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# Persistence of *DNMT3A* R882 mutations during remission does not adversely affect outcomes of patients with acute myeloid leukaemia

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## Summary

Somatic mutation of the *DNMT3A* gene at the arginine R882 site is common in acute myeloid leukaemia (AML). The prognostic significance of *DNMT3A* R882 mutation clearance, using traditional diagnostic next generation sequencing (NGS) methods, during complete remission (CR) in AML patients is controversial. We examined the impact of clearing *DNMT3A* R882 mutations at diagnosis to the detectable threshold of <3% during CR on outcome in 56 adult AML patients. Mutational remission, defined as clearance of pre-treatment *DNMT3A* R882 and all other

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**Authorship contributions**

B.B., A.-K.E., K.M., J.C.B. and C.D.B. contributed to the design and analysis of the study and the writing of the manuscript; A.-K.E. and S.O. performed laboratory-based research; D.N., J.K., and J.B. performed statistical analysis; J.C.B., W.B., R.L., J.K., R.S. and C.D.B. were involved directly or indirectly in the care of patients and/or sample procurement. All authors read and agreed on the final version of the manuscript.

**Disclosure of conflicts of interest**

The authors declare no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Data S1**. Supporting methods.

**Table SI**. Genes mutated at diagnosis and remission in individual patients.

**Table SII**. Standard curve detection sensitivity study of *DNMT3A* R882 mutated versus *DNMT3A* wild-type cell lines.

**Table SIII**. Comparison of pretreatment clinical characteristics of *de novo* AML patients who at CR cleared *DNMT3A* R882 and all other mutations with patients who retained only a *DNMT3A* R882 mutation and patients who retained a *DNMT3A* R882 mutation and had one or more other mutations.

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AML-associated mutations to a variant allele frequency <3%, occurred in 14 patients whereas persistent *DNMT3A* R882 mutations were observed in 42 patients. There were no significant differences in disease-free or overall survival between patients with and without *DNMT3A* R882 mutation clearance. Patients with persistent *DNMT3A* R882 who cleared all other AML mutations and did not acquire new mutations ($n = 30$), trended towards longer disease-free survival (1·6 vs. 0·6 years, $P = 0·06$) than patients with persistence of *DNMT3A* R882, in addition to other mutations or acquisition of new AML-associated mutations, such as those in *TET2, JAK2, ASXL1* and *TP53* ($n = 12$). These data demonstrate that *DNMT3A* R882 mutations, as assessed by traditional NGS methods, persist in the majority of AML patients in CR.

**Keywords**

acute myeloid leukaemia; DNA (cytosine-5)-methyltransferase 3 alpha; mutation clearance; survival; prognosis

Acute myeloid leukaemia (AML) is a clinically and biologically heterogeneous disease characterized by cytogenetic, molecular and epigenetic alterations. Cytogenetic risk stratification of AML is well established (Grimwade *et al*, 1998; Byrd *et al*, 2002; Mrózek *et al*, 2004). Other prognostic factors including mutational aberrations that are identified with next generation sequencing (NGS) methods are utilized to predict outcome or direct therapy. One such aberration, mutation of DNA (cytosine-5)-methyltransferase 3 alpha (DNMT3A), is reported to occur in 23–36% of patients (Thol *et al*, 2011; Hou *et al*, 2012; Marcucci *et al*, 2012; Renneville *et al*, 2012; Ribeiro *et al*, 2012; Gaidzik *et al*, 2013; Gale *et al*, 2015). Nearly two-thirds of these *DNMT3A* mutations affect the R882 codon in exon 23 of the gene's methyltransferase domain (Ley *et al*, 2010; Thol *et al*, 2011; Hou *et al*, 2012; Marcucci *et al*, 2012; Renneville *et al*, 2012; Ribeiro *et al*, 2012; Gaidzik *et al*, 2013; Gale *et al*, 2015). The *DNMT3A* R882 mutations exert a dominant-negative effect, which inhibits the residual *de novo* methyltransferase activity of the wild-type DNMT3A enzyme, leading to aberrant methylation patterns and genomic instability (Kim *et al*, 2013; Russler-Germain *et al*, 2014). In contrast, the mechanism of myeloid transformation for mutations not involving codon R882 of *DNMT3A* is not well defined. The *DNMT3A* gene itself is ubiquitously expressed in embryonic stem cells and known to be an important mediator of haematopoietic stem cell (HSC) differentiation (Challen *et al*, 2012; Mayle *et al*, 2015).

Acute myeloid leukaemia patients with *DNMT3A* mutations have distinct clinical features, including older age, association with cytogenetically normal AML (CN-AML), as well as concurrent presence of internal tandem duplications of the *FLT3* gene (*FLT3*-ITDs) and *NPM1* and *IDH1/IDH2* mutations (Ley *et al*, 2010; Thol *et al*, 2011; Hou *et al*, 2012; Marcucci *et al*, 2012; Renneville *et al*, 2012; Ribeiro *et al*, 2012; Gaidzik *et al*, 2013; Gale *et al*, 2015). Additionally, most (Ley *et al*, 2010; Thol *et al*, 2011; Hou *et al*, 2012; Marcucci *et al*, 2012; Renneville *et al*, 2012; Ribeiro *et al*, 2012; Gale *et al*, 2015), but not all (Gaidzik *et al*, 2013), studies have shown *DNMT3A* mutations to be associated with inferior overall survival (OS) in AML patients. Recent reports describe *DNMT3A* mutations to be present in preleukaemic HSCs that are capable of surviving chemotherapy and persisting in remission samples (Corces-Zimmerman *et al*, 2014; Shlush *et al*, 2014). Mutations in *DNMT3A*, as

