DNMT3A and *IDH* mutations in acute myeloid leukemia and other myeloid malignancies: associations with prognosis and potential treatment strategies

AP Im[1], **AR Sehgal**[1], **MP Carroll**[2], **BD Smith**[3], **A Tefferi**[4], **DE Johnson**[1], and **M Boyiadzis**[1]

[1]Division of Hematology/Oncology, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

[2]Division of Hematology and Oncology, University of Pennsylvania, Philadelphia, PA, USA

[3]The Sidney Kimmel Comprehensive Cancer Center, Department of Oncology at the Johns Hopkins University, Baltimore, MD, USA

[4]Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, MN, USA

Abstract

The development of effective treatment strategies for most forms of acute myeloid leukemia (AML) has languished for the past several decades. There are a number of reasons for this, but key among them is the considerable heterogeneity of this disease and the paucity of molecular markers that can be used to predict clinical outcomes and responsiveness to different therapies. The recent large-scale sequencing of AML genomes is now providing opportunities for patient stratification and personalized approaches to treatment that are based on individual mutational profiles. It is particularly notable that studies by The Cancer Genome Atlas and others have determined that 44% of patients with AML exhibit mutations in genes that regulate methylation of genomic DNA. In particular, frequent mutation has been observed in the genes encoding DNA methyltransferase 3A (DNMT3A), isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2), as well as Tet oncogene family member 2. This review will summarize the incidence of these mutations, their impact on biochemical functions including epigenetic modification of genomic DNA and their potential usefulness as prognostic indicators. Importantly, the presence of *DNMT3A*, *IDH1* or *IDH2* mutations may confer sensitivity to novel therapeutic approaches, including the use of demethylating agents. Therefore, the clinical experience with decitabine and azacitidine in the treatment of patients harboring these mutations will be reviewed. Overall, we propose that understanding the role of these mutations in AML biology will lead to more rational therapeutic approaches targeting molecularly defined subtypes of the disease.

Correspondence: Dr AP Im, Division of Hematology/Oncology, University of Pittsburgh Cancer Institute, 5150 Centre Avenue, Cancer Pavilion 5th floor, Pittsburgh, PA 15232, USA. imap@upmc.edu.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

CURRENT TREATMENT PARADIGMS FOR AML

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of myeloid precursors and the most common acute leukemia diagnosed in adults.[1] The median age at diagnosis is 68 years.[2] Although some progress has been made in characterizing AML at diagnosis, substantial advances in initial therapy, prevention of relapse and improvement of overall survival (OS) have been limited.

The goal of induction chemotherapy in AML is complete remission (CR) with restoration of normal hematopoiesis.[3] Achievement of CR is an important first goal, as CR is closely associated with improved survival. The combination of an anthracycline (that is, daunorubicin or idarubicin) with cytarabine has been the cornerstone of initial induction therapy for adult AML for the past four decades. CR can be expected in 60–70% of newly diagnosed AML patients with current induction regimens. However, without additional therapy, most patients relapse. The use of consolidation therapy with high-dose cytarabine or allogeneic hematopoietic cell transplantation (HCT) improves outcomes in AML patients. For patients under the age of 60, cure rates have continued to rise and now approach 50%. However, for individuals over the age of 60, the cure rates for AML remain unacceptably low.[4,5]

The value of risk stratification by cytogenetic abnormality in AML has been demonstrated by analyses of patients enrolled in prospective clinical trials.[6,7] In addition, a few recurring gene mutations and overexpressed genes with prognostic relevance in AML have been identified and have been incorporated into current prognostication models.[8] Table 1 indicates the cytogenetic and molecular abnormalities used in current clinical practice to provide prognostication for AML at initial diagnosis. Key among the molecular alterations are mutations in the genes encoding FLT3, NPM1 and c-Kit.

FLT3 is a receptor tyrosine kinase that spans the plasma membrane and has an important role in proliferation, survival and differentiation of hematopoietic progenitor cells. FLT3-mutant proteins with internal tandem duplications (ITDs) in the juxta-membrane region (FLT3-ITD mutants) exhibit constitutive or enhanced tyrosine kinase activity.[9] The incidence of FLT3-ITD mutations in AML is ~25% (28–34% in patients with normal cytogenetics), varying somewhat according to age and clinical risk, and being less common in pediatric AML and in AML arising from an antecedent myelodysplastic syndrome (MDS). When treated with conventional chemotherapy, the prognosis for AML harboring FLT3-ITD mutations is significantly worse compared with AML without FLT3 mutations. Several FLT3 inhibitors are in various stages of development. Increasing evidence indicates that allogeneic HCT is of benefit for patients with FLT3-ITD mutations.

Nucleophosmin (*NPM1*) is one of the most frequently mutated genes in AML.[8] Mutations in the NPM1 protein are found in 25–35% of adult AML and are particularly frequent in cytogenetically normal AML (45–64%). AML-associated mutations in NPM1 result in disruption of a carboxy-terminal nuclear localization signal and generation of a new nuclear export signal, leading to aberrant cytoplasmic localization of the protein. Patients with NPM1 mutations, particularly those without concurrent FLT3-ITD mutations, consistently

exhibit a superior outcome. The favorable prognostic impact of NPM1 mutation applies to both younger and older patients.

The identification of the above mutations and their incorporation into risk stratification along with cytogenetic data has advanced our treatment approaches for patients with newly diagnosed AML. However, additional mutations have recently been discovered and our knowledge of their prognostic impact continues to evolve. In particular, a new class of mutations that impact epigenetic mechanisms has emerged. In this review, we highlight these mutations, their impact on prognosis, and their potential role in prognostication and treatment approaches.

