg/m²/day on days 5–7. In this cohort of relapsed patients with predominantly AML, eight of 30 patients (27%) treated with amsacrine and 15 of 33 patients (45%) treated with idarubicin had a CR, although the latter group had greater gastrointestinal and infectious toxicity.

In summary, decitabine has demonstrated single-agent efficacy in acute leukemia, modest activity in progressing CML and tolerability in combined regimens. Studies are ongoing in cohorts with leukemia and myelodysplastic syndrome (K. Mettinger, personal communication).

Azacitidine

**Preclinical studies.** Azacitidine differs from the nucleoside cytidine only by a nitrogen in place of the fifth carbon (Figure 2) [92]. Unfortunately, the drug is hydrolysed in neutral and basic media so that fresh drug must be prepared regularly during prolonged infusions. In models of solid and hematological malignancy, azacitidine has demonstrated activity and it has improved the lifespan of leukemic mice [93–96]. It has also induced both demethylation and differentiation of leukemic cell lines [97, 98]. In another line of evidence supporting the activity of demethylation, azacitidine caused hypomethylation of the γ-globin gene and induction of fetal hemoglobin production in a human and baboons [99, 100].

**Phase I trials.** Several phase I trials have been performed with azacitidine. While their methodologies were not standard, most trials found that myelosuppression was dose limiting, delayed and prolonged. Nausea and vomiting was generally worse in the trials of bolus rather than infusion therapy. Excretion is almost entirely renal [93, 101].

In a trial employing an i.v. bolus regimen daily for 10–15 days, Weiss et al. [102] determined that doses beyond 12–16 mg/kg caused high levels of hematological toxicity,
more commonly granulocytopenia than thrombocytopenia. Myelosuppression typically occurred 20–30 days after the start of therapy and lasted 1–5 weeks. Nausea was common and usually mild to moderate, with less severe diarrhea and stomatitis across various dosing levels. Encouragingly, seven of 11 patients with measurable recurrent breast cancer in soft tissue, two of five patients with cutaneous melanoma and two of six patients with colon cancer had PRs.

Vogler et al. [103] recommended 150 mg/m\(^2\) weekly i.v. bolus based on a phase I trial of 25 patients. At this level, moderate to severe nausea and vomiting were ubiquitous and all patients had moderate to severe granulocytopenia. As with the trial of Weiss et al. [105], myelosuppression was delayed and prolonged.

In an effort to avoid the nausea associated with bolus therapy, Lomen et al. [104] employed a 5-day continuous infusion using 150 mg/m\(^2\)/day. No nausea or vomiting was detected but two patients had significant disorientation, somnolence and extreme apathy. Granulocytopenia was the dominant toxicity. In another trial employing infusion, Vogler et al. [105] treated 21 patients with metastatic solid tumors and 45 patients with leukemia with 5-day continuous infusions of 50–200 mg/m\(^2\)/day. Granulocytopenia was excessive in solid tumor patients treated at the 150 mg/m\(^2\) level when patients were treated every 2 weeks but was better every 3 weeks. Thrombocytopenia was minimal. Nausea and vomiting were common, moderate and usually controllable in both solid and hematological malignancies. In AML, responses were seen at doses of 100 mg/m\(^2\)/day and higher. An alternative administration method employed 275–800 mg/m\(^2\)/subcutaneously daily for 10 days and resulted in excessive treatment-related mortality [106]. Two patients had lethal thrombocytopenic bleeding and three died with hepatic coma, although all three had bulky liver involvement of tumor. Although other studies have demonstrated elevated liver enzymes, hepatic coma is exceptional.

Phase II trials in solid tumors. Table 2 shows the disappointing outcomes of phase II studies, where response rates have generally failed to exceed 10%. The exceptions include PRs seen in six of 29 patients with breast cancer [107], three of 14 patients with lymphomas [107] and two of two patients treated for embryonal cell carcinomas [108].

Phase I and II trials in pediatric acute leukemia—first-line treatment. In contrast, activity has been demonstrated in several trials in pediatric leukemias (Table 3). In an early report, Hrodek and Vesely [109] recorded results on 20 newly diagnosed leukemic children treated with prednisone and azacitidine 1–2 mg/kg daily, usually for 2 weeks. Complete responses were obtained in 10 of 12 children with ALL and one of two children with AML, but not in seven children treated with azacitidine alone.