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well as in *ASXL1*, *JAK2*, *TP53*, *TET2* and several other genes, have also been detected in healthy persons with evidence of clonal haematopoiesis that have a predisposition towards developing malignant haematological disorders including AML (Genovese *et al*, 2014; Jaiswal *et al*, 2014; Steensma *et al*, 2015). As such, mutation of *DNMT3A* is hypothesized to be a founding mutation, with serial acquisition of other mutations, such as *FLT3*-ITD, *NPM1* (Krönke *et al*, 2013) and *IDH1/IDH2* occurring at later time points and ultimately leading to clinical evidence of AML. These findings have raised important questions regarding the necessity of *DNMT3A* R882 mutational clearance for achievement of long-term remission following induction chemotherapy.

To date, only four studies have indirectly addressed these questions and report conflicting results regarding the frequency of *DNMT3A* mutation persistence during complete remission (CR) and the associated prognostic significance of these persistent mutations (Hou *et al*, 2012; Pløen *et al*, 2014; Debarri *et al*, 2015; Klco *et al*, 2015). Thus, the impact of eliminating *DNMT3A* R882 mutations following treatment is unclear. Herein, we examine the largest, to our knowledge, cohort of serially monitored *DNMT3A* R882 patients and demonstrate that persistence of *DNMT3A* R882 during remission at a detectable level (i.e. sensitivity of 3%) with traditional NGS does not adversely impact long-term outcome.

## Methods

### Patients and treatment

This study examined 57 *de novo* AML patients, median age 51 years (range, 28–72 years) with a dominant *DNMT3A* R882 mutation at time of diagnosis (variant allele frequency [VAF] ≥40%), who attained a first complete remission (CR1) and had at least one CR1 bone marrow (BM) sample available for analysis. Most CR samples were collected within 1 week of attainment of morphological CR, and the majority was collected on the same day that CR was documented. Nineteen patients had CR samples collected more than a month after CR was confirmed. Fifty-six out of the 57 patients could be successfully analysed for their mutational status in CR and were included in the statistical analyses. The sequencing data quality of one patient did not allow accurate variant calling, and the sample was excluded from all further statistical analyses (Patient 57; see Table SI for details). All patients received intensive cytarabine and daunorubicin-based induction treatment on Cancer and Leukemia Group B (CALGB) trials. Cancer and Leukemia Group B is now part of the Alliance for Clinical Trials in Oncology. Of the 56 patients included in the outcome analyses, those younger than 60 years were treated on CALGB/Alliance protocols 10503 ($n = 17$) (Blum *et al*, 2012), 19808 ($n = 14$) (Kolitz *et al*, 2010), 10603 ($n = 9$) (Stone *et al*, 2012), 9222 ($n = 2$) (Moore *et al*, 2005), and 9621 ($n = 2$) (Kolitz *et al*, 2004). Patients 60 years or older were treated on CALGB 10201 ($n = 6$) (Marcucci *et al*, 2007), 9720 ($n = 5$) (Baer *et al*, 2002) and 10502 ($n = 1$) (Attar *et al*, 2013). Specific details regarding these trials and dosing schedules are provided in the Supporting Information.

Three patients received allogeneic haematopoietic stem-cell transplantation (HSCT) in CR1 and had remission marrow samples obtained before the transplant. Seventeen patients underwent autologous HSCT in first CR and all but four patients had remission marrow samples collected before transplant.

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Institutional review board approval of all protocols was obtained before any research was performed. All patients gave written informed consent for treatment and research use of their specimens, in accordance with the Declaration of Helsinki.

## Cytogenetic and mutational analyses

Pre-treatment cytogenetic analyses of BM and/or blood samples were performed in the CALGB institutional cytogenetics laboratories and the results were confirmed by central karyotype review as previously reported (Mrózek *et al*, 2008). Viable cryopreserved BM or blood cells of patients enrolled onto the CALGB 9665 tissue bank protocol were stored for future analyses prior to starting treatment. Mononuclear cells from BM or blood were enriched by Ficoll-Hypaque gradient and cryopreserved in liquid nitrogen until thawed at 37°C for analysis. DNA extractions were performed using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). The mutational status of 34 genes (see Supporting Information for list of genes), the details of which are provided in Table SI, was determined by targeted amplicon sequencing using the Miseq platform (Illumina, San Diego, CA). Briefly, DNA library preparations were performed according to the manufacturer's instructions. Samples were pooled and run on the Miseq machine using the Illumina Miseq Reagent Kit v3. Sequenced reads were aligned to the hg19 genome build using the Illumina Isis Banded Smith-Waterman aligner. Single nucleotide variant and indel calling were performed using MuTect and Varscan2 (Broad Institute, Cambridge, MA), respectively (DePristo *et al*, 2011; Cibulskis *et al*, 2013), and these calls were sorted and aggregated with Mucor (Kroll *et al*, 2016). All called variants underwent visual inspection of the aligned reads using the Integrative Genomics Viewer (Broad Institute) (Robinson *et al*, 2011). In addition to the genes analysed with the targeted sequencing panel, testing for the presence or absence of biallelic *CEBPA* mutations was performed as previously reported (Marcucci *et al*, 2008). Thus, both methods combined enabled us to analyse the mutational status of 35 genes. The lower limit of detection for the *DNMT3A* R882 mutation in our NGS method of sequencing was determined by titration assays of a cell line (MOLM-13) containing this aberration. Depth of coverage at *DNMT3A* R882 in the standard curve ranged from 298 to 606 reads with a median of 396 reads. All standard curve samples below 3% VAF were not detectable by our methods (see Table SII). A VAF of ≥3% was therefore used as a cutoff for analyses related to detecting *DNMT3A* and other mutations. Variants were considered mutations if they were not reported in the 1000 Genome database (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/), dbSNP137 or dbSNP142 (http://www.ncbi.nlm.nih.gov/SNP/).

