**DNMT3A Mutational Status Affects the Results of Dose-Escalated Induction Therapy in Acute Myelogenous Leukemia**

Alison R. Sehgal, Phyllis A. Gimotty, Jianhua Zhao, Jing-Mei Hsu, Robert Daber, Jennifer D. Morissette, Selina Luger, Alison W. Loren, and Martin Carroll

**Abstract**

**Purpose:** DNA methyltransferase 3A (DNMT3A) is one of the commonly mutated genes in acute myelogenous leukemia (AML). Reports on the prognostic significance of DNMT3A mutations have been inconsistent, and most of the data are available only for patients 60 years of age or younger. We hypothesized that this inconsistency is due to an interaction between the dose of anthracycline used in induction therapy and DNMT3A status. We studied whether patients with DNMT3A-mutated AML treated with standard dose anthracyclines had an inferior survival compared with patients with other mutation profiles or those who received high-dose therapy.

**Experimental Design:** A total of 152 patients in this retrospective cohort study (median age, 54 years) with de novo AML underwent induction therapy and next-generation sequencing of 33 commonly mutated genes in hematologic malignancies, including DNMT3A, FLT3-ITD, NPM1, and IDH1/2. Cox regression was used to know whether those with DNMT3A mutations who were treated with standard dose anthracycline had inferior survival.

**Results:** DNMT3A mutations, found in 32% of patients, were not associated with an inferior survival. Dose escalation of anthracycline in the induction regimen was associated with improved survival in those with DNMT3A mutations but not those with wild-type DNMT3A. Patients with DNMT3A mutations who received standard dose induction had shorter survival time than other patient groups (10.1 months vs. 19.8 months, *P* = 0.0129). This relationship remained significant (HR, 1.90; *P* = 0.006) controlling for multiple variables.

**Conclusions:** Patients with DNMT3A-mutated AML have an inferior survival when treated with standard-dose anthracycline induction therapy. This group should be considered for high-dose induction therapy. Clin Cancer Res; 21(7); 1614–20. ©2015 AACR.

**Introduction**

The choice of induction and postremission therapy in acute myelogenous leukemia (AML) is guided by certain prognostic factors. Karyotype has historically been the largest determinant of prognosis (2, 3), but this inadequately predicts outcome in a large proportion of patients, particularly those with no karyotypic abnormalities. Recurrent gene mutations in NPM1 and CEBPA, and internal tandem duplications (ITD) in FLT3 have been recognized as important in AML pathogenesis and prognosis (4). More recently, an additional class of genes recurrently mutated in AML genomes has been identified that normally function in the epigenetic regulation of transcription. These include DNA methyltransferase 3A (DNMT3A), TET2, IDH1/IDH2, and ASXL1 (1, 5–10). A growing body of evidence supports a pathogenic role for these mutations in AML (11).

DNMT3A is one of the most commonly mutated genes in AML genomes and has been the topic of significant analysis since it was first noted by Ley and colleagues (12). It encodes one of the DNA methyltransferases, and along with DNMT3B, is responsible for adding a methyl group to cytosine/guanine residues. The prevalence of mutations in DNMT3A ranges from 18% to 36% and is enriched in normal karyotype AML (8, 13–18). The most frequently mutated residue of the DNMT3A gene occurs in the methyltransferase domain at Arginine 882, leading to decreased methylation activity in vitro (15) as well as decreased methylation levels in select genomic regions (12, 15). Additional mutations seen throughout the gene have also been described and are thought to also disrupt normal methylation activity. However, it has not been consistently associated with an altered gene expression pattern (12).

Despite an incomplete understanding of the functional changes induced by DNMT3A mutations, the initial studies of this gene mutation consistently showed that it conferred a poor prognosis (8, 12, 14). However, more recent studies have contradicted this finding, and have shown no difference in overall survival (OS) based on DNMT3A mutational status in large, homogenously treated patient cohorts (1, 13, 18, 19). Although the differences in prognostic significance in these studies may be due to a number of causes, including both patient factors and the
DNMT3A Affects Response to Dose-Escalated AML Induction Therapy

Translational Relevance

The emergence of comprehensive mutational testing in acute myelogenous leukemia (AML) has led to significant excitement in the leukemia community but also some concern for how to use the wide array of new genetic tests. One gene of interest is the DNA methyltransferase 3A (DNMT3A) gene. Previous work by Patel and colleagues (1) suggested that patients <60 years old with AML that express mutant DNMT3A require higher doses of anthracycline in their induction regimen to obtain equivalent results as DNMT3A wild-type patients. However, this observation has not previously been reproduced. Here, we describe our retrospective cohort study that confirms that mutated DNMT3A can predict for both overall and relapse-free survival with standard doses of anthracycline induction therapy, including patients ≥60 years. This decreased prognosis can be overcome by treating patients with higher doses of anthracycline. This confirms that patients with DNMT3A-mutated AML should be treated with higher doses of anthracycline.