Two further combination trials have studied the first line use of azacitidine. Employing the VAPA regimen in AML, Weinstein et al. [110] induced remission with vincristine, doxorubicin, prednisolone and ara-C, and consolidated and maintained the remission with an intensive 14 months of combination therapy including azacitidine. Sixty-one children were treated, with a complete remission rate of 74%. For patients <18 years of age, 56% of responders were disease free at 3 and 5 years, whereas for those ≥18 years of age, the figures were 45% at 3 years and 27% at 5 years. Baehner et al. [111], treating acute non-lymphocytic leukemia (ANLL) in 163 children, used the D-ZAPO regimen, which included induction with daunomycin, azacitidine, ara-C, prednisone and vincristine, and maintenance with 6-thioguanine replacing daunomycin and prednisone. Of 163 children, 56% achieved <5% marrow blasts with induction, with a median remission length of 11 months. In an extension of this study, a further 166 children with ANLL were treated with D-ZAPO and 63% achieved a marrow blast count of <5% [112]. The maintenance regimen, which was expanded to include intrathecal methotrexate and continuous rather than intermittent oral 6-thioguanine, appeared to improve maintenance of CR, with a rate of 32.5% after 2 years versus 17.9% in the previous study.

While these trials were sizeable and employed combination chemotherapy, no adequate phase III study has been performed, making the true contribution of azacitidine difficult to estimate.

Phase I and II trials in pediatric acute leukemia—relapsed or refractory disease. Refractory and relapsed leukemia have also been studied in the pediatric population. Using a 5-day course of bolus azacitidine in a phase I trial, Karon et al. [113] found the maximal acceptable dose to be between 150 and 200 mg/m\(^2\). Among 37 previously treated children, five of 15 children with AML achieved a CR, as did one of 22 children with ALL.

In previously treated children with ANLL, a combination of weekly vincristine, azacitidine daily for 4 days and prednisone produced eight CRs and seven PRs in 53 evaluable children [114]. In 38 children with refractory ANLL previously treated with both anthracyclines and ara-C, Look et al. [115] gave etoposide daily for 3 days followed by azacitidine daily for 2 days, and repeated cycles every 9–16 days. Since out of the first 16 patients only one achieved a CR, the next 22 patients were treated with double the dose of drugs, resulting in 10 CRs but also two deaths during induction. Further intensifying this regimen, Hakami et al. [116] treated 96 children with relapsed or refractory ANLL with two to three sequential courses of etoposide 250 mg/m\(^2\) daily for 3 days followed by azacitidine 300 mg/m\(^2\) daily for 2 days. Thirty-two of these patients had previously received azacitidine. Forty-three children (45%) achieved complete durations with expected toxicities of 100% severe neutropenia (<1000/µl), 86% severe thrombocytopenia (<50000/µl), 39% severe nausea and vomiting and 15% severe mucositis.
After demonstrating feasibility and some success in a pilot study [117], Steuber et al. [118] conducted a randomized phase II trial comparing amsacrine 100 mg/m2/day on days 1–5 and etoposide 200 mg/m2/day on days 1–3 with the same regimen plus azacitidine 250 mg/m2/day over 1 h on days 4–5. The overall complete response rate was 28% for the combination of amsacrine and etoposide and 39% when azacitidine was added. The difference was only statistically significant for the subgroup of patients with refractory but not relapsed disease.

Unfortunately, no adequately powered randomized trial has explored azacitidine in the setting of relapsed pediatric leukemia.

Phase I and II trials in adult acute leukemia—first-line treatment. Azacitidine has been extensively tested against leukemias in adults (Table 3). In a small study, Cassileth et al. [119] found that azacitidine did not add to the CR rate in nine of 19 patients who failed to achieve CR after two cycles of daunorubicin and ara-C, while sequential consolidation therapy with all three drugs generated a remission duration of 10 months, similar to prior studies.

As discussed in the pediatric section, Weinstein et al. [110] used the VAPA induction regimen followed by consolidation and maintenance including azacitidine, achieving a 65% CR rate in adults. Disease-free survival rates were 45% at 3 years and 27% at 5 years.