MUTATIONS IMPACTING EPIGENETIC MECHANISMS

Whole-genome and -exome sequencing efforts have led to the identification of a broader panel of recurrently mutated genes in AML. Many of these mutations occur in genes that are involved in epigenetic regulation of transcription. Mutations in the genes encoding DNA methyltransferase 3A (DNMT3A), isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2), in particular, result in alterations in DNA methylation. Mutations in *DNMT3A* and *IDH1/2* are among the most commonly occurring mutations found in AML, especially in the intermediate- or normal cytogenetic risk category.[10] The Cancer Genome Atlas Research Network has revealed that 44% of AML primary specimens contain a nonsynonymous mutation in DNA methylation-related genes.[11] These mutations may have both mechanistic and prognostic implications, as studies of large AML patient cohorts have determined that aberrant DNA methylation is a hallmark feature of AML and is a likely mechanism of carcinogenesis.[12,13] Moreover, different AML subtypes exhibit different and specific patterns of DNA methylation, with distinct differences in regulation of gene expression.[12,14] Thus, there has been interest in elucidating the role of mutations in epigenetic modifying enzymes in the pathogenesis of AML.

DNMT3A

DNA methylation is an essential epigenetic modification of the genome that is involved in the regulation of gene expression. DNA methylation occurs primarily at the 5 position of cytosine, in particular in the context of CpG islands, which occur in and regulate gene promoters. Cancer genomes are most commonly characterized by global DNA hypomethylation. However, cancer cells also typically exhibit distinct regions of DNA hypermethylation, which are particularly well characterized in the CpG islands of promoter regions of tumor-suppressor genes. Methylation of histones, as well as histone acetylation, is other epigenetic modification by which gene transcription is regulated. Methylation of DNA and histones alters chromatin compaction and alters recruitment of co-activators and co-repressors. Hypermethylation of CpG islands and histones, and deacetylation of histones, leads to inactivation of transcription and, hence, silencing of these tumor-suppressor genes.

DNMT3A belongs to a family of DNA methyltransferases including DNMT1, DNMT3A and DNMT3B, whose role is to catalyze the addition of methyl groups to cytosine residues of CpG nucleotides.[15] DNMT3A is specifically involved in *de novo* DNA methylation, and functions independently of replication. Somatic mutations in *DNMT3A* were initially

identified by three groups of investigators, who observed these mutations in 4–22% of AML patients.[16–18] The majority of *DNMT3A* mutations are missense mutations that occur at residue R882 near the carboxyl terminus of the DNMT3A protein (Figure 1). However, a number of less common nonsense, frameshift and splice site mutations have been found throughout the DNMT3A coding sequence (Figure 1). Thus, mutations in DNMT3A are often classified as R882 versus non-R882 mutations. To date, no clinical difference has been described separating these two classes and the reason for the high prevalence of R882 mutations is unclear.

The function and biological consequences of DNMT3A mutations have yet to be fully elucidated. These mutations are almost always heterozygous, which suggests a gain of function effect, although this remains controversial. Yan *et al.* have reported that DNMT3A mutated at R882 exhibits substantially reduced enzymatic activity *in vitro* compared with wild-type DNMT3A.[17] The non-R882 DNMT3A mutations result in expression of a truncated protein, and thus are believed to disrupt enzymatic function. Challen *et al.*[19] sought to examine the effects of DNMT3A deficiency on hematopoiesis in *DNMT3A* knockout mice, and found that in comparison with wild-type hematopoietic stem cells (HSCs), serial transplantation of the *DNMT3A*-null HSCs resulted in loss of peripheral blood differentiation and expansion of the HSC population in the bone marrow. Moreover, loss of *DNMT3A* resulted in areas of increased, and areas of decreased, DNA methylation at distinct loci in the *DNMT3A*-null HSCs, as well as incomplete repression of HSC-specific genes. Hypomethylation was observed in genes that are commonly overexpressed in AML, such as *Runx1*, *Erg*, *Myc*, *Smad3* and so on, concomitant with upregulation of genes commonly expressed in HSCs and downregulation of differentiation factors. Interestingly, recipients of *DNMT3A*-null HSCs did not develop apparent myeloproliferative neoplasms (MPN), indicating that additional cooperating mutations may be required for disease development.

Despite the apparent biological consequences of *DNMT3A* loss in normal hematopoietic cells, the impact of *DNMT3A* mutations on DNA methylation and specific gene expression in AML remains controversial. Ley *et al.*[16] have reported that there are no differences in the total expression levels of DNMT3A protein or the mean 5-methylcytosine content of cells harboring mutant DNMT3A versus wild-type DNMT3A. This study also reported that although AML patients with mutated DNMT3A contained genomic regions with significantly different levels of methylation, there was no correlation between any of the differentially methylated regions and altered expression of nearby genes. Moreover, there were no clearly defined gene expression patterns that were associated with *DNMT3A* mutation status. Other studies have supported the lack of correlation between DNA methylation and *DNMT3A* mutational status, and have also reported an inability of gene and microRNA expression signatures to predict for *DNMT3A* mutational status.[20,21] In contrast, mRNA expression profiling of primary AML specimens by Yan *et al.*[18] determined that reduction of DNMT3A enzymatic activity as a result of mutation leads to enhanced expression of several genes in the *HOX* family, a family of genes that have important roles in normal hematopoiesis and are dysregulated in AML. In addition, several other studies have demonstrated a clear correlation between DNA methylation and *DNMT3A* mutational status. Hájková *et al.*[22] found significantly lower levels of global DNA methylation and

simultaneous hypermethylation of specific promoter sequences in patients with mutated *DNMT3A*. The lower levels of DNA methylation correlated with higher relapse rates and worse OS in the patients with mutant *DNMT3A*. Lower levels of *HOX* gene methylation were also observed, similar to the findings of Yan *et al.* Additional studies by Ribeiro *et al.*,[23] although failing to find a predictive methylation or gene expression signature associated with *DNMT3A* mutation, identified a single methylation cluster that was enriched in *DNMT3A*-mutated cases. Specifically, this methylation clustered was observed primarily in patients with NPM1 and FLT3-ITD mutations, which were also characterized by *HOX* gene overexpression. Methylation of the tumor-suppressor gene growth arrest and DNA-damage-inducible alpha (*GADD45A*) has also been observed in AML with DNMT3A mutations.[24] These discordant results may be due to differences in the patient populations or differences in methodologies for assessing DNA methylation. DNA methylation assays are still highly varied and the conflicts highlighted here are likely to be clarified, as more robust and uniform assays are applied to these questions.

