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A tale of two siblings: two cases of AML arising from a single pre-leukemic DNMT3A mutant clone

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A tale of two siblings: two cases of AML arising from a single pre-leukemic DNMT3A mutant clone

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Acute myeloid leukemia (AML) patients with DNA methyltransferase 3A (*DNMT3A*) mutations in remission after induction chemotherapy may have hematopoiesis arising from a putative pre-leukemic clone lacking the full spectrum of mutations seen at AML diagnosis.[1,2] *DNMT3A* mutations in hematopoietic stem cells lead to increased self-renewal capacity and dominance of the stem-cell compartment.[2] The common DNMT3A^{R882H} mutation is usually heterozygous and results in loss-of-function with a dominant-negative effect. *DNMT3A* mutations are thought to occur early in AML ontogeny,[3] and are found in around 20% of *de novo* AML and in a higher proportion of secondary AML cases.[4,5] In population studies clonal mutations of *DNMT3A*[6] and other epigenetic regulators are more common with increasing age, and may contribute to a proportion of cases of age-related skewing of X-chromosome inactivation.[1,7] Two recent reports from large cohorts identified *DNMT3A*, Tet methylcytosine dioxygenase 2 (*TET2*) and additional sex combs like transcriptional regulator 1 (*ASXL1*; epigenetic modifying genes) as the most commonly mutated genes in peripheral blood samples,[8,9] with a combined prevalence of $\geqslant$10% in individuals aged over 65 years. One study provided evidence that individuals with clonal hematopoiesis are more likely to develop hematopoietic malignancy, and examined in detail subsequent myeloid malignancy diagnosis samples together with the initial asymptomatic blood samples for three cases.[8] All three cases were found to have multiple (2–6) known pathogenic mutations at the time of asymptomatic blood sampling suggesting that clonal hematopoiesis could be attributed to outgrowth of early leukemic cells. Importantly, a number of the driver mutations were only found upon detailed genetic analysis of the initial blood sample. A similar observation was recently reported in chronic myeloid leukemia, where a DNMT3A mutation that was discovered in molecular remission after treatment was also present in the leukemic clone at diagnosis.[10] Hence, although these studies imply that, at least in some cases, a pre-leukemic clone carrying a single epigenetic founder mutation may be present for some time prior to the development of neoplasia, they do not provide direct evidence of its pathogenicity.

Here we report direct evidence to fill this knowledge gap that a pre-leukemic clone can evolve to AML. Clonal hematopoiesis (*DNMT3A*^{R882H}) was transplanted from an apparently healthy marrow donor to his brother, with subsequent evolution to genetically distinct AML clones in each brother. A healthy 53-year-old man with normal blood counts and bone marrow morphology donated steady-state bone marrow to his 49-year-old brother for treatment of relapsed lymphoma. Fourteen months after the bone marrow transplant, the donor developed normal karyotype AML with mutations in *DNMT3A*, nucleophosmin 1 (*NPM1*), and fms-like tyrosine kinase 3 (*FLT3*). Four months later (18 months post-allograft) the recipient developed normal karyotype AML with identical mutations in *DNMT3A* and *NPM1*, but wild-type *FLT3*. Chimerism analysis confirmed donor-derived leukemia. Both brothers received induction chemotherapy and both relapsed after a short-lived remission.

Whole-exome sequencing and targeted massively parallel sequencing of 29 genes recurrently mutated in myeloid malignancies (Supplementary Information) identified a somatic DNMT3A^{R882H} mutation that dominated donor hematopoiesis (allelic burden 46%), despite normal blood counts and morphology. This clone was transplanted to the recipient, who later developed AML with a mutational profile distinct from that seen in the donor (Figure 1 and Supplementary Table 1). The *DNMT3A* mutation persisted at transformation to AML and at relapse. Of the multiple somatic mutations, other than *DNMT3A*, only NPM1 (W288Cfs*12) was identical in both brothers. This mutation was not detected in an aliquot of the transplanted bone marrow harvest product (sensitivity $<$0.1%). W288Cfs*12 is the most common *NPM1* mutation in AML, accounting for almost 80% of variants.[11] Most DNMT3A-mutant AML cases also have an *NPM1* mutation,[12] and the latency period was longer than that predicted from the known kinetics of *NPM1* AML,[13] so the presence of this mutation in both brothers likely reflects the propensity for these mutations to co-occur in AML.

