Review Article
DNA Methylation Events as Markers for Diagnosis and Management of Acute Myeloid Leukemia and Myelodysplastic Syndrome

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Received 8 May 2017; Revised 17 July 2017; Accepted 30 July 2017; Published 6 September 2017

During the onset and progression of hematological malignancies, many changes occur in the cellular epigenome, such as hypomethylation or hypermethylation of CpG islands in promoter regions. DNA methylation is an epigenetic modification that regulates gene expression and is a key event for tumorigenesis. The continuous search for biomarkers that signal early disease, indicate prognosis, and act as therapeutic targets has led to studies investigating the role of DNA in cancer onset and progression. This review focuses on DNA methylation changes as potential biomarkers for diagnosis, prognosis, response to treatment, and early toxicity in acute myeloid leukemia and myelodysplastic syndrome. Here, we report that distinct changes in DNA methylation may alter gene function and drive malignant cellular transformation during several stages of leukemogenesis. Most of these modifications occur at an early stage of disease and may predict myeloid/lymphoid transformation or response to therapy, which justifies its use as a biomarker for disease onset and progression. Methylation patterns, or its dynamic change during treatment, may also be used as markers for patient stratification, diagnosis prognosis, and response to treatment. Further investigations of methylation modifications as therapeutic biomarkers, which may correlate with therapeutic response and/or predict treatment toxicity, are still warranted.

1. Introduction
Cancer is generally defined as a group of diseases governed by an accumulation of genetic mutations that are considered to be the major cause of uncontrolled cellular growth [1]. However, epigenetic mechanisms, which alter gene expression without affecting the genetic sequence itself, are also significantly involved in cancer development [2, 3]. Genetic modifications comprise mutations in tumor suppressor genes and oncogenes, both of which skew the balance towards dysregulated cellular proliferation. Epigenetic events are more complex, requiring modifications in chromatin structure or interference with RNA transcripts, and mostly include DNA methylation, histone modifications, nucleosome remodeling, and noncoding RNAs [4]. Thus, during the onset and progression of hematological malignancies, many changes can occur in the cellular epigenome, such as hypomethylation or increases in the methylation of CpG islands in promoter regions of key genes [5].

DNA methylation occurs by the addition of a methyl group (CH₃) to the 5' carbon of cytosines that are followed by guanines (CpG sites), resulting in 5-methylcytosine (5-mC). This event is catalyzed by members of the DNMT (DNA methyltransferases) family, mainly DNMT1, DNMT3A, and DNMT3B. DNMT1 is localized in the replication fork during DNA replication, where the new DNA strand is
formed. Therefore, this enzyme binds to the daughter strand and methylates it to precisely mimic the original methylation pattern before replication [6, 7]. DNMT3A and DNMT3B present structural and functional similarities. These enzymes are able to introduce methylation into naked DNA, being associated with de novo DNA methylation and, thus, demonstrating an important role in normal development and disease [7, 8]. Methyltransferase of promoter CpG islands usually occurs in or near promoter regions and may disturb the binding of transcription factors. This alone not only contributes to the regulation of gene expression but may also contribute to tumor suppressor gene silencing [9]. Not only that, loss of preserved epigenetic patterns can lead to activation or inhibition of different cellular signaling pathways, which can, invariably, lead to cancer, and it is known that genes that control cell cycle and DNA repair can be mutated or silenced by hypermethylation of their promoter sites [2, 10].

Several studies have already identified mutations in genes that encode crucial epigenetic regulators of gene transcription, such as IDH1 (isocitrate dehydrogenase 1) and IDH2 (isocitrate dehydrogenase 2), both of which catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate, TET2 (ten eleven translocation 2) which is an α-ketoglutarate-dependent dioxygenase involved in the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) and DNMT3A, all of which have been described in hematological malignancies [11–16]. Moreover, DNA methylation is maintained on subsequent cells by DNMT1, responsible for reproducing the parent strand’s methylation pattern in the daughter strand [8], and mutations in the DNMT family are frequently described in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) [17–19], correlating with poor prognosis [20]. Recently, Spencer and collaborators described that hypomethylation is an initiating event in AML patients with the DNMT3A R882H mutation and DNMT3A-dependent CpG island hypermethylation occurs in consequence of disease progression [21].

The aim of this review is to demonstrate how DNA methylation acts as a potential biomarker for the diagnosis, management, and progression of hematological malignancies, focusing on myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).

1.1. Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukemia (AML). MDS is a heterogeneous condition of clonal hematopoietic disorders characterized by ineffective erythropoiesis, dysplastic features, chromosomal abnormalities, and increased risk of AML progression. It presents a diverse phenotype, being stratified into low-risk or high-risk disease [22]. Hypomethylating agents (such as 5-azacitidine and 5-aza-2'-deoxycytidine, also known as decitabine), or thalidomide analogues (such as lenalidomide), are already employed for the treatment of MDS. Hematopoietic growth factors, immunosuppressive therapy, and hematopoietic stem cell transplantation are also employed, frequently as second-line therapy [23–25]. The choice of treatment is based on each patient’s clinical parameters, such as karyotype, bone marrow blast percentage, and extent of cytopenia, among others [26]. Although bone marrow transplantation is the only choice offering a potentially curative treatment, few patients undergo this procedure because of their advanced age, medical comorbidities, and the limited availability of matching stem cell donors [27]. As supportive therapy, blood and platelet transfusions can be performed, as well as the use of iron chelators and antibiotics [23]. In spite of bone marrow transplantation being the only curative treatment described to date, a randomized phase III trial found that elderly patients with high-risk MDS, complex karyotype, and autosomal monocytes who were treated with decitabine showed higher progression-free survival when compared to patients receiving supportive therapy alone [28].

Decitabine inhibits DNA methylation and, at low doses, may reactivate silenced genes whereas, at high doses, may elicit cytotoxic effects. Studies are conflicting with regard to dose, treatment effectiveness, and patient eligibility, since hypomethylating agents, such as decitabine and 5-azacitidine, are widely used in the clinic even though they yield low complete remission rates, ranging from 15 to 20%. In this context, the treatment eligibility criteria are questioned, as the requirements for patient selection, dose strategy, and treatment duration are not clear, as well as latency and disease response or progression [29]. Dose comparison in decitabine treatments demonstrated more effectiveness at low doses [30]. However, low-dose treatments presented low efficacy and adverse toxic events when compared to treatment with cyclosporine in patients with low- or intermediate-risk MDS [31]. Therefore, a better understanding of response-to-treatment determinants is necessary to improve the therapeutic regimen with hypomethylating agents. In addition to the isolated pathology, other comorbidities should be evaluated in order to recognize which dose each patient should receive [32].

In general, MDS arises from abnormal gene expression, and this expression pattern will define the disease phenotype. Abnormal gene expression stems from different genetic mutations or epigenetic events, which can modify the expression levels of some genes. As an overall rule, these mutations induce cellular growth or inhibit apoptosis and may also block cellular differentiation, resulting in progression to acute leukemia [33]. In fact, the progression of MDS to AML is an example of the multistep theory of carcinogenesis. Kitamura and collaborators presented a new working hypothesis about the molecular bases of hematological malignancies employing the combination of mutations that could influence the phenotype and determine disease. Besides mutations that favor cellular proliferation and that block cellular differentiation, other phenomena in this multistep process were included, such as signal transduction events and epigenetic factors that are associated with dysregulated expression of genes, culminating in cellular immortalization, lack of differentiation, and increased cell survival and growth. Therefore, it is suggested that events that induce cellular immortalization and that favor a less differentiated phenotype are associated with the development of MDS; the addition of events that dysregulate cellular survival and
growth provides enough genetic advantages which allows the progression from MDS to AML [34].

AML is a heterogeneous disease, with different molecular signatures, therapeutic responses, and survival rates. It is a result of abnormal blast accumulation in the bone marrow, an event that, eventually, contributes to bone marrow failure. Blasts of the myeloid lineage are also found in peripheral blood at a concentration of approximately 20%. Different DNA methylation signatures have been described as markers for leukemogenesis and prognosis, and these also contribute to the understanding of disease development [35, 36].

Typically, AML treatment is divided into three phases: induction, consolidation, and maintenance. The rationale is to eliminate leukemic cells from the circulation with cytotoxic chemotherapy (induction) and then to eliminate residual leukemic cells from the circulation (consolidation and maintenance) [37]. Anthracyclines and cytarabine arabinoside (AraC) are the main drugs for most of the therapeutic regimens, aiming for complete remission and increased patient survival. Treatments utilizing a combination of these drugs show response rates with complete remission of 70 to 80% for patients under 60 years old [29, 30]. Refractory AML presents a therapeutic challenge, since standard treatment with AraC yields complete response rates of 17 to 20%. One clinical study for refractory AML aimed at achieving better treatment response by combining AraC with lenalidomide but did not present superior results when compared to AraC alone [29]. Moreover, complete remission in AML is generally not sufficient to increase overall survival [31, 32]. This, in part, can be explained by the fact that the presence of mutated genes in AML affects disease progression and prognosis stratifications, making it necessary to understand and validate its effects in order to assist in the clinical management of these patients [35].

Myelosuppression and febrile neutropenia are serious toxic events that arise during treatment and require great attention because of their effect on patient outcome [36, 37]. Although the use of small inhibitory molecules (such as imatinib and dasatinib) and monoclonal antibodies (such as rituximab) allow for longer treatments with lower toxicity rates, studies have already demonstrated that they may lead to serious grade 3 and 4 toxic events [38–40]. Therefore, it is important to establish optimal targets for each disease and to define when and how targeted therapies should be administered in order to establish a better and safer therapeutic regimen [41]. To this effect, determining the methylated genes that are associated with leukemogenesis and disease progression may also be important for selecting new therapeutic targets.