## Definition of clinical endpoints and statistical analysis

Definitions of clinical endpoints, i.e., CR, disease-free survival (DFS) and OS, are provided in the Supporting Information. The main objective of this study was to evaluate clinical outcomes in *DNMT3A* R882-mutated AML patients in whom *DNMT3A* R882 and other AML mutations either persisted at the time of CR achievement or, within 1 year, were no longer detectable above a VAF cut-off of 3%. Demographic and clinical features of patients in whom *DNMT3A* R882 mutations cleared below the VAF cut-off of 3% at CR (Group 1) were compared with those of patients whose *DNMT3A* R882 mutations persisted at a VAF ≥3% at CR (Group 2) using the Fisher's exact and Wilcoxon rank-sum tests for categorical

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and continuous variables, respectively (Vittinghoff *et al*, 2005). Group 2 was further subdivided as follows: Group 2a comprised patients with persistence of *DNMT3A* R882 at a VAF cut-off of ≥3% but no other AML-associated mutations during CR, and Group 2b included patients who, in addition to persistent *DNMT3A* R882 at a VAF cut-off of ≥3%, had (i) persistence of one or more other AML-associated mutations, or (ii) acquired additional AML mutations that were not present at diagnosis. Demographic and clinical features of these two groups were also compared using the Fisher's exact and Wilcoxon rank-sum tests for categorical and continuous variables, respectively (Vittinghoff *et al*, 2005). Estimated probabilities of DFS and OS were calculated using the Kaplan–Meier method (Kaplan & Meier, 1958), and the log-rank test evaluated differences between survival distributions. Cox proportional hazards model was used to calculate hazard ratios (HR) for DFS and OS (Vittinghoff *et al*, 2005). All analyses were performed by the Alliance for Clinical Trials in Oncology Statistics and Data Center on a database locked on 8 July 2015.

## Results

### Clinical and molecular characteristics of patients who cleared DNMT3A R882 mutations at CR and of those who did not

Among 56 AML patients with *DNMT3A* R882 at diagnosis, 14 cleared the *DNMT3A* R882 mutation, as well as all other AML mutations, below the VAF cut-off of 3% during CR (Group 1), whereas in 42 patients, *DNMT3A* R882 persisted at a VAF ≥3% (Group 2; see Table SI for details). This 3% cut-off was derived from our sensitivity studies described in the methods. The demographic and clinical features of patients assigned to Groups 1 and 2 are shown in Table I. Molecular data for individual patients are provided in Table SI. There were no differences between groups with respect to age, sex, race, platelet counts, white blood cell counts or percentages of presenting blood and BM blasts (Table I). Consistent with prior reports (Ley *et al*, 2010; Thol *et al*, 2011; Hou *et al*, 2012; Ribeiro *et al*, 2012; Gaidzik *et al*, 2013; Gale *et al*, 2015) the majority of patients, 86% in both groups, had CN-AML, whereas eight patients had an abnormal karyotype at diagnosis. With regard to the European LeukaemiaNet (ELN) genetic categories (Döhner *et al*, 2010; Mrózek *et al*, 2012) all patients in Group 1 ($n = 14$) were classified in the Favourable ($n = 7$) or Intermediate-I ($n = 7$) ELN Genetic Group, whereas three (8%) of the 39 classifiable patients in Group 2 were included in the ELN Intermediate-II or Adverse Genetic Group. Ten (71%) patients in Group 1 underwent either allogeneic ($n = 2$) or autologous ($n = 8$) HSCT in first CR, compared with 18 (43%) patients in Group 2 who underwent HSCT in first CR; all except one patient in Group 2 received autologous HSCT. The median VAFs of *DNMT3A* R882 for Groups 1 and 2 at diagnosis were similar [45% (range, 40–53%) vs. 46% (range, 41–50%), $P = 0{\cdot}58$; see Table SI for details). Among patients in Group 2, VAFs of *DNMT3A* R882 mutations were generally lower at time of CR than at diagnosis.

### Disease outcomes of patients who cleared DNMT3A R882 mutation and all other studied AML mutations at CR and of those who did not

We next sought to determine if clearance of *DNMT3A* R882 and mutations in 34 other AML-associated genes included in our panel was associated with an improved outcome.

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With a median follow-up time for living patients of 5·5 years (range: 3·7–11·2 years), no significant differences in DFS (median, 0·6 vs. 1·1 years, $P = 0·52$) or OS (median, 1·3 vs. 3·1 years; $P = 0·47$) were observed between patients in Group 1 and Group 2, respectively (Table II, Fig 1A, B). Because a minority of patients had BM samples collected later than 1 month after achieving a CR, we performed a subset analysis of only those 37 patients for whom BM samples were collected within 1 month of documented CR to exclude a potential bias related to the time of sample collection. As shown in Table III, this analysis again did not demonstrate any difference in DFS or OS between 12 patients with and 25 without clearance of all mutations (Table III, Fig 1C, D).