IDH1/2

IDH1 and its mitochondrial homolog, IDH2, are enzymes involved in citrate metabolism, a critical step in the Krebs cycle.[25] Mutations in the *IDH1/2* genes are well described in lower-grade gliomas (grade II and III astrocytomas and oligodendrogliomas) and secondary glioblastomas, where the mutations have an incidence of more than 70%.[26] These gliomas are characterized by distinctive genetic and clinical characteristics. IDH1 and IDH2 normally function to catalyze the oxidative decarboxylation of isocitrate, producing α-ketoglutarate (α-KG) in an nicotinamide adenine dinucleotide phosphate-dependent manner. In AML, *IDH1/2* mutations result in amino acid changes that are highly restricted, occurring primarily at residue R132 in IDH1 and R140 or R172 in IDH2 (Figure 1).[27] *IDH1/2* mutations are heterozygous, suggesting that the mutations result in an enzymatic gain of function. In fact, *IDH1/2* mutations give rise to proteins with newly acquired and distinct enzyme activity, where the new activity of the enzymes is able to catalyze nicotinamide adenine dinucleotide phosphate hydrogen-dependent reduction of α-KG to 2-hydroxyglutarate (2HG).[28,29] This results in a decrease in α-KG and an increase in 2HG, with 2HG subsequently acting as a competitive inhibitor of α-KG-dependent reactions. Accumulation of this putative oncogenic metabolite has been observed in malignant gliomas and may be related to the pathogenesis of malignant brain tumors.[30] In AML, increased cellular 2HG levels contribute to epigenetic mechanisms of pathogenesis by inhibiting α-KG-dependent enzymes that are important for normal DNA methylation.

An important clue regarding the role of *IDH1/2* mutation and 2HG overproduction in the modulation of DNA methylation lies in the relationship of the IDH enzymes with Tet oncogene family member 2 (TET2; Figure 2). TET2 is an α-KG-utilizing enzyme that hydroxylates 5-methylcytosine as an important step in the demethylation of DNA.[31] TET2 mutations, which lead to loss of function, and *IDH1/2* mutations appear to be mutually exclusive in AML, suggesting an overlapping biologic effect.[25] The lack of α-KG and the presence of the competitive inhibitor 2HG in cells expressing mutant IDH1 or IDH2 serve to attenuate TET2 activity and, thereby, TET2-dependent demethylation of genomic DNA.[32] In fact, Figueroa *et al.*[25] have shown that TET2 loss of function mutations and IDH1/2

mutations are associated with similar epigenetic defects, and expression of IDH1/2-mutant proteins leads to impaired TET2 catalytic function in cells. In addition, expression of either IDH1/2- or TET2-mutant proteins impairs myeloid differentiation and increases the expression of stem/progenitor cell markers, suggesting a role for these mutations in AML differentiation blockade.

In contrast to AML associated with mutated *DNMT3A*, AML with mutations in *IDH1/2* has been clearly associated with a distinct methylation profile. The Cancer Genome Atlas data has revealed extensive gains of methylation in primary AML samples containing *IDH1/2* mutations.[11] In addition, studies by Figueroa *et al.*[25] have shown that AML samples with *IDH1/2* mutations display global DNA hypermethylation and a specific hypermethylation signature. These studies also showed that *IDH1-* and *IDH2*-mutated AML samples displayed similar DNA methylation profiles, consistent with the observation that these are mutually exclusive mutations and likely have a similar biologic effect. Moreover, expression of IDH1/2-mutant proteins in established and primary hematopoietic cells resulted in decreased expression of GATA1 (involved in myeloid differentiation), upregulation of c-Kit and impaired myeloid differentiation with an increase in stem/progenitor cells. Of note, the effects of mutated IDH proteins may not be limited to DNA methylation, as it has also been shown that IDH1/2 mutants capable of producing 2HG act to inhibit the histone demethylation that is necessary for terminal differentiation of lineage-specific progenitor cells.[33] Moreover, mutant *IDH1* cooperates with *HoxA9* gene to promote leukemogenesis in a mouse model.[34] Collectively, these, and other, studies confirm that expression of mutant IDH1 or IDH2 proteins leads to DNA hypermethylation, and that this epigenetic effect contributes to AML pathogenesis, in part, through impairment of hematopoietic differentiation.

INCIDENCE AND PROGNOSTIC IMPACT OF *DNMT3A* AND *IDH1/2* MUTATIONS IN AML

Emerging evidence has linked the presence of *DNMT3A* and *IDH1/2* mutations with clinical outcomes, particularly in cytogenetically normal AML, and it is likely that this will have a significant impact on risk stratification in the future. Subsequent to the initial descriptions of *DNMT3A* mutations in AML, multiple studies have retrospectively evaluated *DNMT3A* mutations in different patient populations (Table 2).[11,17,19–22,35–46] Overall, the incidence of *DNMT3A* mutations ranges from 12 to 35%, with mutations more commonly found in cytogenetically normal AML, and almost never described in AML with favorable cytogenetics. Older age, higher white blood cell and platelet counts, normal cytogenetics and the presence of *NPM1*, *FLT3-ITD* and *IDH1* mutations have been found to be more common in patients with *DNMT3A* mutations versus wild-type *DNMT3A* in the majority of studies.

Although the exact biochemical effects of *DNMT3A* mutations are still being elucidated, their impact on prognosis is more evident. Most studies have shown a negative impact on outcomes such as CR rates, relapse rates, event-free survival, relapse-free survival and OS. The negative impact on prognosis has been shown to be even more pronounced in specific populations, such as older patients, patients with normal cytogenetics and patients in a 'molecular high-risk' group, variably defined but including *FLT3-ITD* mutations or wild-

type *NPM1*.[25,27,47–52] However, not all studies have demonstrated a negative impact on clinical outcomes, perhaps due to different baseline characteristics or treatment courses.[38,45] Prospective studies on the impact of *DNMT3A* mutations in specific, well-defined patient populations are warranted, as well as studies evaluating the ability of specific therapies to abrogate the negative prognosis. Furthermore, the pattern of association of *DNMT3A* mutations with other molecular abnormalities such as *NPM1*, *FLT3* and *IDH* mutations suggests an interplay in the pathogenesis of AML that has yet to be fully delineated.

