REGULAR ARTICLE

blood advances

NPM1 mutated AML can relapse with wild-type *NPM1*: persistent clonal hematopoiesis can drive relapse

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Key Points

- AML with *NPM1* mutation can relapse with *NPM1*^{wt}, which could prevent MRD detection.
- The majority of comutations persist in molecular remission and at *NPM1*^{wt} relapse, suggestive of clonal hematopoiesis driving AML relapse.

Acute myeloid leukemia (AML) with *NPM1* mutation (*NPM1*^{mut}) defines a World Health Organization entity. Absence of minimal residual disease (MRD) following induction chemotherapy is associated with an excellent prognosis. Data are conflicting on *NPM1*^{mut} AML relapsing with wild-type *NPM1 (NPM1*^{wt}*)*. We analyzed 104 paired samples of *NPM1*^{mut} AML patients with relapse and identified 14/104 that relapsed with *NPM1*^{wt} AML. Blood counts at diagnosis differed significantly between patients with *NPM1*^{mut} and *NPM1*^{wt} relapse (median white blood cell count, 30 vs 3×10^9/L, $P = .008$; platelet count, 66 vs 128×10^9/l, $P = .018$). *NPM1*^{mut} relapse occurred significantly earlier than *NPM1*^{wt} relapse (14 vs 43 months, $P = .004$). At diagnosis, *FLT3*-ITD were more frequent in patients with *NPM1*^{mut} relapse ($P = .029$), whereas *DNMT3A* mutations were more frequent in patients with *NPM1*^{wt} relapse ($P = .035$). Sequencing analysis of paired samples at diagnosis, molecular remission, and *NPM1*^{wt} relapse identified cooccurring mutations that persist from diagnosis throughout remission and at relapse, suggestive of a preexisting clonal hematopoiesis. We provide evidence that AML relapsing with *NPM1*^{wt} is a distinct disease and that initial leukemia and relapse potentially arise from a premalignant clonal hematopoiesis.

Introduction

Acute myeloid leukemia (AML) with *NPM1* mutation (*NPM1*^{mut}) is the most common AML with normal karyotype and *NPM1*^{mut} defines a World Health Organization category with good prognosis in the absence of concomitant *FLT3*-ITD mutations.[1,2] There is conflicting data if *NPM1*^{mut} AML can relapse with *NPM1* wild-type (*NPM1*^{wt}) disease following intensive chemotherapy.[3,4] Here we report on 14/104 patients with initial *NPM1*^{mut} AML that relapsed with *NPM1*^{wt} AML. Analyzing those patients at diagnosis and at relapse provides evidence of molecular features suggestive of premalignant clonal hematopoiesis driving initial AML and relapse.

Patients and methods

Patients

All patients gave their written informed consent for scientific evaluations. The study was approved by the internal review board and adhered to the tenets of the Declaration of Helsinki. Between 2006 and 2017, we investigated a total of 104 intensively treated patients with *NPM1*^{mut} AML who attained a complete molecular remission (CMR, absence of *NPM1* transcripts; sensitivity, 0.001%) and finally had a hematologic relapse (Table 1).

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Table 1. Patient characteristics

	NPM1^{mut} relapse	*NPM1*^{wt} relapse	*P*
Total, n	90	14	
Sex, n (%)			
Female	52 (58)	7 (50)	NS
Male	38 (42)	7 (50)	NS
Age, median (range), y	59 (22-82)	62 (41-72)	NS
AML subtype, n (%)			
M0	2 (2)	—	NS
M1	36 (40)	8 (57)	NS
M2	21 (23)	3 (21)	NS
M4	22 (23)	2 (14)	NS
M5a	1 (6)	1 (7)	NS
M5b	4 (4)	—	NS
M6	1 (1)	—	NS
Not specified	4 (4)	—	NS
Initial blast count in BM, median (range), %	76 (21-99)	84 (26-90)	NS
Leukocyte count, median (range), $\times 10^9$/L	30 (1-213)	3 (0.9-51)	.008
Hemoglobin, median (range), g/dL	9.4 (4-16)	9.8 (8-13)	NS
Platelet count, median (range), $\times 10^9$/L	66 (9-1100)	128 (32-267)	.018

BM, bone marrow; NS, not significant.