In a large, complex, multi-armed randomized study, Vogler et al. [120] induced remission in 335 of 508 patients (66%) with AML by administering daunorubicin daily for 3 days and infusing ara-C over 7 days. Patients in CR or PR were then randomized to one of three consolidation regimens: (A) continuous infusional azacitidine; (B) azacitidine as in ‘A’ plus bolus β-deoxythioguanosine; or (C) ara-C, thioguanine and daunorubicin, for 5 days. There was no difference in the relapse rates in the three arms during the consolidation period. After consolidation, patients in continued remission were randomized to one of three maintenance regimens: (D) daunorubicin and infusional ara-C each 13 weeks for 1 year; (E) subcutaneous BCG for 1 year; or (F) a combination of ‘D’ and ‘E’. Regimens containing BCG were significantly inferior, with median remission durations of 9 months compared with 17 months. It was not assessed whether patients who received daunorubicin and ara-C during maintenance fared better if they received azacitidine during consolidation. This trial was unable to determine whether azacitidine consolidation contributed to long-term disease-free survival because of the second randomization.

Phase I and II trials in adult acute leukemia—relapsed or refractory disease. As a single agent, azacitidine was tested in 28 patients with refractory or relapsed acute leukemia [121]. In this early study, 400 mg/m2/day i.v. bolus on days 1–5 induced a CR in three of 18 patients (18%) with AML and one of 10 patients with ALL, with fatal drug-induced hypotension occurring in one patient. In a small randomized phase II study, Levi et al. [122] compared azacitidine to guanzole among previously treated patients with ANLL. Five CRs were seen among 18 patients receiving azacitidine 200–250 mg/m2/day bolus on days 1–5, while none were seen among the patients receiving guanzole.

Two larger studies by Saiki et al. [123, 124] examined several single-agent schedules of azacitidine in patients with relapsed acute leukemia. The tolerable dose was a continuous infusion of 150–200 mg/m2/day for 5–10 days. Unfortunately, CR rates did not exceed 24% at best, and were extremely poor for patients with ALL.

Studies of azacitidine in refractory and relapsed disease have also demonstrated activity in combination with several agents. Adding an amsacrine 150 mg/m2 i.v. push to the same dose of azacitidine daily for 5 days, Kahn et al. achieved a CR in seven of 12 patients (58%) who had previous remissions with daunorubicin and ara-C [125]. None of six ANLL patients without previous remission were successfully treated. Among patients with AML in first relapse, another small report noted seven CRs among 10 patients with this combination [126]. In a third study using this combination, Winton et al. [127] achieved 10 CRs in 52 patients (19%) with relapsed AML, three CRs in 28 patients (11%) with refractory AML and one of 12 patients with refractory ALL, with a cost of some added mucositis, hepatic and cardiac toxicity. While the higher tested doses of 200 mg/m2/day CIV azacitidine on days 1–4 and amsacrine 125–150 mg/m2 over 2–3 h on days 5–8 appeared optimal, the high remission rate of Kahn’s study could not be replicated.

Based on a pilot study demonstrating reasonable activity in patients with relapsed AML [128], Omura et al. [129] studied the combination of azacitidine 150 mg/m2/day CIVI and the purine analogue β-2′-deoxythioguanosine in patients with relapsed leukemia. Among 81 assessable patients with AML, 16 CRs were achieved (20%), while none of six patients with ALL had a CR. With toxicity being principally hematological, the authors concluded that the results were not superior to their experience with azacitidine alone.

A study of 29 patients treated with 5 days of azacitidine 200 mg/m2/day and 175 mg/m2/day of the anthracycline zorubicin revealed some activity in this cohort who had received previous ara-C and anthracyclines [130]. Seven of 10 relapsed patients reached a CR as opposed to one of 19 patients with refractory disease.

A number of trials have shown little benefit of combination therapy. Goldberg et al. [131] concluded that adding azacitidine to relapsed patients having an inadequate marrow response to mitoxantrone did not improve remission rates, although these patients would by definition be refractory. Adding pyrazofurin, an inhibitor of pyrimidine biosynthesis, to azacitidine has failed to improve outcomes and generated prohibitive mucositis and dermatitis [132–134]. Three further studies have tested azacitidine with other agents. In studies with carboplatin, with methyl-GAG, and with vinblastine and
etoposide, outcomes have not been sufficiently favorable to warrant further study [135–137].