Comorbidities may also influence the therapeutic choices available, to the extent where some cases are considered ineligible for certain therapies because of previous or ongoing toxic events [42]. For patients older than 60 years of age, high-dose chemotherapy is poorly tolerated and treatment is rarely curative. Thus, treatment is directed towards increasing overall survival and quality of life [34]. This poses a challenge, and new approaches are needed in order to improve clinical outcome, contributing not only to better therapeutic responses, overall survival, and disease-free survival but also decreasing toxic events that may be fatal to the patient. Moreover, the development of new therapies demands time and incurs high costs [43]. Therefore, employing a molecular approach may optimize the existing therapeutic regimens, improving response rates, prognosis and, possibly, reducing toxic events.

2. Methods

The literature relating DNA methylation and staging/management of MDS and/or AML was reviewed and evaluated, with the goal of verifying which DNA methylation modifications, or changes in gene expression of epigenetic-modulating genes, were most present in disease onset, progression, staging, and toxic events.

The search terms were (biomarker or biomarkers) AND (DNA methylation) AND (acute myeloid leukemia) OR (myelodysplastic syndrome). Eligible literature was identified from PubMed, Science Direct, Web of Science, and Clinical Trial databases, and relevant data were extracted. Unpublished data, comments, letters, and conference proceedings were excluded from this search. A total of 65 articles and clinical trials with methylated genes (or mutations in epigenetic-modulating genes) suggested as marker for diagnosis, management, and prognosis of AML, and/or SMD patients were employed for this review.

3. DNA Methylation as an Epigenetic Biomarker

Cancer is characterized by its heterogeneity, given that each patient presents a variable molecular profile, which results in different molecular and physiological characteristics that contribute to development, prognosis, and response to treatment. In this context, the tumor microenvironment plays a fundamental role in which epigenetic components are associated with and contribute to tumorigenesis [44–47]. Epigenetic events, such as DNA methylation, are commonly identified in tumors, and these phenomena may aid in the understanding of the carcinogenic process since it is widely accepted that DNA methylation is related to cancer development and progression [48–51]. Moreover, these changes may be traced back and associated with disease staging and aggressiveness, allowing them to be employed as diagnostic and prognostic biomarkers. For this reason, studies seek to elucidate the interaction between these epigenetic modifications in chromatin remodeling, DNA replication and transcription, and the regulation of genes whose dysregulation is involved in carcinogenesis [52, 53].

Leukemias are a heterogeneous group of malignant neoplasms arising from the myeloid and/or lymphoid lineage, according to the dysplastic cell type, and which affects bone marrow, peripheral blood, and lymphoid tissues [54]. Aberrant epigenetic mutations have been demonstrated in different leukemia subtypes [48, 49, 55], and the number of identified changes is rising, including genes involved in a plethora of signaling pathways and cellular processes [56, 57]. Association between epigenetic changes, such as DNA methylation, and clinical outcome among leukemia types suggests that these modifications should be explored.
in order to develop a method that could improve patient stratification [55].

DNA methylation is an extensively studied epigenetic phenomenon, and different gene methylation patterns in tumor cells are used not only as markers for diagnosis but also as therapeutics targets. Different clinical trials have validated the ability of 5-azacytidine, a demethylating agent, in reducing global DNA methylation in vivo [58–60]. In this context, inhibitors of DNMT and histone deacetylases (HDAC) demonstrate clinical efficacy in treating hematological malignancies. Fandy and collaborators studied the methylation patterns of p15INK4B (cyclin-dependent kinase inhibitor 2B), a cell growth regulator; CDH-1 (cadherin 1), a calcium-dependent cell-cell adhesion molecule; DAPK-1 (death-associated protein kinase 1), a positive mediator of gamma interferon-induced programmed cell death; and SOCS-1 (suppressor of cytokine signaling 1), which acts downstream of cytokine receptors participating in the negative feedback of cytokine signaling, in the bone marrow of 30 patients with MDS or AML. After treatment with 5-azacytidine and entinostat, an HDAC inhibitor, reversal of promoter methylation was observed but was not associated with clinical response [58]. In another study, administration of hypomethylating agents, such as decitabine, prior to allogeneic stem cell transplants improved patient outcome, all the while without increasing treatment toxicity in MDS patients [59]. The identification of factors that predict response to therapy could help increase treatment efficacy, while, at the same time, reducing its toxicity. For example, Achille and collaborators investigated global DNA methylation and gene expression of CDKN2A (cyclin-dependent kinase inhibitor 2A), CDKN2B (cyclin-dependent kinase inhibitor 2B), both regulators of the cell cycle at the G1 checkpoint; HIC1 (transcriptional repressor 1), a growth regulatory molecule that acts as a tumor suppressor; RARB (retinoic acid receptor beta), a retinoic acid nuclear receptor which also mediates cellular signalling, growth, and differentiation; CDH1; and APAF1 (apoptotic peptidase activating factor 1), an apoptosis initiator by cleavage of caspase 9, before and during hypomethylating therapy, with the purpose of observing whether early changes could predict clinical response. Although global DNA methylation was not associated with clinical response, decreased CDKN2A promoter methylation was observed in patients achieving complete remission, and decreased CDKN2B, RARB, and CDH1 promoter methylation was observed in responders [60].

In addition to these applications, DNA methylation can also be used as a biomarker for metastatic tumor screening [61, 62], cancer stage detection [63], malignant progression assessment [64], treatment response [65], and detection of minimal residual disease [66].

The importance of epigenetic modifications can be exemplified by the fact that patients who relapse after frontline therapy, or those stratified as high risk, may present lineage exchange, a phenomenon that occurs when an acute leukemia from the myeloid or lymphoid lineage at diagnosis presents a "switch" to the opposite lineage on relapse [67–70]. This process can be attributed to the original cellular clone, which may present morphological heterogeneity or high plasticity, or to a new leukemic clone. Hypotheses have already been raised in order to explain this event, but its mechanism has not yet been fully elucidated. However, since physiological plasticity is defined as the ability to modify a particular cellular target without altering its genotype, it may be inferred that epigenetic factors participate in mechanisms involved with phenotype regulation mechanisms and with responses to the cellular niche [67–70].

Since DNA methylation can alter gene function and drive malignant cell transformation, and because aberrant methylation modifications usually occur at an early stage of neoplastic development, different DNA methylation patterns may be investigated not only to identify markers for early tumor detection and risk stratification but also to predict treatment response and prognosis [71]. Several studies can be used to illustrate this application: Zhang and colleagues evaluated the clinical relevance of DLX4 (distal-less homeobox 4) methylation, which plays a role in determining the synthesis of hemoglobin S, in patients diagnosed with MDS. It was found that this gene was significantly hypermethylated in MDS patients when compared to healthy controls. Moreover, patients with hypermethylated DLX4 had a significantly shorter overall survival compared to patients with hypomethylated DLX4 [72]. Similarly, GPX3 (glutathione peroxidase 3) methylation, an enzyme that protects cells from oxidative damage, was identified in the bone marrow of patients diagnosed with MDS and AML, which associated with shorter overall survival compared to patients with unmethylated GPX3 [73]. Wang and collaborators examined the methylation patterns of Wnt antagonist genes in 144 patients diagnosed with MDS. Survival analysis showed that methylated sFRP1, sFRP4, and sFRP5 (secreted frizzled-related protein) were associated with a shorter overall survival. The frizzled-related family has a role in regulating cell growth and differentiation, besides modulating Wnt signaling through direct interaction [74]. In another study, Chaubey and colleagues investigated the effects of the methylation of the supressor of cytokine signaling gene (SOCS-1), a negative regulator of the cytokine pathway. A total of 100 patients diagnosed with MDS were evaluated, and methylation was observed in 53% of the cohort. Progression-free survival and median overall survival were shorter in patients in which SOCS-1 was methylated, in comparison to those with unmethylated SOCS-1 [75]. Overall, these studies present evidence that the methylation pattern of some genes may influence the course of disease, including with regard to prognosis and survival.

Generally, methylation patterns seem not to be directly related to general clinical data but have demonstrated a direct association to disease classification and stratification. For example, there are reports showing that methylation patterns were not different when compared to gender, age, tumor location, and other clinical parameters, such as white blood cell count [76–78]. Even so, it is important to investigate these methylation patterns across different clinical characteristics in order to observe if there are significant associations or correlations to clinical parameters. Therefore, there is still room to investigate methylation patterns as potential biomarkers for different lineages, as well as for predicting
prognosis, response to therapy, and/or toxicity to treatment. Many groups have investigated DNA methylation patterns in these contexts, and their findings are summarized in Table 1.

3.1. DNA Methylation as a Biomarker for Diagnosis and Prognosis. Epigenetic modifications, such as DNA methylation, may occur before histopathological changes and, for this reason, may be used as biomarkers for early diagnosis and risk assessment. It is important to note that many types of hematological malignancies are asymptomatic until they reach advanced stages, and, therefore, a thorough characterization of the biomarker is crucial in order for it to be employed for early detection and prediction of tumor progression [114].

Estrogen receptors (ER) regulated by DNA methylation have been reported to play a key role in leukemogenesis. In 40 patients diagnosed with leukemia and evaluated after one year of chemotherapy, it was observed that patients with ER-α methylation perceived no symptomatic relief, whereas patients without ER-α methylation obtained effective relief with treatment. This data suggest that methylation of ER-α could be further investigated as a biomarker for diagnosis and prognosis, since this gene is present in 95% of all evaluated leukemia cases and is related to a lower response to treatments directed towards symptom relief [90].