Materials and Methods

Patient samples and treatment

Between January 2001 and August 2011, 172 patients with newly diagnosed AML consented to donation of their bone marrow or peripheral blood samples to the tissue bank at our institution. All patients consented to genetic analysis and clinical assessment on the basis of an Institutional Review Board-approved protocol with accompanying HIPAA authorization, and 167 underwent next-generation sequencing on the basis of available leukemia cell DNA.

Of these 167 patients, 152 underwent induction therapy and all analyses were restricted to this group (Supplementary Fig. S1). The regimen selected for each patient was based on treating physician preference, but generally included 3 days of an anthracycline and 7 days of cytarabine. Patients without adequate cytoreduction at the day 14-marrow assessment were retreated at their nadir with the same drugs unless they were felt to have failed therapy. For the purposes of this study, we defined induction therapy as high-dose for those who received a cumulative dose of ≥270 mg/m² of daunorubicin as either a single induction of 90 mg/m²/day or a double induction with 45 mg/m²/day-60 mg/m²/day daunorubicin or 72 mg/m² of idarubicin, given as 12 mg/m²/day initiated on day 1 and again day 14. All other regimens were classified as standard dose therapy.

Cyto genetic analysis

All patients underwent cytogenetic analysis. Karyotype results were classified as good, intermediate, or poor risk according to the Medical Research Council criteria (20). Patients with missing cytogenetic data, including those with failed cytogenetic testing, were classified as unknown.

Next-generation sequencing

Mutational analysis was performed using a targeted next-generation sequencing panel [ASXL1, ATM, BRAF, CBL, CDKN2A, DDX3X, DNMT3A, ETV6, EZH2, FBXW7, FLT3 (ITD and TKD) GNAS, IDH1, IDH2, JAK2, KIT, KHLH6, KRAS, MAPK1, MYD88, NOTCH1, NPM1, PTEN, PTPN11, PHF6, RUNX1, SF3B1, TET2, TP53, WT1, XPO1, ZMYM3]. In short, DNA was quantified using a fluorescent based measurement (Qubit, Life Technologies) and 20 to 250 ng of DNA was used for custom target enrichment. Following library preparation with the TruSeq Amplicon assay (Illumina), libraries were pooled and sequenced on the MiSeq to an average depth of coverage greater than 1,000 ×. This mean depth allowed for the most challenging amplicon to reach a minimum depth of coverage of 250 reads at all copy neutral loci. Data were then processed using a custom analysis pipeline composed of commercial, publicly available, and in house developed tools (21).

Statistical analysis

All hypothesis tests were two sided with statistical significance set as P < 0.05. All analyses were performed in STATA Version 12.0 (StataCorp). Baseline characteristics were compared between the mutated and wt DNMT3A status using the χ² test for categorical variable and the Wilcoxon rank-sum test for continuous variables.

Survival distributions for OS and relapse-free survival (RFS) were computed using the Kaplan–Meier method and compared using the log-rank test to determine statistical differences in the distributions for the exposure groups. A Cox regression model was used to adjust for covariates including age over 60 years, cytogenetic risk group, sex, allogeneic transplant, and FLT3-ITD. A backward elimination procedure was used to create the final multivariate model. Because an interaction between high-dose therapy and DNMT3A status was noted, an interaction term defined as DNMT3A-mutated treated with standard-dose therapy compared with all other groups (DNMT3A wt or DNMT3A-mutated treated with high-dose therapy) was retained in the multivariate model. We anticipated a sample size of 175 patients, with 22 (12.5%) in the DNMT3A-mutated/standard-dose group. A post hoc calculation using bootstrap methods was used to estimate the power of the log-rank test used to test the hypothesis that there was a difference in survival among patients with a DNMT3A mutation who received standard-dose anthracycline (n = 33, 3-year survival rate = 13.1%) compared with those without a DNMT3A mutation and those with a DNMT3A mutation who received high-dose anthracycline (n = 119, 3-year survival = 33.9%). The estimate of the power of the test was 73% (95% confidence interval, 70%–76%).
Results

Patient cohort

This patient cohort included all patients with a diagnosis of AML seen at the Hospital of the University of Pennsylvania (Philadelphia, PA) between January 2001 and August 2011 who provided adequate tissue and gave informed consent for these studies (Supplementary Fig. S1). Patient, disease, and treatment information is detailed in Table 1. Of note, the age range for this study was 19 to 86 years with a median age of 55 years, and 44% were ≥60 years. All cytogenetic risk groups are represented, with the intermediate risk group representing the largest fraction at 62%. High-dose induction therapy (as defined above) was given to 32% of all patients. The median follow-up time was 12.6 months.