In addition known pathogenic mutations were identified in *FLT3* and Isocitrate dehydrogenase 1 (*IDH1*) in the donor AML, as well as a novel *NOTCH4* mutation. Mutations in *NOTCH1* and *NOTCH2* are found in hematological malignancies,[14] but *NOTCH4* mutations have not previously been reported in AML. In the recipient we identified an additional mutation in the Structural Maintenance of Chromosomes 1A (*SMC1A*) gene, which is a member of the cohesin complex: recurrent mutations in the cohesin complex were recently identified in ~12% of AML cases, and are associated with *NPM1* mutations.[15]

DNMT3A-mutant hematopoiesis persisted in both brothers at remission after chemotherapy (Figures 1a and b), consistent with the reported chemoresistance of DNMT3A-mutant clones. The high DNMT3A^{R882H} allelic burden (likely $>$90% of cells carried the mutation), and the disappearance of additional pathogenic mutations indicated reversion to the pre-leukemic clone in remission. In addition, both brothers had minimal residual disease (MRD). In the donor this was evident retrospectively at AML relapse with all earlier mutations plus an additional known pathogenic Wilms' Tumor 1 (WT1^{R394W}) mutation (Figure 1a) which is associated with poor outcome. The recipient was not tested at relapse (sample not available), but in remission the *NPM1* mutation was detected using very deep sequencing at ~0.01% (Figure 1b), consistent with MRD. There was no evidence from the sequencing data of a familial genetic predisposition to cancer: no known pathogenic germline mutations were identified.

We also studied the persistence of *DNMT3A* mutations in remission in *de novo* AML by sequencing remission samples from 12 additional patients with a *DNMT3A* mutation at diagnosis. We identified persistence of the mutation in morphological remission in 8/12 patients (75%; Supplementary Table 2 and Figure 2) whereas all concurrent pathogenic AML-associated mutations were undetectable in available remission samples (Supplementary Table 2), consistent with previous reports.[1,2,16] Interestingly, of the four patients with a persistent *DNMT3A* mutation in multiple remission samples, the mutant allelic burden expanded over time, whereas peripheral blood counts and morphology remained normal. If polyclonal hematopoiesis is not restored in remission, the re-emergence of AML may be due either to failure to eradicate