In contrast to DNMT3A mutations, more is known about the biochemical impact of IDH1/2 mutations (particularly as it pertains to elevated production of 2HG), but the impact on prognosis is less clear. The frequency of *IDH* mutations in AML is well described, with an incidence of 6–16% for *IDH1* and 8–19% for *IDH2* (Table 2).[11,27,28,34,43,45–59] As with *DNMT3A*, the higher incidences are seen in AML populations with normal cytogenetics. The clinical characteristics that are commonly found with *IDH1/2* mutations compared with wild-type are older age, higher platelet levels, normal cytogenetics and the presence of *NPM1* mutations. In addition, as stated above, *IDH1/2* and *TET2* mutations are known to be mutually exclusive. The frequent association with *NPM1* mutation is again suggestive of an interaction in the pathogenesis of AML.

The prognosis of patients with gliomas that harbor *IDH1/2* mutations has been shown to be significantly better than the prognosis of patients with wild-type *IDH1/2*.[26] However, the data in AML has been conflicting. Different studies, comprised of large patient cohorts, have shown that *IDH1/2* mutations are associated with a worse prognosis, a better prognosis or have no association at all (see Table 2). It appears likely that the impact of *IDH1/2* mutations on clinical outcomes may depend on the specific patient population. For example, in a study by Patel *et al.*,[43] mutational analyses were performed on 398 patients enrolled in an Eastern Cooperative Oncology Group clinical trial, evaluating high- versus low-dose daunorubicin for patients with AML. It was shown that patients with *IDH1/2* mutations had a better OS if they also had concurrent *NPM1* mutations. On the other hand, results from Mardis *et al.*,[55] Paschka *et al.*[50] and Ravandi *et al.*[51] have suggested that *IDH1/2* mutations confer a worse prognosis in cytogenetically normal AML, and this was observed in patients with *NPM1* mutations as well. Wagner *et al.*[52] have reported that mutations of R132 in *IDH1*, the most common *IDH* mutation, did not have an impact on survival. Koszarska *et al.*[48] have reported that *IDH1/2* mutations overall are not prognostic for survival, although the *IDH1* R132H mutation is associated with a worse OS. For now, the impact of *IDH1/2* mutations on clinical outcomes remains unclear.

It is interesting that serum levels of 2HG have recently been shown to predict the presence of *IDH* mutations.[60,61] As noted, 2HG is the oncometabolite produced by leukemic cells expressing mutant IDH. It is released into the serum, although little is known about regulation of 2HG steady-state levels in the serum. Interestingly, serum 2HG levels were found to be associated with shorter OS and response to treatment in patients with *IDH* mutations.[60,61] Thus, measurement of serum 2HG levels may have significant value for screening and prognostic purposes. This may become particularly relevant as mutant IDH inhibitors are brought into clinical applications.

Prognostic models for AML that are based on molecular mutations have been proposed by different groups.[36,39,40,43,62,63] These models provide better understanding of the impact of gene mutations in the context of coexisting gene mutations and may allow for comprehensive prognostication. For example, Patel *et al.*[43] proposed a prognostic stratification model based on findings that *IDH1/2* mutation was associated with improved OS in patients with mutated *NPM1*, and in patients who were *FLT3-ITD* positive, while *DNMT3A* mutations led to worse OS than in those who were *DNMT3A* wild type. Using this revised model, the intermediate-risk/normal cytogenetic risk group could be further divided into six distinct risk groups, each of which were described as molecularly favorable, intermediate or unfavorable. Other groups have proposed alternative models and there is not yet a consensus as to how to integrate the growing number of molecular abnormalities associated with AML into a single robust prognostication schema. Clearly, mutational profiling as a means to redefine prognostic risk stratification need to be studied and validated prospectively, but increasing evidence suggests that integration of molecular profiling with cytogenetic risk factors will lead to an improved risk stratification approach for AML patients.

INCIDENCE AND PROGNOSTIC IMPACT OF *DNMT3A* AND *IDH1/2* MUTATIONS IN MDS AND MPN

Myelodysplastic syndrome

Following the discovery of *DNMT3A* and *IDH1/2* mutations in AML, the mutational status of these genes has been evaluated in MDS. *DNMT3A* mutations occur in 2.6–13% of MDS cases, and like AML, the most common mutation is a heterozygous mutation at the R882 location that occurs most frequently in patients with normal cytogenetics (Table 2).[45,64–68] The presence of mutant DNMT3A in MDS appears to confer an inferior survival and a higher likelihood of progression to secondary AML compared with wild-type DNMT3A patients.[64,67,68] This has led to speculation that *DNMT3A* mutations may be involved in leukemic transformation in patients with MDS, although it is important to note that the observed *DNMT3A* mutations occurred in all French-American-British subtypes and across a range of International prognostic scoring system risk categories.[68]

Similarly, *IDH1/2* mutations have been detected in 4–12% of MDS cases in several studies.[65,69–71] One series of 88 patients with isolated 5q deletion MDS failed to identify any cases with *IDH1* or *IDH2* mutations.[72] The two largest studies of patients with MDS who were analyzed for *IDH* mutations identified *IDH1* but not *IDH2* mutations as inferring a worse prognosis with a higher rate of transformation to AML.[70,71] These findings raise the possibility that *IDH* mutations may be involved in the mechanism of progression to AML.