Frequency and spectrum of DNMT3A mutations

Of the 152 patients assessed for mutation status, 52 (31.1%) harbored mutations in the DNMT3A gene (Supplementary Table S1). As expected, missense mutations in the R882 codon were the most common change, found in 57.7% (30/52) of those with DNMT3A mutations. Of those 30 patients, one also had a concurrent non-R882 mutation. An additional 21 patients had single non-R882-DNMT3A mutations and one additional patient had two non-R882 mutations. For subsequent analyses, DNMT3A mutated included both missense mutations in the R882 codon as well as the non-R882 mutations.

Association of DNMT3A with patient, disease, and treatment characteristics

The association of DNMT3A mutations with patient, disease, and treatment characteristics is detailed in Table 1 and Supplementary Fig. S1. At diagnosis, patients with DNMT3A mutations were younger and less likely to be male compared with DNMT3A wt (33% vs. 50% were ≥60 years and 44% vs. 63% were male). More patients with DNMT3A mutations were in the intermediate cytogenetic risk group (79% vs. 54%). The mean WBC count at diagnosis was also higher in those with DNMT3A mutations (74,700 vs. 51,500).

DNMT3A mutations occurred concomitantly with FLT3-ITD, NPM1, and IDH1 mutations more frequently than with wt DNMT3A, as seen in Table 1. When analysis was restricted to those with intermediate-risk cytogenetics, only NPM1 remained...

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Table 1. Patient, disease, and treatment characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Full cohort (n = 152)</th>
<th>DNMT3A mutant (n = 49, 32%)</th>
<th>DNMT3A wt (n = 103, 68%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, median, y (range)</td>
<td>54 (19–79)</td>
<td>54.4 (26–78)</td>
<td>54.1 (19–79)</td>
<td>0.6759</td>
</tr>
<tr>
<td>Age ≥ 60</td>
<td>39%</td>
<td>29%</td>
<td>44%</td>
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</tr>
<tr>
<td>Male</td>
<td>57%</td>
<td>45%</td>
<td>63%</td>
<td>0.034</td>
</tr>
<tr>
<td>WBC at diagnosis (mean)</td>
<td>58,232</td>
<td>76,569</td>
<td>49,509</td>
<td>0.021</td>
</tr>
<tr>
<td>WBC ≥ 100,000</td>
<td>21%</td>
<td>31%</td>
<td>17%</td>
<td>0.057</td>
</tr>
<tr>
<td>Cytogenetics risk groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>13%</td>
<td>0%</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>64%</td>
<td>82%</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>15%</td>
<td>10%</td>
<td>17%</td>
<td></td>
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<tr>
<td>Unknown</td>
<td>7%</td>
<td>8%</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>FLT3-ITD mutant</td>
<td>32%</td>
<td>43%</td>
<td>26%</td>
<td>0.039</td>
</tr>
<tr>
<td>NPM1 mutant</td>
<td>33%</td>
<td>65%</td>
<td>25%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IDH1 mutant</td>
<td>8%</td>
<td>16%</td>
<td>4%</td>
<td>0.008</td>
</tr>
<tr>
<td>IDH2 mutant</td>
<td>14%</td>
<td>14%</td>
<td>14%</td>
<td>0.908</td>
</tr>
<tr>
<td>High-dose therapy</td>
<td>32%</td>
<td>33%</td>
<td>32%</td>
<td>0.844</td>
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<tr>
<td>Double induction</td>
<td>15%</td>
<td>12%</td>
<td>17%</td>
<td>0.493</td>
</tr>
</tbody>
</table>

Abbreviations: n, number of patients; y, years.

Figure 1.

A, OS stratified by DNMT3A status. B, RFS stratified by DNMT3A status.
associated with DNMT3A (70.9% vs. 33.9%, \(P < 0.001\)). Concomitant mutations in DNMT3A, NPM1, and FLT3-ITD occurred in 18 of 167 patients as compared with the 6 of 167 expected by chance alone (\(P = 0.011\)). This triple-mutant genotype was initially noted by The Cancer Atlas Genome Study for AML and suggests biologic cooperation among these genes (22).