## Disease outcomes of patients with persistence of DNMT3A R882 only and of patients with persistent DNMT3A R882 and other AML mutations at CR

Some patients in Group 2 with persistent *DNMT3A* R882 mutations during CR had no other analysed AML mutations present, whereas others did. We hypothesized that the absence of established, AML-associated mutations, outside of *DNMT3A* R882 mutations, might influence outcome. We performed a subset analysis of Group 2 patients, comparing those who had no other mutations associated with AML at CR (Group 2a, $n = 30$) with those who carried at least one persistent or newly acquired AML-associated mutation in addition to *DNMT3A* R882 (Group 2b, $n = 12$). The pretreatment features of these two patient groups are shown in Table SIII. Patients in Group 2a were younger, more often had normal cytogenetics, and differed by ELN group as compared to Group 2b. The VAFs of *DNMT3A* R882 for Groups 2a and 2b at diagnosis were similar [median, 46% (range 41–50%), vs. median 46% (range 41–50%), $P = 0·90$]. As shown in Table IV and Fig 2, patients with persistent *DNMT3A* R882 who had no other AML mutations displayed a trend toward improved DFS compared to patients with additional mutations (median, 1·6 vs. 0·6 years, $P = 0·06$). Although OS was longer for Group 2a patients than for Group 2b patients, this difference did not meet statistical significance (median, 3·8 vs. 1·3 years, $P = 0·14$).

We next compared the outcome of patients in Group 2a, who had persistence of only *DNMT3A* R882, with outcome of patients who lacked *DNMT3A* R882 mutations in remission (Group 1). No pretreatment feature separated these groups. The outcomes for patients in either group did not differ significantly with respect to either DFS (median, 1·6 vs. 0·6 years, $P = 0·35$) or OS (median, 3v8 vs. 1·3 years, $P = 0·40$).

## Frequent identification of new mutations in remission samples in Group 2B

Six of the 12 patients in Group 2b acquired new AML-associated mutations at a VAF >3% (Pt ID 5, 11, 18, 21, 24, 29), in addition to persistent *DNMT3A* R882, during CR as shown in Table SI. Among these six patients, new mutations involved only *ASXL1* ($n = 2$), only *TET2* ($n = 1$), only *JAK2* ($n = 1$), both *TET2* and *JAK2* ($n = 1$) and only *TP53* ($n = 1$), all of which have been previously associated, not only with AML, but also with clonal haematopoiesis in healthy individuals without haematological disease (Xie *et al*, 2014). Of the six patients with new AML mutations, five ultimately relapsed.

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## Discussion

Herein, we report, to our knowledge, the largest series of sequentially analysed AML patients with *DNMT3A* R882 mutations who attained a CR and had remission samples examined for persistent *DNMT3A* R882 and other concurrent AML mutations with a traditional NGS sequencing assay typically utilized in medical practice. We demonstrate that clearance of other AML-associated mutations is common among patients with extended DFS, but *DNMT3A* R882 typically persists in the majority of patients. In contrast to previous publications suggesting that persistence of *DNMT3A* R882 mutations predicts for poor outcome (Hou *et al*, 2012; Klco *et al*, 2015), we found no significant differences in DFS or OS between patients who had cleared *DNMT3A* R882 mutations at the 3% detection during first CR and those who did not. A subset of our patients had marrow samples taken later than 30 d into treatment and we considered the possibility that this could explain, at least in part, the discordant findings. However, even after limiting our analysis to the 37 patients for whom BM samples were available within a month of their marrow CR date, our results were similar (Table III, Fig 1C, D). In a subset analysis of patients with persistent *DNMT3A* R882 mutations following induction chemotherapy (Group 2), we also demonstrated that patients who do not have other AML-associated mutations during CR (Group 2a) show a trend toward longer DFS compared with patients with new or persistent mutations in addition to *DNMT3A* R882 (Group 2b) (Table IV, Fig 2A). While it is possible that a more sensitive assay for detecting *DNMT3A* R882 mutations might identify a smaller subset whose disease has improved outcome, such assays are not widely utilized in general AML practice. Most importantly, our study demonstrates that persistence of *DNMT3A* R882A at levels of 3% or greater, as typically detected by NGS assays widely used in practice, bear no prognostic significance.

Our findings support those of two other studies in which persistent *DNMT3A* R882 mutations at the time of remission did not affect clinical course (Pløen *et al*, 2014; Debarri *et al*, 2015). Cell sorting experiments performed on non-leukaemic HSCs in the study by Pløen *et al* (2014) demonstrated the presence of mutant *DNMT3A* in T-cells and B-cells, at allele frequencies between 4% and 31%. Further, the *DNMT3A* allele burden in these cells was found to increase over time, thereby supporting the idea that acquisition of *DNMT3A* mutations occurs first in ancestral preleukaemic HSCs, as recently reported by two separate groups, where a growth advantage appears to exist (Corces-Zimmerman *et al*, 2014; Shlush *et al*, 2014).