Thus, while numerous studies have shown that azacitidine has activity both as first-line therapy and as treatment for relapse in leukemia, the lack of phase III trials does not allow conclusive statements about its role in this disease.

**Clinical studies in chronic myelogenous leukemia in blast crisis.** Chronic myelogenous leukemia in blast crisis is a highly refractory disease with poor survival. Azacitidine has added little to the treatment of this disease. In a toxic regimen composed of azacitidine, daunorubicin, 6-thioguanine and ara-C, only four PRs were observed among 30 patients [138]. Schiffer et al. [139, 140] studied the combination of azacitidine 250 mg/m²/day CIVI and daily i.v. etoposide. Myelosuppression was severe and only one patient achieved a CR. In a more successful study, Dutcher et al. [141] treated 40 patients in blast crisis with mitoxantrone daily times three plus azacitidine daily times five. Five CRs and two PRs were achieved, but this was not better than previous trials using mitoxantrone alone or in combination with ara-C. In addition, a number of studies included patients with CML in blast crisis among larger cohorts with acute leukemias and found poor responses in the CML subgroup [121, 123–125, 127–129, 131].

Outside of accelerated stage CML, studies continue to be performed with azacitidine. Phase I and II trials are evaluating azacitidine in combination with phenylbutyrate, a histone deacetylase inhibitor, in solid and hematological malignancies. No single-agent study is currently being conducted on solid tumors in North America [142].

**Fazarabine**

**Preclinical studies.** Fazarabine was synthesized in the 1970s as a molecule with the arabinose sugar of ara-C and the triazine ring of azacitidine (Figure 2) [143]. Like ara-C, fazarabine is activated by deoxycytidine kinase and cross-resistance has been demonstrated [144]. Cell lines have been shown to exhibit hypomethylation when exposed to fazarabine [145]. Therapeutic activity was seen in murine leukemic and solid tumor xenograft models, including colon, lung, medulloblastoma and mammary tumors [145, 146].

**Phase I and II clinical studies.** Phase I studies have examined bolus administration and intravenous infusions. The dose-limiting toxicity with these schedules was predominantly granulocytopenia with some thrombocytopenia, while moderate nausea and vomiting was a secondary problem. Rare responses were seen, with stable disease being slightly more common [147–153].

As shown in Table 2, several phase II studies of fazarabine in solid tumors have been published, with little evidence of activity. Using a daily bolus for 5 days, no response was seen in studies of high-grade gliomas or advanced head and neck cancer, although six of 18 patients in the latter study exhibited stable disease [154, 155]. Two studies in gynecological cancers employed the same bolus regimen using 30 mg/m²/day. Nine of 19 patients with advanced ovarian cancer exhibited stable disease, as did seven of 19 patients with advanced cervical cancer, although the duration was unspecified and no response was seen [156, 157].

Infusions have also been disappointing, with no response seen in studies of advanced non-small-cell lung, breast, colorectal or pancreatic cancer [158–162].

The poor activity of this drug in several phase II trials has arrested further investigation. The paucity of clinical studies in hematological malignancies is perhaps secondary to both the known cross resistance with ara-C and the poor outcomes in solid tumor studies.

**DHAC**

**Preclinical studies.** To bypass the problem of instability of azacitidine in aqueous solution, Beisler et al. [163] reduced the 5,6-imino double bond to produce DHAC (Figure 2). The resulting stable water-soluble compound allowed a slow infusion, with the hope of avoiding acute toxicities. In vitro, DHAC has inhibited RNA synthesis and methylation and has slowed cell cycle progression in leukemic and ovarian cell lines [164, 165]. In comparison to azacitidine and decitabine, DHAC has shown less inhibition of DNA methylation [166]. It has shown reproducible activity in both murine leukemias and xenografts [167].

**Phase I and II clinical studies.** In their phase I study, Curt et al. [167] demonstrated a dose-limiting toxicity of grade 3 non-cardiac chest pain at a 24 h infusional dose of 7 g/m². Lesser toxicities included nausea and vomiting, but no other toxicity was seen, including myelosuppression. Transient responses were seen in two patients with heavily treated diffuse histiocytic lymphoma, but both relapsed prior to cycle two at day 28. One patient with progressive Hodgkin’s disease had stable disease for 7 months.