Myeloproliferative neoplasms

MPN are known to harbor JAK2, MPL and TET2 mutations.[73] More recently, mutations in *DNMT3A* and *IDH1/2*, as well as other genes, have been discovered. *DNMT3A* mutations occur at a low frequency (1–5%) in chronic phases of MPN, but at significantly higher frequencies (17–20%) in MPN-derived AMLs. Heterozygous mutations in the R882 location are again the most frequent *DNMT3A* mutations found in MPN (Table 2).[65,74–76]

IDH1/2 mutations are also more common in MPN-derived AML (21–31%), but occur in the chronic phase as well (2–4%), mostly in patients with primary myelofibrosis.[69,77–80] Although the presence of *DNMT3A* mutations has not been determined to have prognostic value in chronic phase MPN, it appears that *IDH* mutations may help to identify patients more likely to develop leukemic transformation.[79] Indeed, in the largest cohort of primary myelofibrosis patients to date, including both a European cohort and a cohort of patients from the Mayo Clinic, patients with mutant *IDH1* (and *IDH2* in the European cohort) but not *DNMT3A* had a shorter leukemia-free survival than those without *IDH* mutations.[81] Interestingly, Tefferi *et al.* recently reported the outcomes from a large cohort of patients with primary myelofibrosis and determined that *IDH* mutation was associated with increased leukemic transformation in patients with JAK2*V617F* mutation compared with patients without this mutation. This suggests potential cooperation of JAK2*V617F* and *IDH* mutations in promoting leukemia development.[79]

APPLICATION OF EPIGENETIC MODIFIERS IN AML WITH *DNMT3A* OR *IDH1/2* MUTATIONS: FUTURE DIRECTIONS

Although molecular mutations provide valuable prognostic information, they may also predict responsiveness to different therapies or may represent potential targets for novel therapeutic agents. As mutations in DNMT3A and IDH1/2 affect DNA methylation, there is particular interest in evaluating the impact of hypomethylating agents in AML patients harboring these mutations. Table 3 summarizes clinical trials in which the presence of *DNMT3A* and *IDH1/2* mutations has been assessed for association with response to hypomethylating agents.

Application of hypomethylating agents in AML

Hypomethylating agents, such as decitabine and azacitidine, are DNA methyltransferase inhibitors, and FDA approved agents for treatment of high-risk MDS. Decitabine is a deoxycytidine analog that is incorporated into DNA during S-phase of the cell cycle and binds to DNA methyltransferase, rendering it inactive. Azacitidine is a cytidine analog that primarily is incorporated into RNA, inhibiting RNA processing and function. To a lesser extent, it is incorporated into DNA, similarly to decitabine. The use of these agents for treatment of patients with AML who are unfit for standard induction chemotherapy has been an area of great interest. Decitabine as a single agent has been evaluated in single-arm phase II and randomized controlled phase III studies in older patients with AML who were not candidates for intensive chemotherapy, and has shown CR rates of 18–47%, with a median survival of 7.7–12.6 months.[82–84] Azacitidine was shown to be effective in a specific population of older AML patients with 20–30% bone marrow blasts, with a CR rate of 18% and median survival of 24.5 months.[85] By comparison, older patients who did not receive treatment have an estimated median survival of 1–4 months. As there is no standard of care for older patients who are unfit for induction chemotherapy, decitabine and azacitidine are now commonly used agents in this challenging patient population.

Relationship between response to hypomethylating agents and the status of DNMT3A and IDH1/2

A few studies have retrospectively assessed the impact of *DNMT3A* and *IDH1/2* mutations in response to hypomethylating agents. In a single-arm phase II trial evaluating the use of decitabine alone as upfront therapy in elderly AML, Blum *et al.*[82] described a CR rate of 47%, and found that higher pretreatment levels of *miR-29b*, a microRNA that targets DNA methyltransferases, were associated with clinical response. Moreover, responders exhibited a trend toward lower pretreatment levels of DNMT3A mRNA compared with non-responders. Methylation and gene expression analyses revealed that treatment with decitabine significantly reduced global DNA methylation, with significant concentration of the hypomethylated regions in chromosome subtelomeric regions. This suggests differential activity of decitabine in distinct chromosome regions.[86] The concentrated impact of decitabine on specific chromosomal regions, including regions that are important for regulation of hematopoietic cell differentiation, has been seen in preclinical studies as well.[87] Further evidence supporting an inverse relationship between *miR-29b* and DNMT3A levels in AML has been provided by Garzon *et al.*[88] who showed that enforced expression of *miR-29b* in AML cell lines resulted in marked reduction of DNMT3A mRNA and protein levels, which in turn led to global hypomethylation. This suggests that strategies to increase *miR-29b* levels, such as the use of synthetic *miR-29b* oligonucleotides, may represent a viable therapeutic option in AML expressing either wild-type or, particularly, mutant DNMT3A. In this regard, treatment with the histone deacetylator AR-42 has been shown to increase *miR-29b* levels and downregulate DNMT3A in leukemia cell lines.[89] Sequential treatment with AR-42 followed by decitabine generated a stronger anti-leukemic effect than either agent alone, both *in vitro* and *in vivo* (nonobese diabetic/severe combined immunodeficiency mice), although reduced potency was seen using the reverse sequence of administration.

DiNardo *et al.*[90] have evaluated the mutational status of *DNMT3A* and *IDH1/2* in 68 older patients treated with decitabine alone, azacitidine alone or a combination regimen incorporating one of these agents in front-line therapy. Overall, a CR rate of 25% was seen among all of the regimens. Patients with *DNMT3A* mutations had a 40% CR versus 22% in wild-type patients, although this was not a statistically significant difference; no association between *IDH1/2* mutation and response was observed. Interestingly, the presence of neither *IDH1/2* nor *DNMT3A* mutations was associated with event-free survival or OS. One hypothesis for the absence of prognostic impact of *DNMT3A* mutations in this study is that the use of hypomethylating agents may have abrogated the poor prognosis that is usually seen in patients with *DNMT3A* mutations. Supporting this hypothesis, Traina *et al.*[91] evaluated *DNMT3A* and *TET2* mutations in 92 MDS patients treated with decitabine or azacitidine, and found that not only was the presence of mutations predictive of CR to these agents, but that patients with these mutations had a better PFS than wild-type patients. Again, this suggests that the poor prognosis conferred by these mutations may be lessened by the use of epigenetic therapies. Finally, Metzeler *et al.*[92] evaluated 46 elderly patients with AML treated with decitabine with or without the histone deacetylase inhibitor vorinostat, and found a significantly higher CR rate in patients with *DNMT3A* mutations (75%) versus wild-type *DNMT3A* (34%). No association was observed between *TET2* and

IDH1/2 mutations and response, similar to that observed by DiNardo *et al.*[90] Collectively, these studies, although small in number, suggest a strong predictive value of *DNMT3A* mutations, but an unclear predictive value of *IDH1/2* mutations, for response to hypomethylating agents. They support the need for larger studies conducted in a prospective manner.