Our study and that of Pløen *et al* (2014) have different conclusions relative to the importance of persistent *DNMT3A* R882 mutations at the time of remission than those of another recently published study Klco *et al* (2015). That study, in a manner similar to ours, used deep digital sequencing of paired samples obtained at diagnosis and remission in 50 AML patients and showed that mutation clearance in remission samples was associated with significantly improved event-free survival (EFS) (median, 17·9 vs. 6·0 months, hazard ratio [HR], 3·67) and OS (median, 42·2 vs. 10·5 months, HR, 2·86), compared with patients with persistent mutations in ≥5% bone marrow cells at CR. Within this group, 12 of 13 patients whose samples collected at remission had persistent *DNMT3A* mutations (type not specified) at a VAF >2·5% relapsed. Although the authors reported no association between

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EFS and mutation status of *DNMT3A*, OS was improved for patients, particularly those with intermediate-risk cytogenetics, who had cleared the *DNMT3A* mutation. Reasons for the discordant results between studies are uncertain but may reflect the fact that 10 of 23 (43%) patients with *DNMT3A* mutations analysed by Klco *et al* (2015) harboured non-R882 *DNMT3A* mutations, whereas our study and that of Pløen *et al* (2014) exclusively evaluated *DNMT3A* R882 mutations. Similar to the aforementioned study, we observed resolution of other common AML mutations in all but two of our patients with long DFS; these two patients had a *JAK2* mutation (V617F) or two distinct mutations of the *TET2* gene persisting at a high VAF.

Further, we did in fact see mutations in *TET2, ASXL1, TP53* or *JAK2* emerge during remission in six patients, all six of whom also harboured *DNMT3A* R882 mutations. In such a case, the non-DNMT3A R882 clone would be more likely to contribute to relapse. Analysis of relapse samples (which were not available in our series) would be required to determine this. Interrogation of this potential change with new primary ancestral clonal cells, and clonal architecture contributing to relapse should be considered as part of prospective AML trials in the future.

Limitations of our study include the retrospective nature of the analysis and the limited sample size, although ours is the largest patient cohort with *DNMT3A* R882 mutations followed serially reported to date. Moreover, a minority of our patients were analysed after CR1 was documented, although even when this group was excluded from the analysis, the results were similar.

In conclusion, we demonstrate that persistence of *DNMT3A* R882 mutations during CR does not adversely impact long-term DFS and OS in AML patients. Future studies examining other possible mechanisms that are modulated by persistent *DNMT3A* R882 expression have the potential to provide insights regarding its significance.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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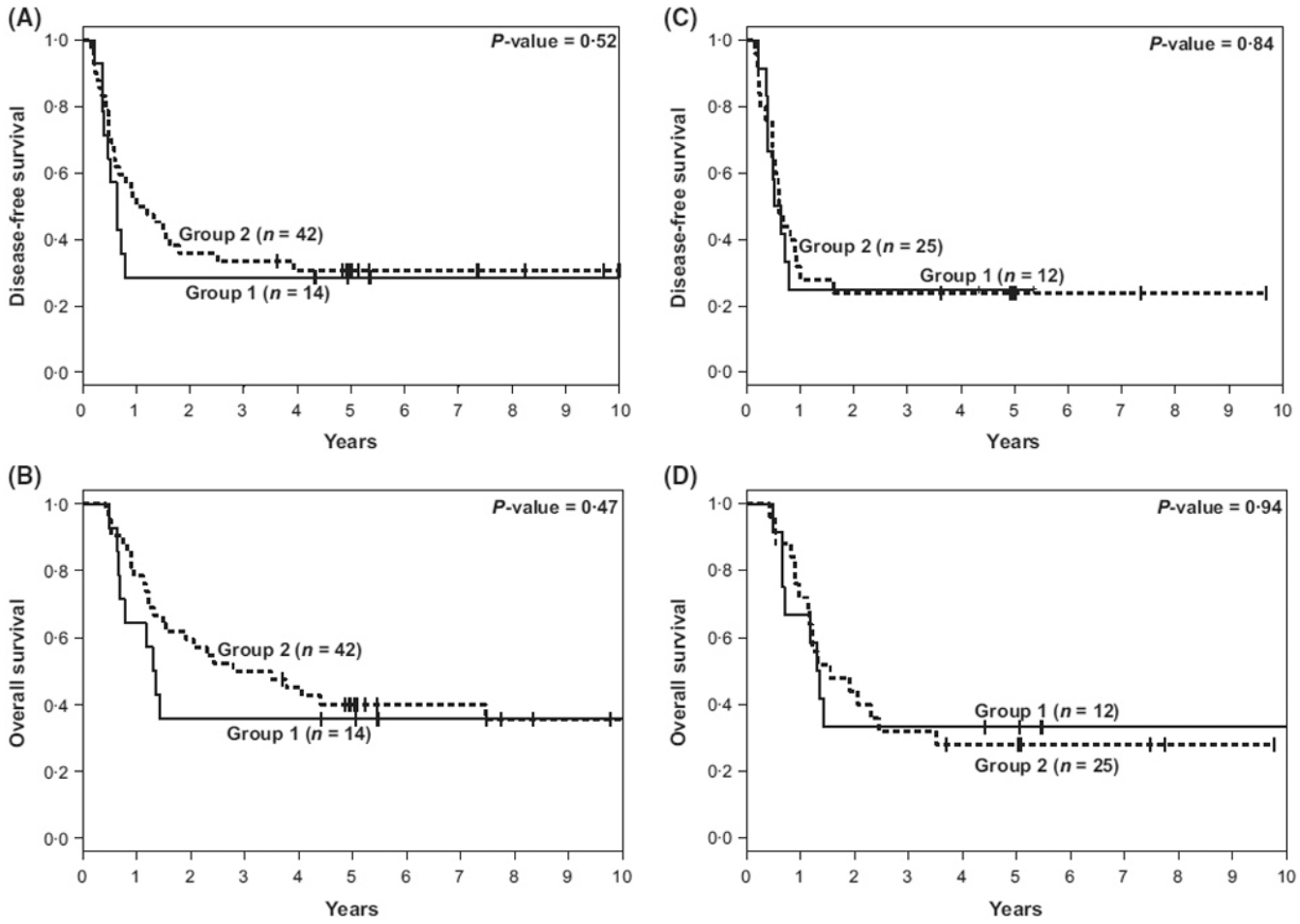