Unfortunately, subsequent phase II studies were not fruitful (Table 2) [168, 169]. The greatest efforts have been directed towards mesothelioma, but neither single-agent DHAC nor the addition of cisplatin have proven useful [170–172].

Dihydro-5-azacytidine has shown limited activity in solid tumors and there are no published studies in leukemia. Clinical research with DHAC has ceased.

**MG98**

MG98 is an antisense oligodeoxynucleotide directed against the 3′ untranslated region of DNMT1 mRNA. This agent has shown an ability to inhibit DNMT1 expression without effecting DNMT3, and to cause demethylation with re-expression of p16 in bladder and colon cancer cell lines [173]. In nude mice bearing human lung and colon xenografts, MG98 caused tumor growth inhibition [173]. Effects may be synergistic in
combination with decitabine [174]. Work in Cynomolagus monkeys demonstrated weight loss only at the highest bolus dose, with no other sign of toxicity [175].

In a phase I study employing a 21-day infusion, an 80 mg/m²/day dose was established as tolerable [176; Davis AJ, Gelmon KA, Siu LL and colleagues, unpublished data]. However, dose-limiting toxicities of transaminits, thrombocytopenia and fatigue prohibited higher doses and it was suggested that future studies might better employ an intermittent schedule. A variable decrease in DNMT mRNA levels was demonstrated in six of 10 patients. In a second phase I study, MG98 was administered as a twice weekly 2-h infusion for 3 of every 4 weeks [177; Stewart DJ, Donehower R, Eisenhauer E and colleagues, unpublished data]. Transaminitis was again seen, as were low grade PTT prolongations, anemia, weakness, fever, confusion, nausea and anorexia. The recommended phase II study dose was 360 mg/m²/infusion. One patient with renal cell carcinoma had a PR lasting 9 months. Based on this information, phase II studies are underway with the twice weekly bolus regimen.

Conclusions

While clinical trials continue with DNMT inhibiting agents, it is evident that the deoxycytidine analogs have limited efficacy in solid tumors. In contrast, these analogs have the potential to improve outcomes in acute leukemia. It is extremely disappointing, however, that despite innumerable combination phase II trials in acute leukemia, no well-designed trial of adequate sample size has been conducted to assess the true impact of either decitabine or azacitidine. The antisense oligodeoxynucleotide MG98 requires further study to assess its merits. Other diseases not discussed in this article, such as the myelodysplastic syndrome, also appear to be reasonable targets for further investigation.

Since there is ample evidence to show that these agents reverse methylation in malignant clones and to demonstrate that they inhibit tumor growth, why has this not translated into better clinical outcomes? One can hypothesize that drugs with a more cytostatic than cytotoxic mechanism might be more efficacious in less advanced settings. To date, however, there is no evidence to support this notion. A better question might be whether we are measuring the correct outcome. Two points are notable in this regard: first, it has been shown that there is inconsistency in the relationship between response in phase II trials and survival in phase III trials [178–181], and second, use of the response measure may in fact lead to the premature termination of drug development [182]. To avoid these pitfalls, measures such as time to progressive disease (TPD) or initial progressive disease (PD) rates might be used in non-randomized ‘screening’ studies. Agents with long TPD and low PD rates may have the potential to improve survival when evaluated in subsequent randomized trials. It is interesting that several cases of disease stabilization occurred with decitabine [76] and stabilization was common in a large study with azacitidine [107], although durations were not specified. However, while evidence exists to support the correlation of TPD with survival [179, 183–186], neither endpoint has been adequately validated as a method of screening new agents in the phase II setting [187].

Finally, it may also be the case that DNMT inhibitors are only half of the treatment equation, and combination therapy may be necessary. As discussed, methylation induces recruitment of histone deacetylase, which inhibits transcription, and agents have been devised that inhibit this deacetylase [188]. The combination of a DNMT inhibitor and a histone deacetylase inhibitor might more completely activate tumor suppressor genes. Alternatively, combination with agents affecting completely different mechanisms may be necessary. Ongoing studies are examining these possibilities [142] (K. Mettinger, personal communication).

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