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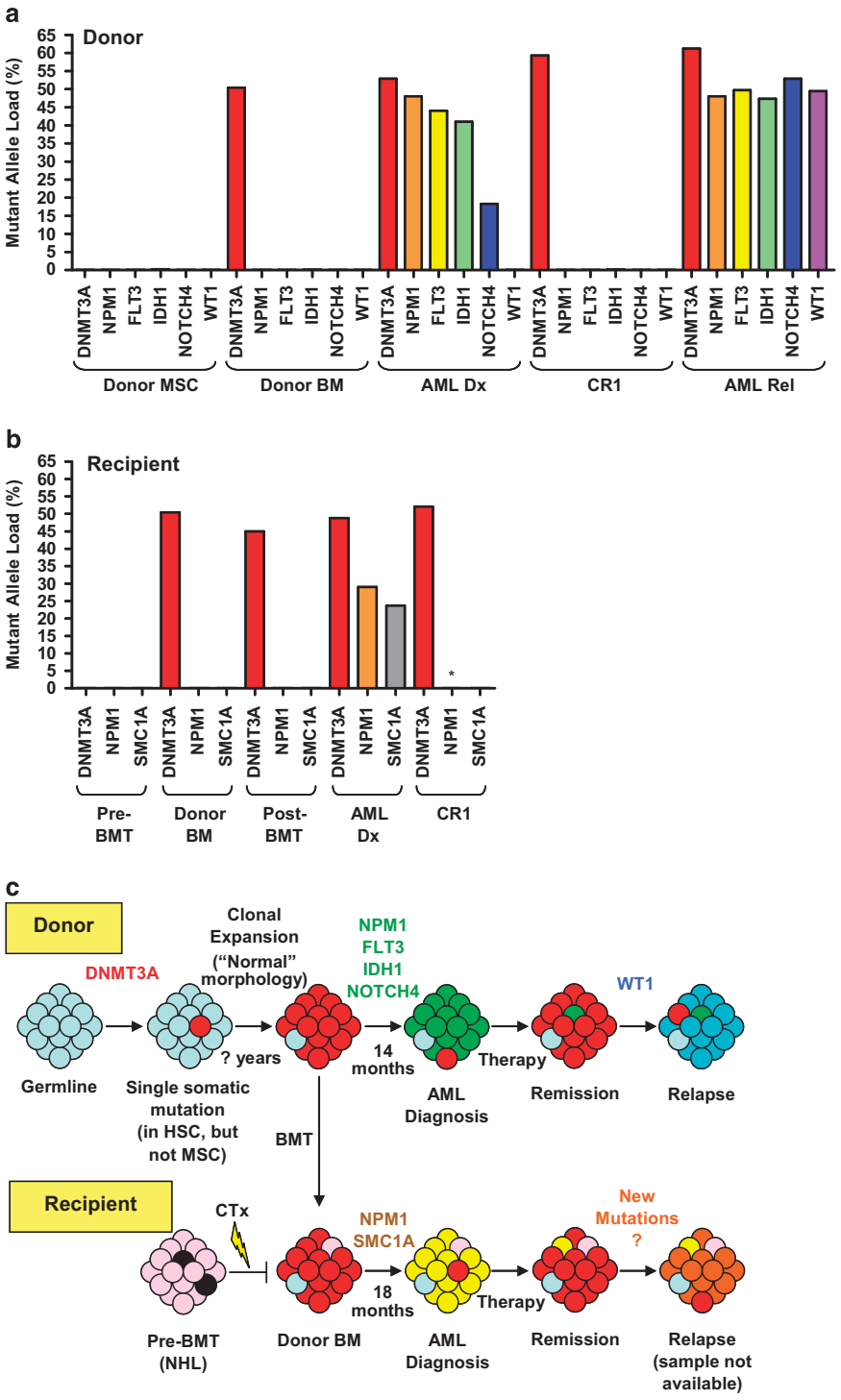

Figure 1. Mutational evolution of independent AMLs in two brothers. Mutation profiles of the donor (**a**) and recipient (**b**) brothers prior to AML diagnosis, at diagnosis, in remission, and at relapse. (**c**). Schematic of mutation acquisition and clonal expansion in donor and recipient bone marrow during independent evolution of their AMLs. All previously acquired mutations persisted in diagnosis samples and donor relapse sample. In the donor, all mutations present in the diagnosis sample persisted at relapse. A relapse sample from the recipient was not available. NPM1 detected at * ~ 0.01%. MSC, mesenchymal stromal cells; Dx, diagnosis; CR1, complete remission; Rel, relapse; BMT, bone marrow transplantation; CTx, conditioning chemotherapy.

MRD or to the independent evolution of a second AML derived from the original pre-leukemic clone. Available data suggest that MRD is the dominant cause of relapse.