Methylation of ID4 (inhibitor of DNA binding 4), a regulator of cell growth, senescence, differentiation, apoptosis, angiogenesis, and neoplastic transformation, was analyzed and suggested as a biomarker for the diagnosis of MDS. Li and collaborators analyzed the methylation status of 100 patients diagnosed with MDS, compared to 31 patients diagnosed with aplastic anemia (AA). ID4 gene promoter methylation status correlated with clinical parameters in MDS and AA, and bisulfite analysis revealed that gene methylation was higher in patients diagnosed with MDS. Finally, the authors suggest that ID4 gene promoter methylation could be a causative agent in hematopoietic disorders and, therefore, could be used to distinguish MDS from AA [96]. Similarly, Kang and colleagues investigated ID4 gene methylation in two patients and in the demethylation-treated MDS cell line (MUTZ1) with bisulfite sequencing PCR. The two MDS patients were treated with decitabine and demonstrated, after treatment, a decrease in methylation. This indicates that this gene may be a biomarker for selection and assessment of effective therapeutic schemes [95].

DNA methylation has also been described as a biomarker for prognosis in hematological malignancies, allowing for a simpler and lower cost analysis than other genetic tests, and also aiding in therapeutic decisions [2, 115–118]. High levels of global DNA methylation are an independent adverse prognostic factor for MDS. Calvo and collaborators, for example, isolated DNA from bone marrow of patients at diagnosis and determined the methylation rate via ELISA. Patients with methylated DNA above 2.73% had a lower overall survival than those with levels below 2.73% and presented a negative trend in terms of leukemia-free survival [119].

Complement C1r (C1R) gene methylation, which encodes a protein that is involved in the complement system, has been shown to be a robust, simple, and cost-effective biomarker for prognosis investigation in 194 AML patients. A comparison of C1R DNA methylation with healthy donor samples and samples from patients diagnosed with AML showed that patients diagnosed with AML with favorable cytogenetic risk scores had higher methylation in C1R and longer overall survival. It was also suggested that DNA methylation of C1R might be of independent prognostic relevance; however, further studies must be carried out in order for this to be validated [76].

In another report, Kurtović and collaborators studied samples of newly diagnosed adults with AML, including de novo AML, secondary AML, AML occurring after MDS, and aplastic anemia presenting different cytogenetic patterns. The DNA methylation status of target promoter sequences of p15 and O-6-methylguanine-DNA methyltransferase (MGMT), an enzyme involved in cellular defense against mutagenesis and toxicity from alkylating agents, was analyzed, and 81% of patients presented methylation in at least one of these two genes. It was not possible to prove that p15 and/or MGMT could predict response to therapy and overall survival; however, it was found that AML patients with methylation in both genes or in p15 alone had a higher frequency of early death and lower frequency of complete remission and presented a trend for shorter overall survival. Moreover, a cluster of abnormalities with adverse prognosis was observed in the group with aberrant methylation of both genes or of p15 alone [77]. Thus, the methylation pattern of these genes may be used for AML patient stratification. In fact, the p15 gene was associated with a tumor suppressor role based on its inactivation through hypermethylation of its promoter region in gliomas and leukemias [120]. In addition, this gene often exhibits hypermethylation in its promoter region in adults and children with both myeloid and lymphoid acute leukemia [121, 122].

Also with regard to prognosis, inhibition by methylation of the secreted frizzled-related protein genes sFRP2 and sFRP5, both members of the Wnt pathway, was associated with poor prognosis in normal karyotype AML patients. The Wnt pathway is of great importance, since it plays an important role in the self-renewal of hematopoietic stem cells and in the development of progenitor cells [123]. In another study, Zhou and collaborators investigated the methylation status of the GPX3 (glutathione peroxidase 3) gene promoter in the bone marrow of 110 MDS patients. Methylation was analyzed by methylation-specific PCR and bisulfite sequencing PCR and was observed in 15% of MDS patients. The methylation rate was higher than those of controls and lower than the methylation rate of AML patients. It was also observed that GPX3 methylation was associated with older age, higher frequency of DNMT3A mutations, and shorter overall survival. The authors conclude that, therefore, GPX3 methylation in bone marrow could be a marker for adverse prognosis and progression to leukemia in MDS patients [124].

3.2. DNA Methylation as a Biomarker for Treatment Response and Toxicity. Both AML and MDS are characterized by an exacerbated proliferation of undifferentiated myeloid cells [29]. Decitabine (5-aza-2′-deoxycytidine) or 5-azacytidine
| Gene          | Disease | Patients (n) | Sample type | Associated factors                                                                                                                                                                                                 | Ref.   |
|--------------|---------|--------------|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|
| AWT1         | AML     | 356          | BM/B        | Classification of myeloid-derived leukemias. Hypermethylation could monitor the recurrence of disease during remission in patients undergoing allogeneic stem cell transfer.                                                 | [79]   |
| BMI1         | AML/MDS | 54           | BM/B        | DNA methylation was associated with poor prognosis.                                                                                                                                                                   | [80]   |
| C1R          | AML     | 194          | B           | DNA methylation was associated with the occurrence of specific genomic mutations that are used for risk stratification.                                                                                             | [76]   |
| CDH          | MDS     | 60           | BM          | DNA methylation was associated with poor prognosis and lower complete remission.                                                                                                                                     | [81]   |
| CDH1         | MDS     | 317          | BM/B        | Aberrant DNA methylation predicts overall survival and progression-free survival.                                                                                                                                     | [82]   |
| CDH13        | MDS     | 37           | BM          | Hypermethylation can contribute to the development and poor outcome of disease.                                                                                                                                      | [83]   |
| CDKN2B       | MDS     | 78           | BM          | DNA methylation was associated with leukemic transformation and disease progression.                                                                                                                                    | [84]   |
| CEBPA        | AML     | 181          | BM          | DNA methylation was associated with pathogenesis and prognosis.                                                                                                                                                      | [85]   |
| CXXC5        | AML     | 529          | BM          | Methylation was associated with better outcome.                                                                                                                                                                       | [86]   |
| DLC-1        | MDS     | 43           | BM/B        | DNA methylation was associated with poor prognosis.                                                                                                                                                                   | [87]   |
| DLX4         | MDS     | 103          | BM          | DNA methylation was associated with poor outcome and shorter overall survival                                                                                                                                        | [72]   |
| DNMT3A       | LMA     | 88           | B           | Methylation was associated with poor prognosis.                                                                                                                                                                       | [89]   |
| ERalpha-A    | MDS     | 317          | BM/B        | Aberrant DNA methylation predicts overall survival and progression-free survival.                                                                                                                                     | [82]   |
| ERalpha-A    | MDS     | 37           | BM          | Hypermethylation can contribute to the development and poor outcome of disease.                                                                                                                                      | [83]   |
| EVI1         | LMA     | 476          | BM/B        | Hipomethylation was associated with poor prognosis.                                                                                                                                                                   | [91]   |
| EZH2         | AML/MDS | 54           | BM/B        | DNA methylation was associated with poor prognosis.                                                                                                                                                                   | [80]   |
| FHIT         | MDS     | —            | B           | DNA methylation was associated with pathogenesis.                                                                                                                                                                      | [92]   |
| GPX3         | MDS     | 110          | BM          | DNA methylation was associated with poor prognosis and progression to leukemia in MDS.                                                                                                                               | [73]   |
| HIC1         | MDS     | 37           | BM          | Hypermethylation can contribute to the development and poor outcome of disease.                                                                                                                                      | [83]   |
| HOXA5        | AML     | 378          | BM/B        | Hypermethylation was frequently observed in all types of leukemia and strongly correlated with progression to blast crisis.                                                                                             | [93]   |
| HRK          | MDS     | 60           | BM          | DNA methylation was associated with advanced stage of MDS and progression.                                                                                                                                             | [94]   |
| ID4          | LMA     | 212          | BM          | DNA methylation was associated with shorter overall survival                                                                                                                                                           | [73]   |
| ID4          | MDS     | 142          | BM          | DNA methylation was suggested as biomarker for diagnosis.                                                                                                                                                               | [95]   |
| ID4          | MDS     | 100          | BM          | DNA methylation was suggested as biomarker for diagnosis.                                                                                                                                                               | [96]   |
| ID4          | AML     | 14           | BM          | DNA methylation was suggested as biomarker for minimal residual disease detection.                                                                                                                                   | [66]   |
Table 1: Continued.