Figure 2.

Figure 3.
A, OS, comparing the DNMT3A-mutant patients who received standard dose therapy to all other patients (DNMT3A-wt and DNMT3A mutant who received high-dose therapy). B, RFS, comparing the DNMT3A-mutant patients who received standard dose therapy to all other patients (DNMT3A-wt and DNMT3A-mutant who received high-dose therapy).
Association of DNMT3A mutations with clinical outcomes
There was no difference in OS or RFS based on DNMT3A status alone, with median survival of 17.3 months and RFS of 13.8 months for DNMT3A-mutant compared with 16 and 13.1 for DNMT3A-wt (P = 0.3297 and P = 0.222, respectively; Fig. 1).
A mutational analysis of the Eastern Cooperative Oncology Group (ECOG) 1900 trial patients demonstrated that the benefit of anthracycline-intensified induction was seen only in those with a particular mutation profile, including DNMT3A mutations (1). We found a similar pattern in our institution’s cohort. Patients with mutated DNMT3A had an improved OS with high-dose therapy (P = 0.017) as compared with those with DNMT3A-wt, who did not benefit from intensified therapy (Fig. 2). Those with a mutated DNMT3A also had improved RFS with high-dose therapy, although this did not meet statistical significance (P = 0.082).

Of the 152 patients who received induction therapy, 33 (21.7%) had both a DNMT3A mutation and received standard-dose induction. We found that patients with this profile had worse prognosis, with a median survival of 10.1 months compared with 19.8 months for all other patients (P = 0.0129; Fig. 3A). Of note, there was no survival difference between the 3 patient subsets (DNMT3A-wt/standard dose; DNMT3A-wt/high dose; DNMT3A-mutant/high dose) that make up the comparator group (Fig. 4; P = 0.845, 0.2637, 0.2767).
This relationship of poorer survival in the DNMT3A-mutant/standard-dose group persisted on multivariate analysis after adjustment for other known prognostic factors, including age >60 years, karyotype, FLT3-ITD, and NPM1 mutations (HR, 1.89; P = 0.006). With the exception of FLT3-ITD, all known prognostic factors were significantly associated with survival in the univariate analyses (Table 2). A similar effect was seen for RFS, with a median RFS of 10.1 months for those patients with a DNMT3A mutation treated with standard-dose therapy and 13.6 months for all others (P = 0.020; Fig. 3B). This relationship was also significant in the multivariate analysis (Table 2).

Discussion
Mutational analysis in AML is being used to supplement traditional cytogenetic analysis to better understand prognosis and guide postremission therapy. It has emerging implications for targeted therapy. The results of this study suggest that it may also help to determine induction chemotherapy. Although DNMT3A mutations have a controversial impact on survival, this appears to be at least partially explained by an interaction with the dose of induction chemotherapy. Our study of a large single institutional cohort of patients with AML confirms that patients with mutated, but not wt, DNMT3A have an inferior prognosis if treated with standard doses of anthracycline chemotherapy during induction therapy.
This finding was consistent throughout our analysis, including our multivariate analysis that adjusted for age, cytogenetics, FLT3-ITD, and NPM1. As this was a retrospective study performed on patients who were not randomized to different doses of anthracyclines, it is possible that the inferior survival seen in DNMT3A-mutant patients who received standard-dose anthracycline was due to selection bias. Indeed, we were not able to collect and adjust for performance status. However, this
finding was seen only in patients with DNMT3A mutations, the minority of the cohort, suggesting that the inferior survival of this group is due to a true biologic effect, not simply selection bias for those with a poor performance status who were unable to tolerate higher doses of anthracycline chemotherapy. Furthermore, even patients with mutated DNMT3A who achieved a complete response with standard-dose anthracyclines had an inferior RFS, demonstrating that the improved survival seen in the patients who received high-dose therapy was not the result of failure to provide a second induction to unfit patients with residual disease.