**Fig 1.**

Kaplan–Meier survival plots of AML patients with pre-treatment *DNMT3A* R882 mutations who cleared *DNMT3A* R882 mutation at complete remission (CR) and those who did not. (A) Disease-free survival. (B) Overall survival. (C) Disease-free survival for patients with remission samples collected within 30 d of morphological CR date. (D) Overall survival for patients with remission samples collected within 30 d of morphological CR date. Group 1 includes patients whose *DNMT3A* R882 mutations cleared below the variant allele frequency (VAF) cut-off of 3% in their remission sample and who had no other AML mutation. Group 2 includes patients with a *DNMT3A* R882 mutation with a VAF ≥3% in their remission sample with or without other AML mutations.

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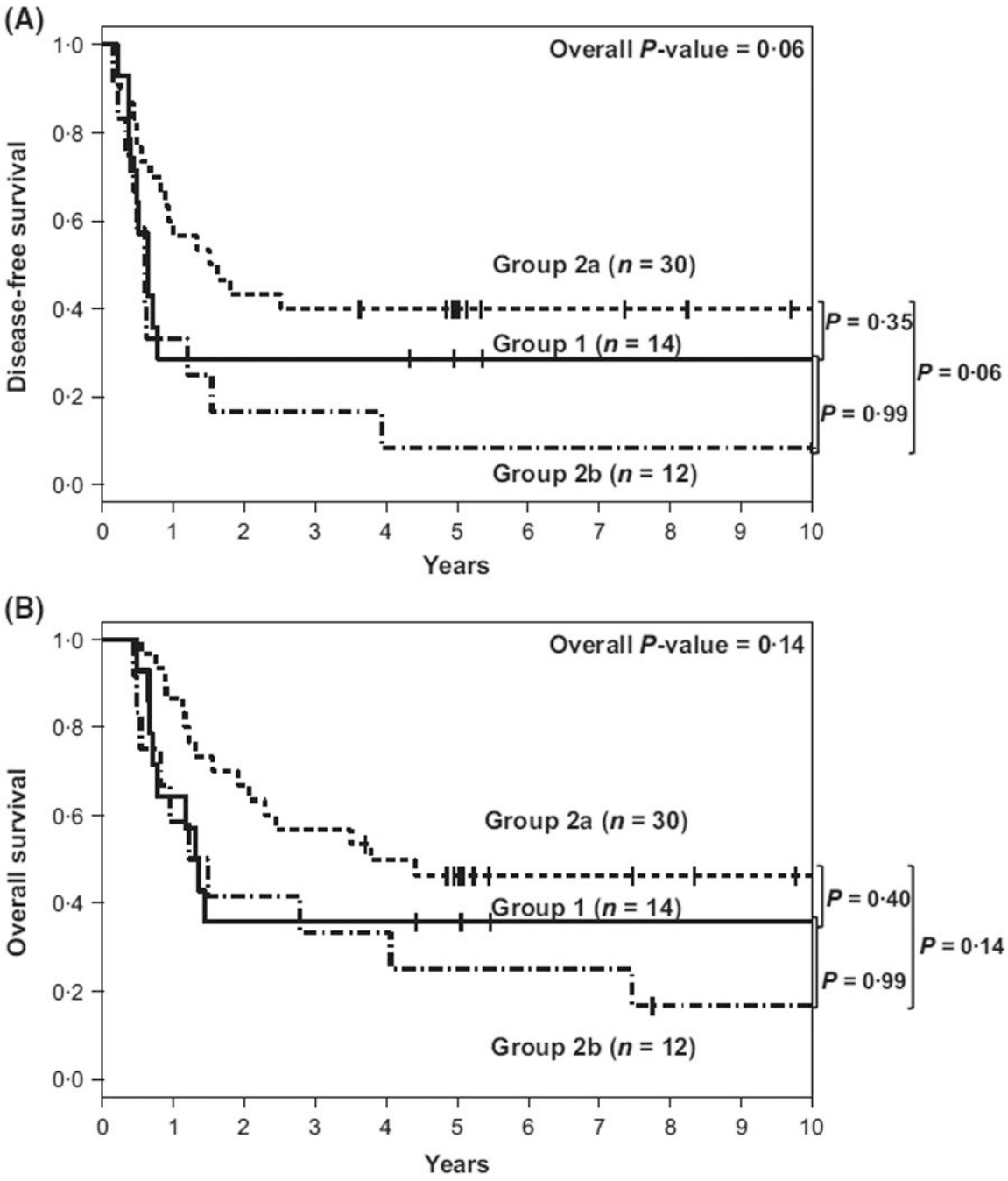


**Fig 2.**

Kaplan–Meier survival plots of AML patients with pretreatment *DNMT3A* R882 mutations by three *DNMT3A* complete remission groups (A) Disease-free survival. (B) Overall survival. Group 1 includes patients whose *DNMT3A* R882 mutations cleared below the variant allele frequency (VAF) cut-off of 3% in their remission sample and who had no other AML mutation. Group 2a includes patients with a *DNMT3A* R882 mutation with a VAF ≥3% in their remission sample but with clearance of all other AML mutations. Group 2b

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includes patients with a *DNMT3A* R882 mutation with a VAF ≥3% and ≥1 other AML mutation in their remission sample.