| Gene  | Disease | Patients (n) | Sample type | Associated factors                                                                 | Ref. |
|-------|---------|--------------|-------------|--------------------------------------------------------------------------------------|------|
| LET-7A-3 | MDS    | 95           | BM          | DNA methylation was associated with poor prognosis.                                  | [97] |
| MGMT   | AML    | 21           | BM/B        | Co-methylation with p15 gene showed high proportion of leukemic blast cells.         | [77] |
| MGMT   | AML    | 30           | BM          | DNA methylation was suggested as biomarker to predict therapeutic outcome in male AML patients. | [98] |
| NOR1   | MDS    | 317          | BM/B        | Aberrant DNA methylation predicts overall survival and progression-free survival.    | [82] |
| NPM2   | MDS    | 317          | BM/B        | Aberrant DNA methylation predicts overall survival and progression-free survival.    | [82] |
| OLIG2  | MDS    | 317          | BM/B        | Aberrant DNA methylation predicts overall survival and progression-free survival.    | [82] |
| p15    | AML    | 21           | BM/B        | DNA methylation was associated with higher frequency of early death. Comethylation with MGMT gene showed high proportion of leukemic blast cells. | [77] |
| p15INK4b | MDS  | 53           | BM          | DNA methylation was associated with worse prognosis increasing with disease evolution to AML. | [99] |
| p15INK4b | t-MDS; t-AML | 81        | BM/B        | DNA methylation presented a significantly shorter survival and correlated with loss of chromosome arm 7q. | [100] |
| p15INK4b | MDS   | 47           | BM          | DNA methylation was associated with pediatric disease evolution.                    | [101] |
| p15INK4b | MDS   | 317          | BM/B        | Aberrant DNA methylation predicts overall survival and progression-free survival.    | [83] |
| p15INK4b | MDS  | 47           | BM          | DNA methylation was associated with pediatric disease evolution.                    | [102] |
| p21    | MDS    | 88           | BM          | DNA methylation could predict clinical outcome.                                     | [103] |
| p73    | MDS    | 88           | BM          | DNA methylation was associated with poor prognosis in de novo MDS.                  | [103, 104] |
| PcG    | AML    | 118          | BM          | DNA methylation was associated with poor prognosis.                                  | [105] |
| PGRA   | MDS    | 317          | BM/B        | Aberrant DNA methylation predicts overall survival and progression-free survival.    | [82] |
| PGRB   | MDS    | 317          | BM/B        | Aberrant DNA methylation predicts overall survival and progression-free survival.    | [82] |
| PLA2R1 | MDS    | 32           | B           | DNA methylation was associated with disease evolution in MDS and leukemogenesis     | [106] |
| PLK    | Onco-hematological diseases | ND | BM          | Promoter methylation correlates with disease and tumorigenesis in blood neoplasms.   | [107] |
| PPARD  | AML    | 344          | BM/B        | DNA methylation was associated with favorable outcome.                              | [108] |
| PSMD2  | AML    | 344          | BM/B        | DNA methylation was associated with favorable outcome.                              | [108] |
| RIL    | MDS    | 317          | BM/B        | DNA methylation was associated with poor prognosis.                                | [80] |
| RING1  | AML/MDS | 54        | BM/B        | DNA methylation was associated with worse overall survival and poor prognosis.      | [74] |
| sFRP1  | MDS    | 144          | BM          | DNA methylation was associated with increased risk of relapse and risk of death, predicting adverse clinical outcome in patients with normal karyotypes. | [74] |
| sFRP2  | AML    | 72           | BM/B        | DNA methylation was associated with worse overall survival and poor prognosis        | [74] |
| sFRP2  | MDS    | 144          | BM          | DNA methylation was associated with increased risk of relapse and risk of death, predicting adverse clinical outcome in patients with normal karyotypes. | [74] |
| sFRP5  | AML    | 72           | BM/B        | DNA methylation was associated with worse overall survival and poor prognosis        | [74] |
| sFRP5  | MDS    | 144          | BM          | DNA methylation was associated with worse overall survival and poor prognosis        | [74] |
is used to treat these diseases, but there is a chance that more than half of patients will develop resistance to these therapies, leading to worse treatment response [125]. The early identification of whether a patient will respond to treatment is still a major obstacle for achieving clinical success. Evaluations of the clinical course, and subsequent follow-ups, are essential for the safety and efficacy of treatment and for disease remission. Therefore, it is of great importance to identify early markers that may predict which patients will be early responders, late responders, or will not respond at all to treatment.

In a study by Shen and collaborators, it was identified that hypermethylation of p53, a vastly studied tumor suppressor gene, and p73, which participates in the apoptotic response to DNA damage and, therefore, also acts as a tumor suppressor, correlated strongly with sensitivity to alkylating agents in several cancer cell lines. Six of which were blood- or bone marrow-derived, suggesting that a DNA methylation profile may be useful to identify sensitivity to cancer therapy. However, it should be noted that this study was performed in cultured cell lines and not with patient samples, and, therefore, further studies need to be carried out in order to understand the role of these markers during a patient’s clinical course [126].

Another study by Shen and colleagues, with 317 MDS patients, demonstrated that CDH1; CHD13; ERα; NOR (oxidored-nitro domain-containing protein isoform 1), a gene that encodes two transcripts and acts as a tumor suppressor; NPM2 (nucleoplasmin 2), involved in chromatin reprogramming; OLG2 (oligodendrocyte lineage transcription factor 2), involved in the chromosomal translocation t(14;21)(q11.2;q22) which is associated with T-cell acute lymphoblastic leukemia; CDKN2B; PGRα (progesterone receptor A), which functions as transcriptional activator or repressor; and RIL (PDZ and LIM domain 4), localized in a region frequently deleted in AML and MDS, were methylated in MDS/AML patients. The methylation pattern before treatment was not associated with clinical response to decitabine. However, methylation reduction after more than four months of treatment correlated with clinical response in 34 patients [127]. In spite of these interesting results, it is important to search for markers that indicate the clinical response before treatment begins or in a shorter time of treatment, in order to aid in choosing the most appropriate therapeutic course for each patient. In a clinical study conducted by Tan and collaborators, it was possible to verify an increase in the acetylation of histones H3 and H4 following treatment with 5-azacitidine combined with panobinostat in AML or MDS patients. The importance of this work stems from the fact that this evaluation was performed utilizing peripheral blood mononuclear cells separated by flow cytometry during the first month of treatment, which is a procedure that could be easily reproduced in other centers [128].

As a matter of fact, genes that have already been related to disease-free survival or disease progression could be reevaluated in peripheral blood in order to corroborate previous findings in bone marrow. For example, the cadherin (CDH) family encodes a calcium-dependent cell-cell adhesion protein, whose loss of function can increase cellular proliferation and invasion, contributing to cancer progression. Other genes, such as p15<sup>ink4b</sup> and other tumor suppressor genes, encode cyclin-dependent kinase inhibitors which contribute to cell growth regulation and controls cell cycle progression. Data suggest that methylation of this gene could allow leukemic cells to escape inhibitory signals from the bone marrow. The methylation patterns of these two gene families have already been related to AML progression in MDS patients, and, therefore, could be investigated in peripheral blood as well in order to verify if these results are corroborated [83, 99]. The discovery and validation methylation markers in peripheral blood can be very helpful in investigating response during treatment.

During follow-up, in addition to the therapeutic response, toxic effects are evaluated in order to guarantee the patient’s safety. Recent studies have sought to correlate epigenetic regulation of cytokines with tumor development [129, 130]. Moreover, cytokine evaluation was suggested as biomarkers for assessing toxicity during treatment, since they are raised significantly in inflammatory responses. However, they present a short serum half-life and lack toxicity-specific expression [131]. Wang and colleagues assessed serum inflammatory cytokines weekly for 15 weeks in patients with

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Table 1: Continued.

| Gene    | Disease | Patients (n) | Sample type | Associated factors                                                                 | Ref. |
|---------|---------|--------------|-------------|-----------------------------------------------------------------------------------|------|
| SOCS-1  | MDS     | 100          | B           | DNA methylation was associated with disease progression and poor survival          | [75] |
| SOX17   | MDS     | 164          | BM          | DNA methylation was associated with poor prognosis.                               | [110]|
| TERTpro/Ex1 | AML     | 43           | BM          | Hypermethylation was associated with inferior patient survival.                   | [111]|
| TERTpro/Ex1 | AML/MDS | 33           | BM/B        | DNA methylation was associated with poor prognosis and inferior patient survival. | [111]|
| VTRNA1–3| MDS     | 140          | BM          | DNA methylation was associated with poor outcome.                                | [112]|
| XPNPEP  | AML     | 344          | BM/B        | DNA methylation was associated with unfavorable outcome.                          | [108]|
| ZO-1    | MDS     | ND           | BM          | DNA methylation was associated with disease progression.                          | [113]|

AML: acute myeloid leukemia; B: peripheral blood; BM: bone marrow; MDS: myelodysplastic syndrome; ND: not declared; t-AML: therapy-related acute myeloid leukemia; t-MDS: therapy-related myelodysplastic syndrome.
non-small-cell lung cancer during concurrent chemoradiation therapy. An increase in serum IL-6 (interleukin 6), a cytokine that plays a role in inflammation and B-cell maturation, was related to pain, fatigue, disturbed sleep, lack of appetite, and sore throat suggesting a role between proinflammatory cytokine and worsening of symptoms in patients undergoing treatment [132]. With regard to leukemia, Tsapogas and collaborators recently reviewed the role of the cytokine Flt3-ligand (Fms-related tyrosine kinase 3 ligand), which stimulates the proliferation, differentiation, and survival of early hematopoietic cells by activating the FLT3 receptor (Fms-related tyrosine kinase 3), in normal and malignant hematopoiesis [133].

Among the adverse events that may occur during treatment, myelosuppression is the main dose-limiting toxicity and is associated with morbidity and mortality [134, 135]. Febrile neutropenia, or the onset of an infection during neutropenia, represents an emergency and requires administration of broad spectrum antibiotics. These complications may result in reduced dose or interruption of chemotherapy, which compromises patient recovery [101, 136–139]. Moreover, these complications generate high costs, including hospitalization, and may lead to death, demonstrating the importance of its prevention [140, 141].

The understanding of the patient’s clinical course, treatment, and risk factors for severe adverse events, such as febrile neutropenia, may allow for preventive actions that reduce the incidence of serious treatment-related complications, all the while reducing the cost of health care [101]. Currently, there are no studies that directly relate and validate changes in epigenetic patterns with the development of toxicity to treatment in hematological malignancies. DNA methylation has already been associated with susceptibility to isoproterenol-induced cardiac pathology in mice. The basal state of the cardiac DNA methylome before and after isoproterenol treatment was compared, and a single-base resolution DNA methylation measurement revealed that treatment decreases global methylation, an event that was associated with heart failure. However, further studies are necessary to investigate this association [142].