A striking feature of this case was the relatively short latency period to the emergence of AML in both brothers (Figure 1c). Whereas it is often assumed that immunosuppression of the transplant recipient may contribute to the risk of donor-derived leukemia,[17] the latency periods in the healthy donor and in the immunosuppressed transplant recipient were similar. We speculate that the high allelic burden of the DNMT3A mutations might have been a contributing factor in these two cases, consistent with the observation from a population study

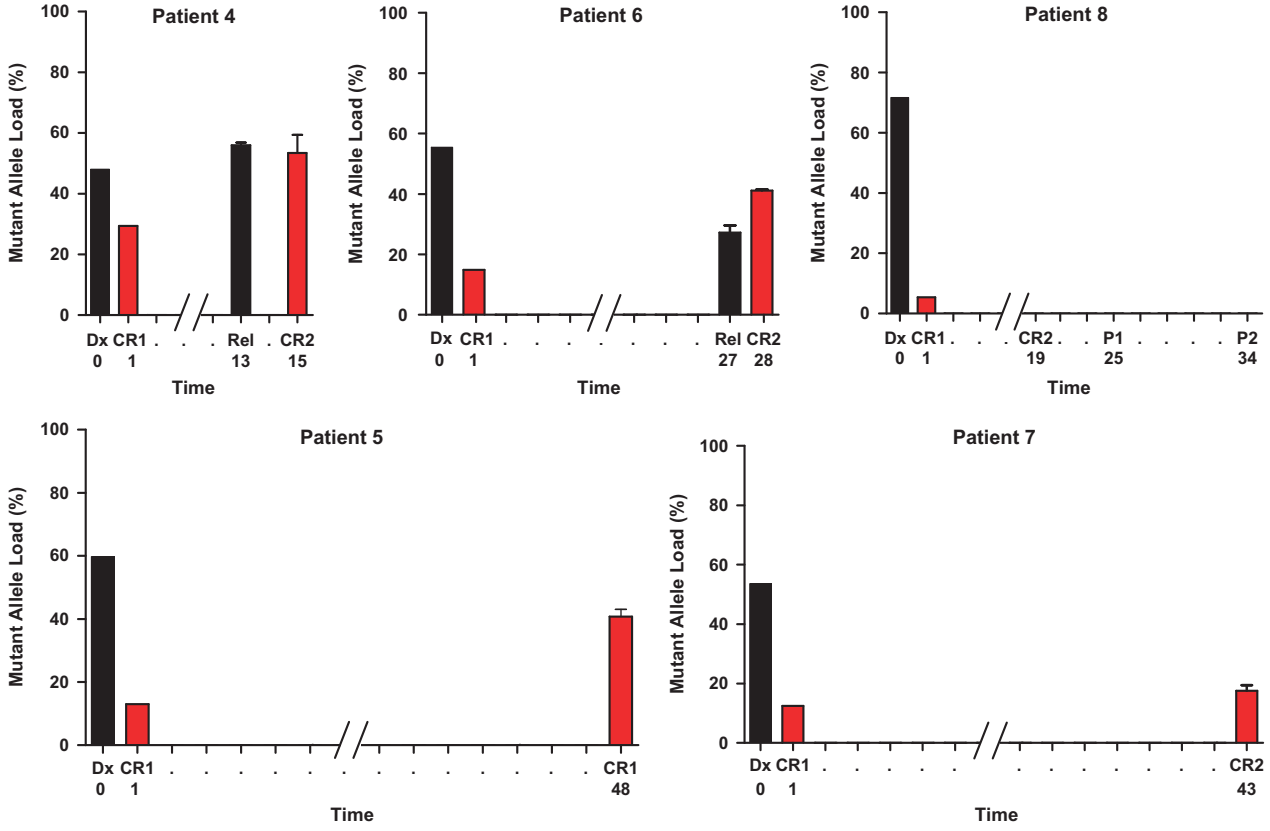

Figure 2. Persistence of *DNMT3A* mutations in complete remission. The allelic burden of DNMT3A (R882H/C) mutation was measured using a custom Sequenom assay in serial samples from a cohort of 12 additional patients with DNMT3A-mutant AML, those in whom the mutation was detected during complete remission are shown. Active AML (black), remission (red). Sample types—Dx (diagnosis), CR1 (first complete remission), CR2 (second complete remission), Rel (relapse), P1 (first sample post-BMT), P2 (second sample post-BMT). Numbers along *x*-axis are time after diagnosis (months).

that higher allelic burdens were associated with an increased risk of subsequent neoplasia.[9] This study provides for the first time direct evidence that a single pre-leukemic mutation can promote evolution to hematopoietic malignancy; in this case, not only one but two genetically distinct AML clones.