The biology driving this relationship is not certain. DNMT3A mutations have been linked to changes in methylation patterns in affected genomes (12, 15) and extensive methylation loss when occurring with FLT3-ITD and NPM1 mutations together (22). It is possible that the pattern of changes in methylation through DNMT3A mutations could affect response to anthracyclines. Alternatively, DNMT3A mRNA and protein have been shown to be upregulated in response to increasing doses of doxorubicin in human colorectal cell lines, and silencing of DNMT3A increased the percentage of senescent cells in response to treatment with doxorubicin (23). DNMT3A mutations, particularly the single amino acid mutation, R882, has been shown to result in decreased function of the methyltransferase enzyme in in vitro studies (15). It is plausible that the decreased function of DNMT3A allows for a better response to high-dose anthracycline chemotherapy.

We defined high-dose anthracycline for this study as either a cumulative dose of $\geq 270$ mg/m$^2$ of daunorubicin (single induction of $90$ mg/m$^2$/day or double induction with $45–60$ mg/m$^2$) or $72$ mg/m$^2$ of idarubicin (double induction of $12$ mg/m$^2$ of idarubicin). Forty-nine patients received this high-dose therapy; this included 24 patients who received “double-induction” with two rounds of standard induction. This was generally performed at the treating physician’s discretion in response to an inadequately ablated day 14 bone marrow biopsy. As such, we considered this a single high-dose regimen. We feel that this is a reasonable and physiologic approach, and previous studies of anthracycline induction have been performed in a “response-adapted” method using double-induction as needed, then using the total dose of anthracycline received to guide subsequent trials (24–26).

Our findings of inferior survival in patients with DNMT3A mutations who receive standard doses of anthracyclines support those of Patel and colleagues in the ECOG 1900 cohort (11). With two studies now revealing this interaction, it seems reasonable to use the findings to guide therapy. Anthracycline escalation led to an improved survival without an increase in toxicity in patients under the age of 60 years in ECOG 1900 (27). Thus, we would not recommend a return to standard-dose induction for those without DNMT3A mutations without further studies. However, in patients over the age of 60 years, the role of dose escalation is uncertain. A cooperative group study published by Lowenberg and colleagues compared $45$ mg/m$^2$ with $90$ mg/m$^2$ of daunorubicin in patients over the age of 60 years with newly diagnosed AML and found that although there was an improved CR rate in those who received high-dose anthracycline, there was no difference in OS or in toxicity profile (28). Similarly, the Acute Leukemia French Association (ALFA)-9801 study found no difference in CR, OS, or EFS for dose-escalated therapy compared with standard-dose idarubicin in patients ages 50 to 70 years with AML (29). As such, high-dose therapy has not been routinely adopted for this age group, although the lack of excess toxicity in these trials suggests that anthracycline induction may not need to be dose-attenuated either (30).

Patients with DNMT3A mutations who are $\geq 60$ years old may be a select group for whom higher dose anthracycline is reasonable. However, DNMT3A mutational analysis is often not feasible before the initiation of induction therapy. For example, current processing time for this test at our center is 7 to 10 days. Our cohort of patients receiving induction therapy ranged from age 19 to 79 years including 59 patients age $\geq 60$. Fourteen patients $\geq 60$ years old received high-dose therapy; 10 of whom received it as a double induction. Therefore, one strategy in older patients may be to give standard-dose induction with $45$ mg/m$^2$, and, if subsequently found to have a DNMT3A mutation, they could receive a second dose of daunorubicin at $45$ mg/m$^2$ on day 14 regardless of bone marrow biopsy results at that time to ensure that they receive full high-dose anthracycline induction.

Mutational analysis of leukemic cells in patients with newly diagnosed AML is becoming more feasible and the number of clinical applications is growing. These results suggest that DNMT3A mutations alter the response to anthracycline chemotherapy, and define a group for whom high-dose therapy is particularly useful. Furthermore, it suggests that chemotherapy dose should be considered in the algorithm when incorporating comprehensive gene mutation signatures into risk-adapted postremission therapy plans. Future studies are necessary to determine the biology that guides this relationship to allow further personalization of treatment plans in this AML subtype. Significantly larger patient cohorts are necessary to define the behavior of rare subtypes—such as the triple DNMT3A, FLT3-ITD, and NPM1 mutant—and their response to chemotherapy.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.R. Sehgal, J.D. Morrissette, M. Carroll

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.R. Sehgal, P.A. Gimotty, J. Zhao, J.-M. Hsu, R. Daber, J.D. Morrissette, S.M. Luger, A.W. Loren, M. Carroll

Writing, review, and/or revision of the manuscript: A.R. Sehgal, P.A. Gimotty, J. Zhao, R. Daber, S.M. Luger, A.W. Loren, M. Carroll

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.R. Sehgal, R. Daber

Study supervision: A.W. Lugen, M. Carroll

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