Another study with ovarian cancer patients analyzed the methylation in peripheral blood via bisulftite pyrosequencing in different genes during treatment with paclitaxel versus docetaxel. It was observed that higher methylation within the estrogen receptor 1 (ESR1) gene was associated with neuropathy on the paclitaxel arm. This was the first cancer study linking DNA methylation in peripheral blood with clinical outcomes, including adverse effects, and suggests that studies evaluating methylation patterns with treatment toxicity in other tumors should also be performed [143]. Another example is the EuroTARGET cohort, a collaborative project that aims to evaluate targeted therapy in renal cell cancer and tumor-related biomarkers for response and toxicity to treatment. Multiplatform “omics,” including the methylome, are being employed to identify biomarkers for toxicity; however, the final data is not yet available [144].

With regard to clinical studies, an ongoing study (NCT02259218; clinicaltrials.gov) aims to identify potential biomarkers that may predict the development of radiation pneumonitis in lung cancer patients and radiation necrosis in brain cancer patients. Metabolic and epigenetic profiles are being studied from blood, urine, and tissue samples in order to find biomarkers that are capable of predicting which patients are more likely to develop adverse effects as a result of radiation treatment [145]. Similar studies should be carried out in order to evaluate biomarkers for toxicity before, during, and after treatment in order to predict early toxic events. It is especially important to investigate these biomarkers in peripheral blood, since samples can be obtained with ease and without need of lengthy preparations for the procedure. This would allow for greater patient safety and drug dose adjustment during treatment, optimizing the therapeutic regimen.

4. Conclusion

With regard to MDS and AML, current treatment challenges include choosing the appropriate combination of treatment modalities and chemotherapeutic regimens, since response to therapy is not always achieved. In addition, different adverse effects may occur during treatment because of the toxic effects of most, if not all, chemotherapeutic agents. This seriously delays treatment, affecting the chances of remission, and may directly harm the patient, even leading to death. Moreover, early diagnosis is important in order to increase the potential for a better clinical response during treatment.

Several DNA methylation events affect gene expression and are related to different types of tumors, including hematological malignancies. However, their potential as biomarkers for early diagnosis, stratification, and prediction of treatment response has yet to be more thoroughly evaluated. Studies have demonstrated a significant relationship between DNA methylation patterns and confirmative diagnosis, prognostic potential, and response to treatment. Because changes in DNA methylation are early manifestations and may also act as potential therapeutic targets, the identification of these patterns becomes essential for clinical success. Thus, it is necessary to undertake more studies involving patient samples in order to discover and validate new biomarkers in this field. It is suggested that studies should investigate DNA methylation patterns in peripheral blood samples, in order to optimize not only early diagnosis but also patient management during treatment, allowing for close monitoring of disease progression, adverse events, and response to treatment without the need for bone marrow collection.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of Interest

The authors hereby declare that they have no conflict of interest.
Acknowledgments

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Grant no. 303567/2016-3 to Ana Lucia Abujamra), by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; to Jayse Alves), by the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS; to Laura Reckziegel), and by Fundação Vale do Taquari de Educação e Desenvolvimento Social (FUVATES; Grant no. 10901246 to Ana Lucia Abujamra and Laura Reckziegel).

References

[1] D. Hanahan and R. A. Weinberg, "Hallmarks of cancer: the next generation," Cell, vol. 144, no. 5, pp. 646–674, 2011.
[2] S. B. Baylin and P. A. Jones, "A decade of exploring the cancer epigenome—biological and translational implications," Nature Reviews Cancer, vol. 11, no. 10, pp. 726–734, 2011.
[3] J. Sandoval and M. Esteller, "Cancer epigenomics: beyond genomics," Current Opinion in Genetics & Development, vol. 22, no. 1, pp. 50–55, 2012.
[4] S. Sharma, T. K. Kelly, and P. A. Jones, "Epigenetics in cancer," Carcinogenesis, vol. 31, no. 1, pp. 27–36, 2010.
[5] J. S. You and P. A. Jones, "Cancer genetics and epigenetics: two sides of the same coin?" Cancer Cell, vol. 22, no. 1, pp. 9–20, 2012.
[6] A. Hermann, R. Goyal, and A. Jettsch, "The Dnmt1 DNA-(cytosine-C5)-methyltransferase methylates DNA processively with high preference for hemimethylated target sites," Journal of Biological Chemistry, vol. 279, no. 46, pp. 48350–48359, 2004.
[7] L. D. Moore, T. Le, and G. Fan, "DNA methylation and its basic function," Neuropsychopharmacology, vol. 38, no. 1, p. 23, 2013.
[8] M. Okano, D. W. Bell, D. A. Haber, and E. Li, "DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development," Cell, vol. 99, no. 3, pp. 247–257, 1999.
[9] M. A. Mcdevitt, "Clinical applications of epigenetic markers and epigenetic profiling in myeloid malignancies," Seminars in Oncology, vol. 39, no. 1, pp. 109–122, 2012.
[10] M. Hatzipostolou and D. Ilipooulos, "Epigenetic aberrations during oncogenesis," Cellular and Molecular Life Sciences, vol. 68, no. 10, pp. 1681–1702, 2011.
[11] G. Marcuccu, K. Maharry, Y. Z. Wu et al., "IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study," Journal of Clinical Oncology, vol. 28, no. 14, pp. 2348–2355, 2010.
[12] S. Abbas, S. Lughath, F. G. Kavelaars et al., "Acquired mutations in the genes encoding IDH1 and IDH2 both are recurrent aberrations in acute myeloid leukemia: prevalence and prognostic value," Blood, vol. 116, no. 12, pp. 2122–2126, 2010.
[13] M. Janin, E. Mylonas, V. Saada et al., "Serum 2-hydroxyglutarate production in IDH1- and IDH2-mutated de novo acute myeloid leukemia: a study by the Acute Leukemia French Association group," Journal of Clinical Oncology, vol. 32, no. 4, pp. 297–305, 2014.
[14] K. Lund, P. D. Adams, and M. Copland, "EZH2 in normal and malignant hematopoiesis," Leukemia, vol. 28, no. 1, pp. 44–49, 2014.
[15] Z. Li, X. Cai, C. L. Cai et al., "Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies," Blood, vol. 118, no. 17, pp. 4509–4518, 2011.
[16] I. Jeziskova, M. Musilova, M. Cilen et al., "Distribution of mutations in DNMT3A gene and the suitability of mutations in R882 codon for MRD monitoring in patients with AML," International Journal of Hematology, vol. 102, no. 5, pp. 553–557, 2015.
[17] T. J. Ley, L. Ding, M. J. Walter et al., "DNMT3A mutations in acute myeloid leukemia," New England Journal of Medicine, vol. 363, no. 25, pp. 2424–2433, 2010.
[18] Cancer Genome Atlas Research Network, "Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia," The New England Journal of Medicine, vol. 368, no. 22, pp. 2059–2074, 2013.
[19] M. J. Walter, L. Ding, D. Shen et al., "Recurrent DNMT3A mutations in patients with myelodysplastic syndromes," Leukemia, vol. 25, no. 7, pp. 1153–1158, 2011.
[20] H. A. Hou and H. F. Tien, "Mutations in epigenetic modifiers in acute myeloid leukemia and their clinical utility," Expert Review of Hematology, vol. 9, no. 5, pp. 447–469, 2016.
[21] D. H. Spencer, D. A. Russler-Germain, S. Ketkar et al., "CpG island hypermethylated mediated by DNMT3A is a consequence of AML progression," Cell, vol. 168, no. 5, pp. 801–816, 2017.
[22] Z. K. Otrock, R. V. Tiu, J. P. Maciejewski, and M. A. Sekeres, "The need for additional genetic markers for myelodysplastic syndrome stratification: what does the future hold for prognostication?", Expert Review of Hematology, vol. 6, no. 1, pp. 59–68, 2013.
[23] B. Faltas, A. M. Zeidan, and U. Gergis, "Myelodysplastic syndromes: towards a risk-adaptive treatment approach," Expert Review of Hematology, vol. 5, no. 5, pp. 611–624, 2013.
[24] O. Salim, T. Toptas, E. Avsar et al., "Azacitidine versus decitabine in patients with refractory anemia with excess blasts – results of multicenter study," Leukemia Research, vol. 45, pp. 82–89, 2016.
[25] Y. Jing, X. Jin, L. Wang et al., "Decitabine-based chemotherapy followed by haploidentical lymphocyte infusion improves the effectiveness in elderly patients with acute myeloid leukemia," Oncotarget, vol. 5, 2016.
[26] A. M. Zeidan, Y. Linhares, and S. D. Gore, "Current therapy of myelodysplastic syndromes," Blood Reviews, vol. 27, no. 5, pp. 243–259, 2013.
[27] G. J. Mufti and V. Potter, "Myelodysplastic syndromes: who and when in the course of disease to transplant," ASH Education Program Book, vol. 2012, no. 1, pp. 49–55, 2012.
[28] M. Lübbert, S. Suciu, A. Hagemeijer et al., "Decitabine improves progression-free survival in older high-risk MDS patients with multiple autosomal monosomies: results of a subgroup analysis of the randomized phase III study 0601 of the EORTC Leukemia Cooperative Group and German MDS Study Group," Annals of Hematology, vol. 95, no. 2, pp. 191–199, 2016.
[29] E. A. Griffiths and S. D. Gore, "Epigenetic therapies in MDS and AML," Advances in Experimental Medicine and Biology, vol. 754, pp. 253–283, 2013.
[30] J. P. J. Issa, G. García-Manero, F. J. Giles et al., “Phase I study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies,” Blood, vol. 103, no. 5, pp. 1635–1640, 2004.

[31] X. Luo, H. Wu, Y. Ding, Y. H. Chen, and A. B. Liang, “Clinical efficacy comparison of ultralow dose of decitabine and cyclosporine on low-risk and intermediate-risk type 1 of myelodysplastic syndrome,” Journal of Experimental Hematology, vol. 24, no. 2, pp. 510–514, 2016.