Strategies for targeting mutant IDH1/2 proteins

The newly acquired and distinct enzyme activity gained on mutation of IDH1 and IDH2 proteins provides an attractive and novel therapeutic target. A number of IDH inhibitors are in various stages of development and have been evaluated in preclinical studies. These inhibitors have been shown to reverse hyper-methylation of both histones and DNA in IDH-mutant-expressing leukemia cell lines, and also lower 2HG levels by >90% in xenograft models.[93] Rohle *et al.*[94] have shown that AGI-5198, a selective R132H-IDH1 inhibitor, blocks the ability of mutant IDH1 to produce 2HG, and in turn impairs the growth of mutant, but not wild-type, glioma cells *in vitro*. Other studies have shown that AGI-5198 reverses DNA methylation induced by mutant IDH1, resulting in re-expression of genes involved in glioma cell differentiation, and can block IDH mutant production of 2HG, leading to restored differentiation and regulation of proliferation in IDH-mutated leukemia cell lines.[95,96] These effects were not seen in cells expressing only wild-type IDH1. Another small molecule inhibitor of mutant IDH1, HMS-101, induces apoptosis and decreases colony formation of human bone marrow cells expressing mutant IDH1.[34] A small molecule inhibitor of IDH2/R140Q (AGI-6780) has also been developed that promotes differentiation of TF-1 erythroleukemia and primary human AML cells *in vitro*.[97] Indirect inhibition of IDH mutations has been studied as well. For example, growth inhibition of IDH-mutant primary AML cells has been demonstrated by inhibition of glutaminase, the enzyme responsible for production of glutamine, and the primary source of α-KG and 2HG in IDH-mutated AML.[98] Future testing is needed to determine whether inhibitors targeting mutant IDH1/2 proteins will demonstrate clinical benefit. In this regard, it is of note that AG-221 is currently in Phase I clinical trials in patients with IDH2-mutant AML (clinicaltrials.gov NCT01915498). Results of this and other ongoing studies should quickly provide insight into how such compounds will be tolerated in the clinic.

Perspectives

The role of specific gene mutations in AML continues to be an area of intense interest, and it is becoming increasingly evident that mutations impacting epigenetic mechanisms, such as mutations in *DNMT3A* and *IDH1/2*, are likely to dictate both prognosis and potential therapeutic responsiveness in intermediate-risk AML. Although there is still much to elucidate about the mechanisms of pathogenesis in *DNMT3A*-mutated AML and the specific prognostic role of *DNMT3A* and *IDH1/2* mutations, there is an abundance of data to support different hypotheses for study in prospective clinical trials. From current evidence, it is possible to foresee a refinement of the intermediate cytogenetic risk group, where the presence or absence of mutations will stratify patients into unique risk categories and aid in determining treatment plans. The impact of hypomethylating agents in *DNMT3A*-mutated AML is intriguing and merits focused investigation. The design of treatment regimens that incorporate hypomethylating agents with standard chemotherapy may also be warranted for

younger patients with *DNMT3A* mutations, a patient population where hypomethylating agents are typically not considered. In addition, being able to define a subset of older patients with AML who might have improved responses and survival with hypomethylating agents may help to better refine our current treatment approach in this challenging patient population with a particularly poor prognosis and limited treatment options. Moreover, aside from the potential predictive utility of *DNMT3A* mutations in the use of hypomethylating agents, the presence of these mutations may be predictive of responsiveness to other types of drugs. For example, the presence of DNMT3A mutations has been shown to be predictive of response in patients receiving high-dose daunorubicin as an induction regimen.[26] Regarding allogeneic HCT, the clinical benefit of HCT in first CR for patients with *DNMT3A* mutations has not been clearly established, but is an important question for future studies. Even dietary compounds such as curcumin (turmeric), genistein (soybean), tea polyphenols (green tea), resveratrol (grapes) and sulforaphane (cruciferous vegetables) have been found to alter DNA methylation and histone modifications, and there is interest in evaluating the impact of these compounds on cancer prevention and treatment.[99,100] Prospective determination of the predictive value of *DNMT3A* mutations for benefit from hypomethylating agents, specific chemotherapeutic regimens or from allogeneic HCT may provide an important step forward for creating individualized therapies based on the molecular profile of individual patients. Finally, as novel therapies such as newer hypomethylating agents (for example, sapacitibine and SGI-110) and specific targeted IDH1/2 inhibitors continue to be developed, molecular 'fingerprinting' of AML for these and other mutations may help to guide the use of both conventional and novel agents in this disease. It is to be hoped that such improved subclassification systems for AML will lead to more improvements in AML therapy in the decades ahead than have been seen in recent years.