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**Table I**

Comparison of pre-treatment clinical characteristics of *de novo* AML patients who cleared *DNMT3A* R882 mutations at complete remission and of those who did not.

| Characteristic | Group 1* (*n* = 14) | Group 2* (*n* = 42) | *P* |
|---|---|---|---|
| Age, years | | | |
| Median | 48 | 52 | 0·55 |
| Range | 28–65 | 28–72 | |
| Age group, *n* (%) | | | |
| Younger (<60 years) | 11 (79) | 33 (79) | 1·00 |
| Older (≥60 years) | 3 (21) | 9 (21) | |
| Sex, *n* (%) | | | |
| Male | 8 (57) | 25 (60) | 1·00 |
| Female | 6 (43) | 17 (40) | |
| Race, *n* (%) | | | |
| White | 13 (93) | 39 (97) | 1·00 |
| Non-white | 1 (7) | 1 (3) | |
| Haemoglobin, g/l | | | |
| Median | 83 | 94 | 0·06 |
| Range | 68–132 | 71–251 | |
| Platelet count, $\times 10^9$/l | | | |
| Median | 57 | 81 | 0·12 |
| Range | 13–266 | 16–347 | |
| WBC count, $\times 10^9$/l | | | |
| Median | 30–6 | 45–5 | 0·76 |
| Range | 5·3–131·7 | 2·0–248·0 | |
| Percentage of blood blasts | | | |
| Median | 68 | 62 | 0·64 |
| Range | 6–97 | 0–96 | |
| Percentage of bone marrow blasts | | | |
| Median | 69 | 75 | 0·86 |
| Range | 24–91 | 6–91 | |
| Extramedullary involvement, *n* (%) | 3 (25) | 16 (41) | 0·33 |
| Pretreatment cytogenetic findings, *n* (%) | | | |
| Normal karyotype | 12 (86) | 36 (86) | 0·61 |
| Sole trisomy† | 1 (7) | 2 (5) | |
| Two numerical abnormalities‡ | 1 (7) | 0 (0) | |
| Sole deletion§ | 0 (0) | 3 (7) | |
| Complex karyotype with >5 abnormalities | 0 (0) | 1 (2) | |
| ELN Genetic Group¶ | | | |

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| Characteristic | Group 1* (*n* = 14) | Group 2* (*n* = 42) | *P* |
|---|---|---|---|
| Favourable | 7 (50) | 18 (46) | 1·00 |
| Intermediate-I | 7 (50) | 18 (46) | |
| Intermediate-II | 0 (0) | 2 (5) | |
| Adverse | 0 (0) | 1 (3) | |
| Transplantation in 1st CR, *n* (%) | | | |
| Allogeneic | 2 (14) | 1 (2) | 0·06 |
| Autologous | 8 (57) | 17 (40) | |
| No transplantation | 4 (29) | 24 (57) | |
| Time from 1st CR to 1st CR sample (d) | | | |
| Median | 0 | 0 | 0·17 |
| Range | 0–185 | 0–333 | |
| Time from 1st CR to 1st CR sample, *n* (%) | | | |
| Same day | 10 (71) | 22 (52) | 0·10 |
| Within 1–7 d after CR date | 1 (7) | 3 (7) | |
| Within 7–30 d after CR date | 1 (7) | 0 (0) | |
| More than 30 d after CR date | 2 (14) | 17 (40) | |
| *DNMT3A* R882 VAF % at diagnosis | | | |
| Median | 45 | 46 | 0·58 |
| Range | 40–50 | 41–50 | |

AML, acute myeloid leukaemia; CR, complete remission with remission sample obtained at any time point after morphological remission is achieved; ELN, European LeukaemiaNet; *n*, number; WBC, white blood cell; VAF, variant allele frequency.

*Group 1 is defined as patients whose *DNMT3A* R882 mutation cleared below the VAF cut-off of 3% in their remission sample and who had no other AML mutation, and Group 2 is defined as patients with a *DNMT3A* R882 mutation with a VAF >3% with or without other AML mutations in their remission sample.

†This category includes single patients with sole +4, +8, and +21, respectively.

‡The patient in this category had +Y and +8.

§This category includes single patients with sole add(7)(q22), del(9) (q13q22) and del(20)(q12), respectively.

¶See Döhner *et al*, 2010.