[32] B. Telek, L. Rejtó, P. Batár et al., “Drug treatment of acute myelogenous leukaemia. Current options and future perspectives,” Orvosi Hetilap, vol. 157, no. 22, pp. 843–846, 2016.

[33] D. G. Gilliland and J. D. Griffin, “The roles of FLT3 in hematopoiesis and leukemia,” Blood, vol. 100, no. 5, pp. 1532–1542, 2002.

[34] T. Kitamura, N. Watanabe-Okochi, Y. Enomoto et al., “Novel working hypothesis for pathogenesis of hematological malignancies: combination of mutations-induced cellular phenotypes determines the disease (cMIP-DD),” The Journal of Biochemistry, vol. 159, no. 1, pp. 17–25, 2015.

[35] J. M. Foran, “New prognostic markers in acute myeloid leukemia: perspective from the clinic,” ASH Education Program Book, vol. 2010, no. 1, pp. 47–55, 2010.

[36] E. H. Estey, “Acute myeloid leukemia: 2014 update on risk-stratification and management,” American Journal of Hematology, vol. 89, no. 11, pp. 1063–1081, 2014.

[37] A. Khwaja, M. Bjorkholm, R. E. Gale et al., “Acute myeloid leukaemia,” Nature Reviews Disease Primers, vol. 10, no. 2, article 16010, 2016.

[38] C. Y. Cheah, L. J. Nastoupil, S. S. Neelapu, S. G. Forbes, Y. Oki, and N. H. Fowler, “Lenalidomide, idelalisib, and rituximab are unacceptably toxic in patients with relapsed/refractory indolent lymphoma,” Blood, vol. 25, no. 21, pp. 3357–3359, 2015.

[39] M. Ban-Hoefen, R. Burack, L. Sievert, and D. Sahasrabudhe, “Pipilumab-induced neutropenia in melanoma,” Journal of Investigative Medicine High Impact Case Reports, vol. 4, no. 3, pp. 1–5, 2016.

[40] A. M. Brunner, D. B. Costa, R. S. Heist et al., “Treatment-related toxicities in a phase II trial of dasatinib in patients with squamous cell carcinoma of the lung,” Journal of Thoracic Oncology, vol. 8, no. 11, pp. 1434–1437, 2013.

[41] A. Rashidi and R. B. Walter, “Antigen-specific immunotherapy for acute myeloid leukemia: where are we now, and where do we go from here?,” Expert Review of Hematology, vol. 9, no. 4, pp. 335–350, 2016.

[42] S. Sauthele, M. P. Krauß, R. Heilmann et al., “Impact of comorbidities on overall survival in patients with chronic myeloid leukemia: results of the randomized CML study IV,” Blood, vol. 126, no. 1, pp. 42–49, 2015.

[43] D. J. Stewart, G. Batist, H. M. Kantarjian, J. P. Bradford, J. H. Schiller, and R. Kurzrock, “The urgent need for clinical research reform to permit faster, less expensive access to new therapies for lethal diseases,” Clinical Cancer Research, vol. 21, no. 20, pp. 4561–4568, 2015.

[44] P. A. Jones, “Overview of cancer epigenetics,” Seminars in Hematology, vol. 42, pp. S3–S8, 2005.

[45] S. Khare and M. Verma, “Epigenetics of colon cancer,” Methods in Molecular Biology, vol. 863, pp. 177–185, 2012.

[46] M. Verma, P. Maruvada, and S. Srivastava, “Epigenetics and cancer,” Critical Reviews in Clinical Laboratory Sciences, vol. 41, no. 5–6, pp. 585–607, 2004.

[47] M. Verma, B. K. Dunn, S. Ross et al., “Early detection and risk assessment proceedings and recommendations from the workshop on epigenetics in cancer prevention,” Annals of the New York Academy of Sciences, vol. 983, no. 1, pp. 298–319, 2003.

[48] S. Y. Kim, D. Y. Shin, S. M. Kim, M. Lee, and E. J. Kim, “Aberrant DNA methylation-induced gene inactivation is associated with the diagnosis and/or therapy of T-cell leukemias,” Leukemia Research, vol. 47, pp. 116–122, 2016.

[49] Y. Zhang, Q. Jiang, X. Kong et al., “Methylation status of the promoter region of the human frizzled 9 gene in acute myeloid leukemia,” Molecular Medicine Reports, vol. 14, no. 2, pp. 1339–1344, 2016.

[50] M. G. S. Aithal and N. Rajeawari, “Role of Notch signalling pathway in cancer and its association with DNA methylation,” Journal of Genetics, vol. 3, pp. 667–675, 2013.

[51] N. Dong, L. Shi, D. C. Wang, C. Chen, and X. Wang, “Role of epigenetics in lung cancer heterogeneity and clinical implication,” Seminars in Cell & Developmental Biology, vol. 64, pp. 18–25, 2017.

[52] M. Verma, “Epigenetic biomarkers in cancer epidemiology,” Methods in Molecular Biology, vol. 863, pp. 467–480, 2012.

[53] M. Verma, M. J. Khoury, and J. P. A. Ioannidis, “Opportunities and challenges for selected emerging technologies in cancer epidemiology: mitochondrial, epigenomic, metabolomic, and telomerase profiling,” Cancer Epidemiology, Biomarkers & Prevention, vol. 22, no. 2, pp. 189–200, 2013.

[54] J. W. Vardiman, J. Thiele, D. A. Arber et al., “The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes,” Blood, vol. 114, no. 5, pp. 937–951, 2009.

[55] C. Florean, M. Schnekenburger, C. Grandjenette, M. Dicato, and M. Diederich, “Epigenomics of leukemia: from mechanisms to therapeutic applications,” Epigenomics, vol. 3, no. 5, pp. 581–609, 2011.

[56] M. Esteller, “Epigenetic gene silencing in cancer: the DNA hypermethylation,” Human Molecular Genetics, vol. 16, no. 1, pp. 50–59, 2007.

[57] S. B. Baylin, “DNA methylation and gene silencing in cancer,” Nature Clinical Practice. Oncology, vol. 2, pp. 4–11, 2005.

[58] T. E. Fandy, J. G. Herman, P. Kerns et al., “Early epigenetic changes and DNA damage do not predict clinical response in an overlapping schedule of 5-azacytidine and entinostat in patients with myeloid malignancies,” Blood, vol. 114, no. 13, pp. 2764–2773, 2009.

[59] L. D. Silva, M. De Lima, H. Kantarjian et al., “Feasibility of allo-SCT after hypomethylating therapy with decitabine for myelodysplastic syndrome,” Bone Marrow Transplantation, vol. 43, no. 11, pp. 839–843, 2009.

[60] N. J. Achille, M. Othus, K. Phelan et al., “Association between early promoter-specific DNA methylation changes and outcome in older acute myeloid leukemia patients,” Leukemia Research, vol. 42, pp. 68–74, 2016.

[61] Z. Liu, J. Zhang, Y. Gao et al., “Large-scale characterization of DNA methylation changes in human gastric carcinomas with and without metastasis,” Clinical Cancer Research, vol. 20, no. 17, pp. 4598–4612, 2014.
[62] M. J. Fackler, Z. L. Bujanda, C. Umbricht et al., “Novel methylated biomarkers and a robust assay to detect circulating tumor DNA in metastatic breast cancer,” Cancer Research, vol. 74, no. 8, pp. 2160–2170, 2014.

[63] A. Schröck, A. Leisse, L. de Vos et al., “Free-circulating methylated DNA in blood for diagnosis, staging, prognosis, and monitoring of head and neck squamous cell carcinoma patients: an observational prospective cohort study,” Clinical Chemistry, vol. 63, no. 7, pp. 1288–1296, 2017.

[64] Y. Li, L. Yang, Y. Pan, J. Yang, Y. Shang, and J. Luo, “Methylation and decreased expression of SHP-1 are related to disease progression in chronic myelogenous leukemia,” Oncology Reports, vol. 31, no. 5, pp. 2438–2446, 2014.

[65] J. S. Tsang, S. Venken, O. Sharaf et al., “Global DNA methylation is altered by neoadjuvant chemoradiation therapy in rectal cancer and may predict response to treatment—a pilot study,” European Journal of Surgical Oncology, vol. 40, no. 11, pp. 1459–1466, 2014.

[66] Y. Zhao, L. Yu, Q. S. Wang et al., “Id4 gene methylation for detection of minimal residual disease in acute leukemia,” Zhonghua Xue Ye Xue Za Zhi, vol. 27, no. 5, pp. 298–301, 2006.

[67] C. Cobaleda, “Reprogramming of B cells. Cellular programming and reprogramming,” Methods and Protocols, vol. 636, pp. 233–250, 2010.

[68] M. Park, K. N. Koh, B. E. Kim et al., “Lineage switch at relapse of childhood acute leukemia: a report of four cases,” Journal of Korean Medical Science, vol. 26, no. 6, pp. 829–831, 2011.

[69] E. Dorantes-Acosta and R. Pelayo, “Free-circulating methylated DNA in blood for diagnosis, staging, prognosis, and monitoring of head and neck squamous cell carcinoma patients: an observational prospective cohort study,” Clinical Chemistry, vol. 63, no. 7, pp. 1288–1296, 2017.

[70] F. Xu, X. Li, L. Wu et al., “Overexpression of the EZH2, RING1 and BMI1 genes is common in myelodysplastic syndromes: relation to adverse epigenetic alteration and poor prognostic scoring,” Annals of Hematology, vol. 90, no. 6, pp. 643–653, 2011.

[71] M. Grövдал, R. Khan, A. Aggerholm et al., “Negative effect of DNA hypermethylation on the outcome of intensive chemotherapy in older patients with high-risk myelodysplastic syndromes and acute myeloid leukemia following myelodysplastic syndrome,” Clinical Cancer Research, vol. 13, no. 23, pp. 7107–7112, 2007.