Acknowledgments

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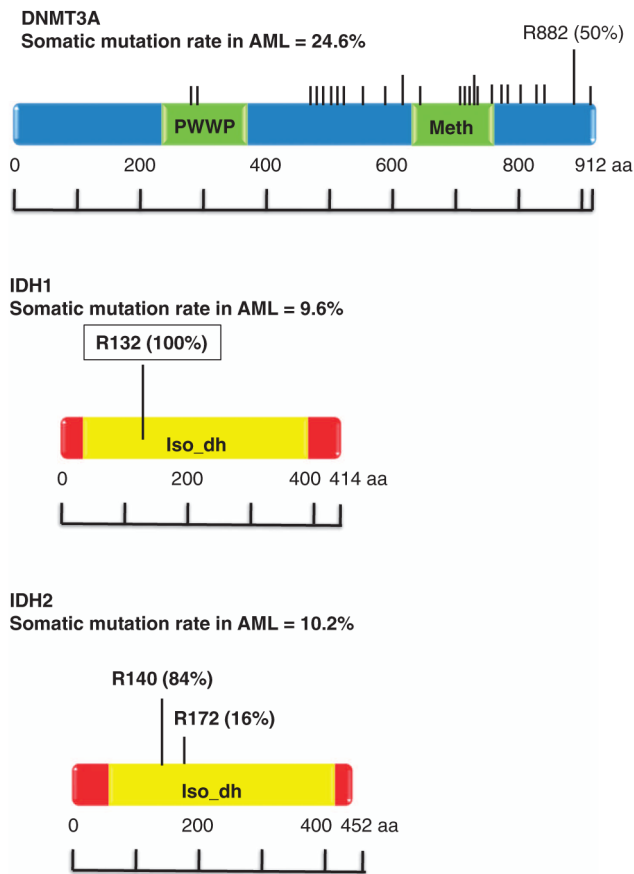
Figure 1.
Location and frequency of DNMT3A, IDH1 and IDH2 mutations as determined by The Cancer Genome Atlas. Mutation data was obtained from the cBio portal.[101]
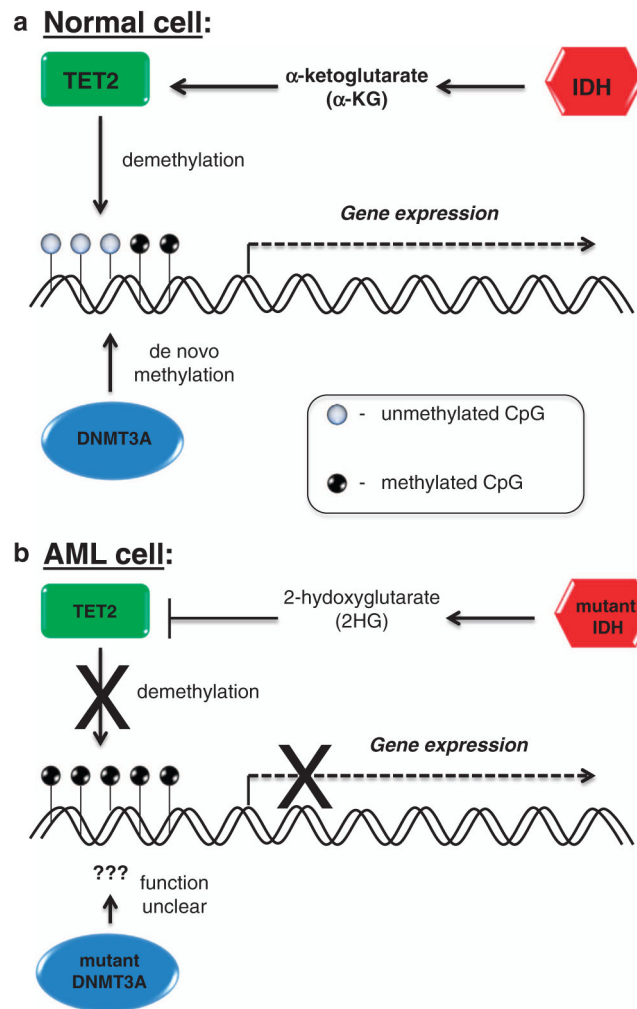
Figure 2.
(**a**) Normal function of IDH1/2, TET2 and DNMT3A enzymes in DNA methylation and gene expression. (**b**) Pathogenic mechanism of mutated IDH1/2 and DNMT3A in gene silencing in AML.

Table 1

Risk status based on cytogenetic and molecular abnormalities

Risk status	Cytogenetics		Molecular abnormalities
Favorable risk	inv(16) or t(16;16) t(8;21) t(15;17)	OR	Normal cytogenetics with NPM1 mutation in the absence of FLT3-ITD
Intermediate risk	Normal cytogenetics +8 t(9;11)	OR	t(8;21), inv(16), t(16;16) with c-Kit mutation
Poor risk	Complex (3 clonal chromosomal abnormalities) −5, 5q-, −7, 7q- 11q23–non t(9;11) inv(3), t(3;3) t(6;9) t(9;22)	OR	Normal cytogenetics with FLT3-ITD mutation

Abbreviation: OR, overall remission.

Table 2

Incidence and prognosis of DNMT3A and IDH1/2 mutations in AML, MDS and myeloproliferative neoplasms

AML

Study	DNMT3A	IDH1	IDH2	Association of mutation with prognosis	Reference
Mardis et al. (n =80)	ND	16% in NC	ND	IDH1: no association with OS; possible worse OS in AML with NC and wild-type NPM1	55
Marcucci et al. (n =358)	ND	14%	19%	IDH1: worse DFS; IDH2 (R172): lower CR	49
Wagner et al. (n =275)	ND	R132: 10.9%	ND	IDH1 R132: no association with prognosis NPM1/mutated FLT3	52
Paschka et al. (n =805)	ND	7.6%	8.7%	IDH1/2: worse RFS and OS in NC with mutated NPM1/wild-type FLT3	50
Abbas et al. (n =893)	ND	6%	11%	IDH1/2: worse OS in NC with wild-type NPM1/wild-type FLT3	47
Caramazza et al. (n =26)	ND	IDH1/2: 19% in AML with trisomy 8	IDH1/2: 19% in AML with trisomy 8	ND	53
Figueroa et al. (n =385)	ND	6.2%	8.6% (IDH1/2: 27.1% in NC)	ND	25
Ley et al. (n =281)	22.1% (33.7% in NC)	ND	ND	DNMT3A: worse OS	16
Patel et al. (n =199)	ND	6%	2% (IDH1/2: 11.8% in NC)	ND	56
Chou et al. (n =446)	ND	6.1%	12.1%	IDH1: no association with prognosis; IDH2: better prognosis, but worse OS in mutated FLT3	27
Yan et al. (n =112)	20.5%	ND	ND	DNMT3A: worse IS, time to treatment failure and lower CR	18
Thol et al. (n =489)	17.8%	ND	ND	DNMT3A: worse OS overall; worse OS and CR in NC; worse OS, RFS and lower CR in NC with wild-type NPM1/wild-type FLT3	20
Shen et al. (n =605)	12.3%	9.3%	9.8%	DNMT3A: worse OS, EFS and lower CR; IDH1/2: no association with prognosis	36
Hou et al. (n =500)	14% (22.9% in NC)	ND	ND	DNMT3A: worse OS and RFS	39
Markova et al. (n =226)	29.6% in NC	ND	ND	DNMT3A: higher relapse rate, especially in mutated FLT3	41
Chotirat et al. (n =230)	ND	8.7%	10.4%	IDH1/2: no association with prognosis	54
Marcucci et al. (n =415)	35.3% in age<60; 33.3% in age ≥60	ND	ND	DNMT3A: worse DFS, trend toward worse OS; Younger patients with non-R882 mutations: worse DFS and OS; Older patients with R882 mutations: shorter DFS and OS	21
Patel et al. (n =398)	23%	7%	8%	DNMT3A: worse OS in mutated FLT3; predictive of response to high-dose daunorubicin	43