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## Table II

Outcomes of *de novo* AML patients who cleared *DNMT3A* R882 mutations at CR and of those who did not.

| End point | *DNMT3A* CR Group 1* (*n* = 14) | *DNMT3A* CR Group 2* (*n* = 42) | *P* | HR (95% CI) |
|---|---|---|---|---|
| Complete remission, *n* (%) | 14 (100) | 42 (100) | – | – |
| Disease-free survival | | | | |
| Median, years | 0·6 | 11 | 0·52 | 1·26 (0·61–2·60) |
| % disease-free at 3 years (95% CI) | 29 (9–52) | 33 (20–48) | | |
| % disease-free at 5 years (95% CI) | 29 (9–52) | 31 (18–45) | | |
| Overall survival | | | | |
| Median, years | 1·3 | 31 | 0·47 | 1·33 (0·62–2·83) |
| % alive at 3 years (95% CI) | 36 (13–59) | 50 (34–64) | | |
| % alive at 5 years (95% CI) | 36 (13–59) | 40 (25–54) | | |

AML, acute myeloid leukaemia; CI, confidence interval; CR, complete remission; HR, hazard ratio; *n*, number.

*Group 1 is defined as patients whose *DNMT3A* R882 mutation cleared below the VAF cut-off of 3% in their remission sample and who had no other AML mutation, and Group 2 is defined as patients with a *DNMT3A* R882 mutation with a VAF >3% with or without other AML mutations in their remission sample.

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## Table III

Outcomes of *de novo* AML patients whose CR sample was analyzed molecularly within a month after CR date according to whether their *DNMT3A* R882 mutations were cleared at CR or not.

| End point | DNMT3A CR Group 1* (*n* = 12) | DNMT3A CR Group 2* (*n* = 25) | *P* | HR (95% CI) |
|---|---|---|---|---|
| Complete remission, *n* (%) | 12 (100) | 25 (100) | – | – |
| Disease-free survival | | | | |
| Median, years | 0·6 | 0·6 | 0·84 | 1·08 (0·49–2·40) |
| % disease-free at 3 years (95% CI) | 25 (6–50) | 24 (10–42) | | |
| % disease-free at 5 years (95% CI) | 25 (6–50) | 24 (10–42) | | |
| Overall survival | | | | |
| Median, years | 1·3 | 1·5 | 0·94 | 1·03 (0·45–2·37) |
| % alive at 3 years (95% CI) | 33 (10–59) | 32 (15–50) | | |
| % alive at 5 years (95% CI) | 33 (10–59) | 28 (12–46) | | |

AML, acute myeloid leukaemia; CI, confidence interval; CR, complete remission; HR, hazard ratio; *n*, number.

*Group 1 is defined as patients whose *DNMT3A* R882 mutation cleared below the VAF cut-off of 3% in their remission sample and who had no other AML mutation, and Group 2 is defined as patients with a *DNMT3A* R882 mutation with a VAF >3% with or without other AML mutations in their remission sample.

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**Table IV**

Outcomes of *de novo* AML patients who at CR cleared *DNMT3A* R882 and all other mutations, patients who retained only a *DNMT3A* R882 mutation and patients who retained a *DNMT3A* R882 mutation and had one or more other mutations.

| End point | *DNMT3A* CR Group 1* (*n* = 14) | *DNMT3A* CR Group 2a* (*n* = 30) | *DNMT3A* CR Group 2b* (*n* = 12) | *P*† | *P*‡ Gl vs. G2a | HR (95% CI) Gl vs. G2a | *P*‡ Gl vs. G2b | HR (95% CI) Gl vs. G2b | *P*‡ G2a vs. G2b | HR (95% CI) G2a vs. G2b |
|---|---|---|---|---|---|---|---|---|---|---|
| Complete remission, *n* (%) | 14 (100) | 27 (100) | 15 (100) | – | – | – | – | – | – | – |
| Disease-free survival | | | | | | | | | | |
| Median, years | 0·6 | 1·6 | 0·6 | 0·06 | 0·35 | 1·63 (0·63–4·21) | 0·99 | 0·68 (0·24–1·95) | 0·06 | 0·42 (0·17–1·05) |
| % disease-free at 3 years (95% CI) | 29 (9–52) | 40 (23–57) | 17 (3–41) | | | | | | | |
| % disease-free at 5 years (95% CI) | 29 (9–52) | 40 (23–57) | 8 (0–31) | | | | | | | |
| Overall survival | | | | | | | | | | |
| Median, years | 1·3 | 3·8 | 1·3 | 014 | 0·40 | 1·67 (0·61–4·53) | 0·99 | 0·78 (0·26–2·35) | 014 | 0·47 (0·18–1·23) |
| % alive at 3 years (95% CI) | 36 (13–59) | 57 (37–72) | 33 (10–59) | | | | | | | |
| % alive at 5 years (95% CI) | 36 (13–59) | 46 (27–63) | 25 (6–50) | | | | | | | |

AML, acute myeloid leukaemia; CI, confidence interval; CR, complete remission; HR, hazard ratio; *n*, number.

*Group 1 (G1) is defined as patients whose *DNMT3A* R882 mutation cleared below the VAF cut-off of 3% in their remission sample and who had no other AML mutation. Group 2a (G2a) is defined as patients with a *DNMT3A* R882 mutation with a VAF >3% in their remission sample but who cleared all other AML mutations. Group 2b (G2b) is defined as patients with a *DNMT3A* R882 mutation with a VAF >3% in their remission sample and persistent or newly acquired other AML mutations.

†Overall *P*-value pertaining to comparison of all three patients groups.

‡Pairwise comparisons are adjusted from multiple comparisons using the Bonferroni method (Westfall *et al*, 1999).