[72] L. Shen, H. Kantarjian, Y. Guo et al., “DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes,” Journal of Clinical Oncology, vol. 28, no. 4, pp. 605–613, 2010.

[73] A. Guillaumet-Adkins, J. Richter, M. D. Odero et al., “Promoter hypermethylation of p15INK4B, HIC1, CDH1, and ER is frequent in myelodysplastic syndromes and acute myeloid leukemia following myelodysplastic syndrome,” Blood, vol. 125, no. 10, pp. 1511–1517, 2010.

[74] E. Dorantes-Acosta and R. Pelayo, “Free-circulating methylated DNA in blood for diagnosis, staging, prognosis, and monitoring of head and neck squamous cell carcinoma patients: an observational prospective cohort study,” Clinical Chemistry, vol. 63, no. 7, pp. 1288–1296, 2017.

[75] T. C. Lin, S. S. Jiang, W. C. Chou et al., “Rapid assessment of the heterogeneous methylation status of CEBPA in patients with acute myeloid leukemia by using high-resolution melting profile,” The Journal of Molecular Diagnostics, vol. 13, no. 5, pp. 514–519, 2011.

[76] C. Cobaleda, “Reprogramming of B cells. Cellular programming and reprogramming,” Methods and Protocols, vol. 636, pp. 233–250, 2010.

[77] R. Kanwal and S. Gupta, “Epigenetic modifications in cancer,” Clinical Genetics, vol. 81, no. 4, pp. 303–311, 2012.

[78] T. J. Zhang, J. D. Zhou, D. Q. Yang et al., “Hypermethylation of DLX4 predicts poor clinical outcome in patients with myelodysplastic syndrome,” Clinical Chemistry and Laboratory Medicine, vol. 54, no. 5, pp. 865–871, 2016.

[79] J. D. Zhou, J. Lin, T. J. Zhang et al., “GPX3 methylation in bone marrow predicts adverse prognosis and leukemia transformation in myelodysplastic syndrome,” Cancer Medicine, vol. 6, no. 1, pp. 267–274, 2017.

[80] H. Wang, R. Fan, X. Q. Wang et al., “Methylation of Wnt antagonist genes: a useful prognostic marker for myelodysplastic syndrome,” Annals of Hematology, vol. 92, no. 2, pp. 199–209, 2013.

[81] R. Chaubey, S. Szazwai, M. Mahapatra, S. Chhikara, and R. Saxena, “Prognostic relevance of aberrant SOCS-1 gene promoter methylation in myelodysplastic syndromes patients,” International Journal of Laboratory Hematology, vol. 37, no. 2, pp. 265–271, 2015.

[82] T. Božić, Q. Lin, J. Frobel et al., “DNA-methylation in C1R is a prognostic biomarker for acute myeloid leukemia,” Clinical Epigenetics, vol. 4, pp. 7–116, 2015.

[83] K. N. Kurtović, M. Krajnović, A. Bogdanović et al., “Concomitant aberrant methylation of p15 and MGMT genes in acute myeloid leukemia: association with a particular immunophenotype of blast cells,” Medical Oncology, vol. 29, no. 5, pp. 3547–3556, 2012.

[84] J. Roman-Gomez, A. Jimenez-Velasco, X. Agirre, F. Prosper, A. Heiniger, and A. Torres, “Lack of CpG island methylator phenotype defines a clinical subtype of T-cell acute lymphoblastic leukemia associated with good prognosis,” Journal of Clinical Oncology, vol. 23, no. 28, pp. 7043–7049, 2005.

[85] A. Guillaumet-Adkins, J. Richter, M. D. Odero et al., “Hypermethylation of the alternative AWT1 promoter in hematological malignancies is a highly specific marker for acute myeloid leukemias despite high expression levels,” Journal of Hematology & Oncology, vol. 7, no. 1, pp. 4, 2014.

[86] R. Chaubey, S. Szazwai, M. Mahapatra, S. Chhikara, and R. Saxena, “Prognostic relevance of aberrant SOCS-1 gene promoter methylation in myelodysplastic syndromes patients,” International Journal of Laboratory Hematology, vol. 37, no. 2, pp. 265–271, 2015.

[87] T. Božić, Q. Lin, J. Frobel et al., “DNA-methylation in C1R is a prognostic biomarker for acute myeloid leukemia,” Clinical Epigenetics, vol. 4, pp. 7–116, 2015.

[88] K. N. Kurtović, M. Krajnović, A. Bogdanović et al., “Concomitant aberrant methylation of p15 and MGMT genes in acute myeloid leukemia: association with a particular immunophenotype of blast cells,” Medical Oncology, vol. 29, no. 5, pp. 3547–3556, 2012.
[91] I. Vazquez, M. Maicas, J. Cervera et al., “Downregulation of EVII is associated with epigenetic alterations and good prognosis in patients with acute myeloid leukemia,” Haematologica, vol. 96, no. 10, pp. 1448–1456, 2011.

[92] Y. F. Deng, L. Zhang, X. Q. Zhang, M. Q. Hu, X. Z. Zhang, and Y. L. Xu, “Methylation of FHT gene promoter region in DNA from plasma of patients with myelodysplastic syndromes and demethylation effect of decitabine,” Zhong-guo Shi Yan Xue Ye Xue Za Zhi, vol. 20, no. 5, pp. 1144–1148, 2012.

[93] G. Strathdee, T. L. Holyoake, A. Sim et al., “Inactivation of HOXA genes by hypermethylation in myeloid and lymphoid malignancy is frequent and associated with poor prognosis,” Clinical Cancer Research, vol. 13, no. 17, pp. 5048–5055, 2007.

[94] F. Zaker, N. Amirizadeh, N. Nasiri et al., “Ploid gene aberrant methylation in the differentiation of myelodysplastic syndrome,” International Journal of Molecular and Cellular Medicine, vol. 5, no. 2, pp. 90–99, 2016.

[95] H. Y. Kang, X. R. Wang, L. Gao et al., “Clinical significance of ID4 gene methylation in demethylation-treated MDS cell line and 2 MDS patients,” Zhonggguo Shi Yan Xue Ye Xue Za Zhi, vol. 23, no. 2, pp. 455–459, 2015.

[96] M. Y. Li, Y. Y. Xu, H. Y. Kang et al., “Quantitative detection of ID4 gene aberrant methylation in the differentiation of myelodysplastic syndrome from aplastic anemia,” Chinese Medical Journal, vol. 128, no. 15, pp. 2019–2025, 2015.

[97] D. H. Wu, D. M. Yao, L. Yang et al., “Hypomethylation of let-7a-3 is associated with poor prognosis in myelodysplastic syndrome,” Leukemia & Lymphoma, vol. 58, no. 1, pp. 96–103, 2017.

[98] Q. Hong, X. Chen, H. Ye et al., “Association between the methylation status of the MGMT promoter in bone marrow specimens and chemotherapy outcomes of patients with acute myeloid leukemia,” Oncology Letters, vol. 11, no. 4, pp. 2851–2856, 2016.

[99] B. Quesnel, G. Guillerm, R. Vereecque et al., “Methylation of the p15(INK4b) gene in myelodysplastic syndromes is frequent and acquired during disease progression,” Blood, vol. 91, no. 8, pp. 2985–2990, 1998.

[100] D. H. Christiansen, M. K. Andersen, and J. Pedersen-Bjerregaard, “Methylation of p15INK4B is common, is associated with deletion of genes on chromosome arm 7q and predicts a poor prognosis in therapy-related myelodysplasia and acute myeloid leukemia,” Leukemia, vol. 17, no. 9, pp. 1813–9, 2003.

[101] G. H. Lyman, M. S. Poniewierski, and E. Culakova, “Risk of chemotherapy-induced neutropenic complications when treating patients with non-Hodgkin lymphoma,” Expert Opinion on Drug Safety, vol. 15, no. 4, pp. 483–492, 2016.

[102] E. F. Rodrigues, C. B. Santos-Rebouças, M. M. Gonçalves Pimentel et al., “Epigenetic alterations of p15INK4B and p16INK4A genes in pediatric primary myelodysplastic syndrome,” Leukemia & Lymphoma, vol. 51, no. 10, pp. 1887–1894, 2010.

[103] Y. Zhao, J. Guo, X. Zhang et al., “Downregulation of p21 in myelodysplastic syndrome is associated with p73 promoter hypermethylation and indicates poor prognosis,” American Journal of Clinical Pathology, vol. 140, no. 6, pp. 819–827, 2013.

[104] Y. S. Zhao, R. Yang, S. C. Gu et al., “Study of aberrant p73 promoter methylation in patients with myelodysplastic syndrome,” Zhonghua Xue Ye Xue Za Zhi, vol. 33, no. 10, pp. 847–851, 2012.

[105] S. Deneberg, P. Guardiola, A. Lennartsson et al., “Prognostic DNA methylation patterns in cytogenetically normal acute myeloid leukemia are predefined by stem cell chromatin marks,” Blood, vol. 118, no. 20, pp. 5573–5582, 2011.

[106] M. Menschikowski, U. Platzbecker, A. Hagelgans et al., “Aberrant methylation of the M-type phospholipase A2 receptor gene in leukemic cells,” BMC Cancer, vol. 12, no. 1, p. 576, 2012.

[107] A. Ward, G. Sivakumar, S. Kanjeekal et al., “The deregulated promoter methylation of the polo-like kinases as a potential biomarker in hematological malignancies,” Leukemia & Lymphoma, vol. 56, no. 7, pp. 2123–2133, 2015.