Study	DNMT3A	IDH1	IDH2	Association of mutation with prognosis	Reference
Ravandi et al. (n =170)	ND	IDH1/2: 30%	IDH1/2: 30%	IDH1/2: better OS in mutated NPM1 / IDH2: better OS	51
Renneville et al. (n =123)	29% in NC	ND	ND	IDH1/2: no association with prognosis; worse OS in mutated NPM1/wild-type FLT3	44
Ribeiro et al. (n =415)	23.1%	ND	ND	DNMT3A: worse OS and EFS	23
Fernandez-Mercado et al. (n =84)	16.7% in NC	12.2% in NC	12.2% in NC	DNMT3A: worse OS and RFS, especially in wild-type NPM1/wild-type FLT3	37
Hájková et al. (n =79)	41%	ND	ND	ND	22
Westman et al. (n =11)	ND	IDH1/2: 12%	IDH1/2: 12%	ND	59
Ostronoff et al. (n =191)	19%	ND	ND	DNMT3A: worse OS, EFS and RFS	42
Koszarska et al. (n =376)	ND	8.5%	7.5%	IDH1/2: no association with prognosis / IDH R132H: worse OS	48
The Cancer Genome Atlas Research Network (n =200)	26%	IDH1/2: 20%	IDH1/2: 20%	ND	11
Gaidzik et al. (n =1770)	20.9%	ND	ND	DNMT3A: no association with prognosis R882: worse RFS non-R882: better OS	38
Roller et al. (n =194)	41% in NC	ND	ND	ND	45
Hou et al. (n =318)	14.9% (19.5% in NC)	ND	ND	DNMT3A: worse OS and EFS	40
Haferlach et al. (n =1291)	30.9%	8.8%	16.4%	ND	46
MDS					
Kosmider et al. (n =100)	ND	2%	3%	IDH1/2: no impact on survival	69
Patnaik et al. (n =88)	ND	0%	0%	ND	72
Rocquain et al. (n =65)	ND	3%	5%	ND	102
Thol et al. (n =193)	ND	4%	0%	IDH1: associated with inferior OS and higher rate of transformation to AML	71
Ewalt et al. (n =100)	5%	ND	ND	ND	66
Brecqueville et al. (n =66)	6%	IDH1/2: 5%	IDH1/2: 5%	ND	65
Walter et al. (n =150)	8.7%	ND	ND	DNMT3A: worse OS and increased progression to AML	68
Lin et al. (n =51)	7.8%	ND	ND	DNMT3A: worse OS	64
Thol et al. (n =193)	2.6%	ND	ND	DNMT3A: increased progression to AML	67
Patnaik et al. (n =277)	ND	3%	9%	IDH1: associated with inferior survival	70
Roller et al. (n =115)	13%	ND	ND	DNMT3A: no impact on survival	45

Study	DNMT3A	IDH1	IDH2	Association of mutation with prognosis	Reference
Myeloproliferative Neoplasms					
Green et al. (n =180)	ND	0%	0%	ND	77
Kosmider et al. (n =100 CMML)	ND	0%	8%	IDH1/2: no impact on survival	69
Pardanani et al. (n =166)	ND	IDH1/2: 3%	IDH1/2: 3%	ND	78
Tefferi et al. (n =1422)	ND	1%	1%	IDH1/2: no impact on survival in 111 patients with primary myelofibrosis	80
Brecqueville et al. (n =135)	1%	0%	0%	ND	65
Abdel-Wahab et al. (n =68 MPN; 15 CMML)	4% (MPN) 0% (CMML)	ND	ND	DNMT3A: no patients underwent leukemic transformation in follow-up time	74
Stegelmann et al. (n =80)	5%	ND	ND	ND	76
Jankowski et al. (n =52 CMML)	4%	ND	ND	DNMT3A: no impact on survival	75
Tefferi et al. (n =130 PMF)	ND	4%	5%	IDH1/2: inferior OS and LFS, more pronounced in presence of JAK2V617F	79
Vannucchi et al. (n =879 PMF)	5.7%	IDH1/2: 2.6%	IDH1/2: 2.6%	IDH1/2: inferior LFS	81

Abbreviations: AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; CR, complete remission; DFS, disease-free survival; EFS, event-free survival; LFS, leukemia-free survival; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; NC, normal cytogenetics; ND, not determined; OS, overall survival; PMF, primary myelofibrosis; RFS, relapse-free survival.

Table 3

Impact of DNMT3A and IDH1/2 mutations in response to hypomethylating agents

Study	Treatment	Response rate overall	Impact of mutation in response to hypomethylating agents	Reference
Blum *et al.* (*n* =53)	Decitabine 20 mg/m^2 × 10 days	CR 47%	Responders had a trend for lower pretreatment DNMT3A compared with non-responders Higher level of pretreatment miR-29b associated with clinical response.	82
Metzeler *et al.* (*n* =46)	Decitabine or decitabine +vorinostat	CR 41%	CR 75% in mutated DNMT3A versus CR 34% in wild-type DNMT3A; DNMT3A mutation was predictive of response TET2 and IDH1/2 mutations were not associated with CR	92
Traina *et al.* (*n* =92)	Decitabine, azacitidine or both	ORR 24%	TET2 and DNMT3A mutations were predictive of response TET2 and DNMT3A independently prognostic for better PFS	91
DiNardo *et al.* (*n* =56)	Decitabine, azacitidine or combination therapies	CR 25%	Neither DNMT3A nor IDH1/2 were associated with CR Neither DNMT3A nor IDH1/2 were associated with EFS or OS	90

Abbreviations: CR, complete remission; EFS, event-free survival; OS, overall survival; ORR, overall remission rate; PFS, progression-free survival.