A number of clinically important questions arise from this case. For AML cases with *DNMT3A* (or functionally similar) mutations, should mutational analysis in remission be used to guide the post-remission therapy? In our series of 12 cases of *de novo* AML, the rate of relapse was high irrespective of the persistence of the *DNMT3A* mutation in remission. Ploen *et al.*[16] reported relapse in only 50% of DNMT3A-mutant AML patients with the mutation present in remission, but allogeneic stem-cell transplantation would be offered to most individuals in this setting. Transplantation may potentially eradicate both AML and its antecedent clone. What is the implication of pre-leukemic mutations for the monitoring of MRD? A founder mutation that is present in a pre-leukemic clone may not be a reliable indicator of AML remission status. Genomic profiling of AML could be used to select the best target(s) for MRD analysis: one or more genes that are part of the evolved clone. Lastly, is there a case for mutation screening for a defined panel of potential pre-leukemic mutations as part of the allogeneic stem-cell donor assessment? On the basis of recent reports,[6,7,9] it would appear that the population prevalence of such mutations might be sufficient for screening to be justified in selected populations (e.g., older donors, or those with a personal or familial history of malignancy). What is needed to inform this decision is a better understanding of the natural history of such clones, and the associated risk of emerging hematopoietic malignancy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

DMR and CNH wrote the manuscript. DMR, AB, RJD, DKH, LBT and IDL treated the patients and/or collected essential samples. CMB and GDC provided essential samples. CNH, DMR, ALB, WTP, JF, AWS and HSS planned and analyzed sequencing experiments. MH, MZ, KLA, YL, MB and GAE performed sequencing experiments. All authors critically reviewed and approved the manuscript.

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Effect of genetic profiling on prediction of therapeutic resistance and survival in adult acute myeloid leukemia

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Therapeutic resistance, that is failure to achieve complete remission (CR) despite not incurring treatment-related mortality (TRM), or relapse after achieving CR, is the principal cause of failure in acute myeloid leukemia (AML). Many clinical, cytogenetic, and molecular characteristics are strongly associated with resistance.[1] However, using areas under receiver operating curves (AUCs) to quantify their predictive ability in >4500 younger and older adults with newly diagnosed AML, we recently demonstrated that such data, even when combined, provided only limited ability (AUC < 0.8) to predict resistance.[2] Greater ability (AUC > 0.90–0.95) to forecast resistance to standard therapy in individual patients might obviate the need to randomize between standard and investigational treatments, optimize care algorithms and ultimately benefit patients.

Our previous analysis included molecular data pertaining only to *NPM1* mutations and FLT3/ITDs. At that time, we hypothesized that consideration of mutational data for other genes might improve predictive accuracy. Indeed, by profiling close to 20 genes, Patel *et al.*[3] were able to separate participants of a recent Eastern Cooperative Oncology Group (ECOG) phase 3 trial (E1900) with cytogenetically defined intermediate-risk AML into three subgroups with significantly different outcomes. Here, we investigated the extent to which prediction of resistance and survival can be improved by inclusion of detailed molecular data in the E1900 study cohort. Our study also offered the opportunity to assess whether CD25, a marker that has shown to improve risk classification independent of other established biomarkers in the E1900 cohort, could further improve the accuracy of outcome prediction in individual patients.[4]

E1900 (NCT00049517) was a randomized trial that compared daunorubicin doses of 45 and 90 mg/m^2 each given on days 1–3 together with standard-dose cytarabine in 657 patients aged 17–60 years with newly diagnosed AML.[5] Of these, 398 patients had sufficient material available for complete genetic profiling (see below).[3] Institutional review boards of participating institutions approved all protocols, and patients were treated according to the Declaration of Helsinki.

For our analysis, early death TRM was defined as death within 28 days after initiating therapy[6] or study registration, if the date of

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