[108] E. Taskesen, F. J. Staal, and M. J. Reinders, “An integrated approach of gene expression and DNA-methylation profiles of WNT signaling genes uncovers novel prognostic markers in acute myeloid leukemia,” BMC Genomics, vol. 16, no. 4, article 54, 2015.

[109] E. A. Griffiths, S. D. Gore, C. Hooker et al., “Acute myeloid leukemia is characterized by Wnt pathway inhibitor promoter hypermethylation,” Leukemia & Lymphoma, vol. 51, no. 9, pp. 1711–1719, 2010.

[110] R. Fan, X. L. Zhao, H. Wang et al., “Abnormal methylation of the sex-determining region Y-box 17 (SOX17) promoter predicts poor prognosis in myelodysplastic syndrome,” Clinical Laboratory, vol. 60, no. 9, pp. 1465–1474, 2013.

[111] X. Zhao, X. Tian, S. Kajigaya et al., “Epigenetic landscape of the TERT promoter: a potential biomarker for high risk AML/MDS,” British Journal of Haematology, vol. 175, no. 3, pp. 427–439, 2016.

[112] A. S. Helbo, M. Treppendahl, D. Aslan et al., “Hypermethylation of the VTRNA1-3 promoter is associated with poor outcome in lower risk myelodysplastic syndrome patients,” Genes, vol. 6, no. 4, pp. 977–990, 2015.

[113] H. Y. Kang et al., “Downregulation of ZC3H12A gene methylation in myelodysplastic syndrome progression,” Zhonggguo Shi Yan Xue Ye Xue Za Zhi, vol. 23, no. 3, pp. 746–749, 2015.

[114] M. Verma, “The role of epigenomics in the study of cancer biomarkers and in the development of diagnostic tools,” Advances in Experimental Medicine and Biology, vol. 867, pp. 59–80, 2015.

[115] N. C. Wong, D. Ashley, Z. Chatterton et al., “A distinct DNA methylation signature defines pediatric pre-B cell acute lymphoblastic leukemia,” Epigenetics, vol. 7, no. 6, pp. 535–541, 2012.

[116] M. Esteller, M. Toyota, M. Sanchez-Cespedes et al., “Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is associated with G to A mutations in K-ras in colorectal tumorigenesis,” Cancer Research, vol. 60, no. 9, pp. 2368–2371, 2000.

[117] S. J. Clark, A. Statham, C. Stirzaker, P. L. Molloy, and M. Frommer, “DNA methylation: bisulphite modification and analysis,” Nature Protocols, vol. 1, no. 5, pp. 2353–2364, 2006.

[118] M. Begemann, I. Leisten, L. Soellner, K. Zerres, T. Eggermann, and S. Spengler, “Use of multilocus methylation-specific single nucleotide primer extension (MS-SNuPE) technology in diagnostic testing for human imprinted loci,” Epigenetics, vol. 7, no. 5, pp. 473–481, 2012.
[119] X. Calvo, M. Nomdedeu, A. Navarro et al., “High levels of global DNA methylation are an independent adverse prognostic factor in a series of 90 patients with de novo myelodysplastic syndrome,” Leukemia Research, vol. 38, no. 8, pp. 874–881, 2014.

[120] J. G. Herman, J. Jen, A. Merlo, and S. B. Baylin, “Hypermethylation-associated inactivation indicates a tumor suppressor role for p15INK4B,” Cancer Research, vol. 56, no. 4, pp. 722–727, 1996.

[121] Y. Oki and J. P. J. Issa, “Epigenetic mechanisms in AML—a target for therapy,” Cancer Treatment and Research, vol. 145, pp. 19–40, 2010.

[122] I. H. I. Wong, M. H. Ng, D. P. Huang, and J. C. Lee, “Aberrant p15 promoter methylation in adult and childhood acute leukemias of nearly all morphologic subtypes: potential prognostic implications,” Blood, vol. 95, no. 6, pp. 1942–1949, 2000.

[123] N. I. Khan and L. Bendall, “Role of WNT signaling in normal and malignant hematopoiesis,” Histology and Histopathology, vol. 21, pp. 761–774, 2006.

[124] J. D. Zhou, T. J. Zhang, X. X. Li et al., “Epigenetic dysregulation of ID4 predicts disease progression and treatment outcome in myeloid malignancies,” Journal of Cellular and Molecular Medicine, vol. 21, no. 8, pp. 1468–1481, 2017.

[125] J. P. J. Issa, “DNA methylation as a therapeutic target in cancer,” Clinical Cancer Research, vol. 13, no. 6, pp. 1634–1637, 2007.

[126] L. Shen, Y. Kondo, S. Ahmed et al., “Drug sensitivity prediction by CpG island methylation profile in the NCI-60 cancer cell line panel,” Cancer Research, vol. 67, no. 23, pp. 11335–11343, 2007.

[127] L. Shen, H. Kantarjian, Y. Guo et al., “DNA methylation predicts survival and response to therapy in patients with myelodysplastic syndromes,” Journal of Clinical Oncology, vol. 28, no. 4, pp. 605–613, 2009.

[128] P. Tan, A. Wei, S. Mithraprabhu et al., “Dual epigenetic targeting with panobinostat and azacitidine in acute myeloid leukemia and high-risk myelodysplastic syndrome,” Blood Cancer Journal, vol. 4, no. 1, p. 170, 2014.

[129] R. Yasmin, S. Siraj, A. Hassan, A. R. Khan, R. Abbasi, and N. Ahmad, “Epigenetic regulation of inflammatory cytokines and associated genes in human malignancies,” Mediators of Inflammation, vol. 2015, Article ID 201703, 8 pages, 2015.

[130] B. T. Joyce, T. Gao, L. Liu et al., “Longitudinal study of DNA methylation of inflammatory genes and cancer risk,” Cancer Epidemiology, Biomarkers & Prevention, vol. 24, no. 10, pp. 1531–1538, 2015.

[131] J. M. Tarrant, “Blood cytokines as biomarkers of in vivo toxicity in preclinical safety assessment: considerations for their use,” Toxicological Sciences, vol. 117, no. 1, pp. 4–16, 2010.

[132] X. S. Wang, Q. Shi, L. A. Williams et al., “Inflammatory cytokines are associated with the development of symptom burden in patients with NSCLC undergoing concurrent chemoradiation therapy,” Brain, Behavior, and Immunity, vol. 24, no. 6, pp. 968–974, 2010.

[133] P. Tsapogas, C. J. Mooney, G. Brown, and A. Rolink, “The cytokine Flt3-ligand in normal and malignant hematopoiesis,” International Journal of Molecular Sciences, vol. 18, no. 6, 2017.

[134] N. M. Kuderer, D. C. Dale, J. Crawford, L. E. Cosler, and G. H. Lyman, “Mortality, morbidity, and cost associated with febrile neutropenia in adult cancer patients,” Cancer, vol. 106, no. 10, pp. 2258–2266, 2006.

[135] M. A. Lewis, A. W. Hendrickson, and T. J. Moynihan, “Onco-logic emergencies: pathophysiology, presentation, diagnosis, and treatment,” CA: a Cancer Journal for Clinicians, vol. 61, no. 5, pp. 287–314, 2011.

[136] G. H. Lyman and D. J. Delgado, “Risk and timing of hospitalization for febrile neutropenia in patients receiving CHOP, CHOP-R, or CNOP chemotherapy for intermediate-grade non-Hodgkin lymphoma,” Cancer, vol. 98, no. 11, pp. 2402–2409, 2003.

[137] G. H. Lyman, D. C. Dale, J. Friedberg, J. Crawford, and R. I. Fisher, “Incidence and predictors of low chemotherapy-dose-intensity in aggressive non-Hodgkin’s lymphoma: a nationwide study,” Journal of Clinical Oncology, vol. 22, no. 21, pp. 4302–4311, 2004.

[138] G. H. Lyman, “Impact of chemotherapy dose intensity on cancer patient outcomes,” Journal of the National Comprehensive Cancer Network, vol. 7, no. 1, pp. 99–108, 2009.

[139] M. Shayne, E. Culakova, M. S. Poniewierski et al., “Dose intensity and hematologic toxicity in older cancer patients receiving systemic chemotherapy,” Cancer, vol. 110, no. 7, pp. 1611–1620, 2007.

[140] J. A. Klastersky and M. Paesmans, “Treatment of febrile neutropenia is expensive: prevention is the answer,” Oncology Research and Treatment, vol. 34, no. 5, pp. 226–228, 2011.

[141] A. M. Hendricks, E. T. Loggers, and J. A. Talcott, “Costs of home versus inpatient treatment for fever and neutropenia: analysis of a multicenter randomized trial,” Journal of Clinical Oncology, vol. 29, no. 30, pp. 3984–3989, 2011.

[142] H. Chen, L. D. Orozco, J. Wang et al., “DNA methylation indicates susceptibility to isoproterenol-induced cardiac pathophysiology and is associated with chromatin states,” Circulation Research, vol. 118, no. 5, pp. 786–797, 2016.

[143] J. M. Flanagan, C. S. Wilhelm-Benartzi, M. Metcalf, S. B. Kaye, and R. Brown, “Association of somatic DNA methylation variability with progression-free survival and toxicity in ovarian cancer patients,” Annals of Oncology, vol. 24, no. 11, pp. 2813–2818, 2013.

[144] L. F. van der Zanden, S. H. Vermeulen, A. Oskarsdottir et al., “Description of the EuroTARGET cohort: a European collaborative project on TARGETed therapy in renal cell cancer–GENetic- and tumor-related biomarkers for response and toxicity,” Urologic Oncology, vol. 35, no. 8, 2017.

[145] A. Chakravarti, “Longitudinal metabolomic and epigenetic profiling of body fluids from patients with lung and brain cancer receiving radiation therapy,” 2014, https://www.clinicaltrials.gov/ct2/show/NCT02259218.