Next-generation sequencing

Unique molecular identifier based sequencing of 63 genes associated with hematological malignancies was performed at diagnosis and for patients with *NPM1*^{wt} relapse in paired samples at diagnosis, CMR, and at clinical relapse. Digital error suppression was applied to follow mutations in up to 1/500 molecules as follows. In short, all samples were analyzed by a gene panel containing *ASXL1*, *ASXL2*, *ATM*, *BCL2*, *BCOR*, *BCORL1*, *BIRC3*, *BRAF*, *BTK*, *CALR*, *CBL*, *CSF3R*, *CSNK1A1*, *CXCR4*, *DNMT3A*, *EGR2*, *ETNK1*, *ETV6*, *EZH2*, *FBXW7*, *FLT3*, *FOXO1*, *GATA1*, *GATA2*, *ID3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KLF2*, *KRAS*, *MAP2K1*, *MPL*, *MYC*, *MYD88*, *NF1*, *NFKBIE*, *NOTCH1*, *NOTCH2*, *NPM1*, *NRAS*, *PHF6*, *PIGA*, *PLCG2*, *POT1*, *PTPN11*, *RAD21*, *RUNX1*, *SAMHD1*, *SETBP1*, *SF3B1*, *SRSF2*, *STAG2*, *STAT3*, *STAT5B*, *TCF3*, *TET2*, *TP53*, *U2AF1*, *UBR5*, *WT1*, *XPO1*, and *ZRSR2*. The library of 63 genes was generated with a TruSeq Custom Amplicon Low Input Kit (Illumina, San Diego, CA) following the manufacturer's protocol. The library contained molecular tags (unique molecular identifiers) that allow the detection and quantification of the individual molecule of each template DNA fragment. This tag was incorporated and sequenced, enabling the accurate detection of true variants with high resolution up to 0.2% variant allele frequency (VAF) as polymerase chain reaction duplicates can be identified and discarded. The library was sequenced and demultiplexed on a Nextseq instrument (Illumina) as described previously.[5] The FASTQ files were further processed using Sequence Pilot software, version 4.3.1, Build 502 (JSI Medical Systems, Ettenheim, Germany), for alignment and variant calling. Analysis parameters were set according to the manufacturer's default recommendation. Validity of the somatic mutations was checked against the publicly accessible COSMIC v78 (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic) and ClinVar databases. Functional interpretation was performed using SIFT 1.03 (http://sift.jcvi.org) and PolyPhen 2.0 (http://genetics.bwh.harvard.edu/pph2).[6] Additionally, *TP53* variants were verified using the International Agency for Research on Cancer repository.[7] Single-nucleotide polymorphisms were annotated according to the National Center for Biotechnology Information dbSNP (http://www.ncbi.nlm.nih.gov/snp; Build 147) and ExAC population frequency database. Variants of uncertain significance were excluded from statistical analyses. *FLT3*-ITD was analyzed by gene scan.

Statistical analysis

Statistical analysis was performed using Prism 7.03 software by GraphPad (La Jolla, CA). Survival differences were calculated by log-rank test and for group comparisons the χ^2 test was used.

Results

We identified 14/104 patients with a hematologic relapse that was *NPM1*^{wt}. At first diagnosis. there was no significant distribution of age, sex, or AML subtype in the group with *NPM1*^{mut} or *NPM1*^{wt} relapse. However, in the subgroup with *NPM1*^{mut} relapse, we observed a significant higher white blood cell count (WBC; median, 30 vs 3 $\times$ 10^9/L, $P = .008$) and significant lower platelet count (median platelet count 66 vs 128 $\times$ 10^9/L, $P = .018$; Table 1). Bone marrow blast count and hemoglobin were comparable (Table 1).

For 90/104 patients with an *NPM1*^{mut} relapse, the median time to relapse was 14 months (range, 3-77). In contrast, for the 14 patients with an *NPM1*^{wt} relapse, the median time to relapse was significantly longer, at 43 months (range, 4-66, $P = .004$; Figure 1A). There was no significant difference in overall survival: at 2 years following relapse, overall survival was 48% (95% confidence interval, 12-77) vs 54% (14-83) for patients with *NPM1*^{mut} relapse and *NPM1*^{wt} relapse, respectively (NS; Figure 1B).

Diagnostic samples were available for sequencing in 61/90 patients with *NPM1*^{mut} relapse and 11/14 patients with *NPM1*^{wt} relapse. The mutational landscape of these patients revealed recurrent comutations in 18 different genes in 62/72 patients. *DNMT3A*, *TET2*, *FLT3*-ITD, *NRAS*, *IDH1*, *IDH2*, *FLT3*-TKD, and *WT1* were mutated in >5% of cases (Figure 2). *FLT3*-ITD mutations were significantly more frequent in patients with *NPM1*^{mut} relapse (analysis available for 104 patients, 33/90 vs 1/14, $P = .029$), whereas *DNMT3A* mutations were significantly more frequent in the cohort of patients with *NPM1*^{wt} relapse (analysis available for 72 patients, 22/59 vs 9/13, $P = .035$). At diagnosis, a median of 2 genes per patient were comutated with *NPM1* (range, 0-5); only 7/72 patients had no cooccurring mutation detected, all of which relapsed with *NPM1*^{mut} AML (11% vs 0%, NS).

For the same 11/14 cases with *NPM1*^{wt} relapse, the relapse samples were also available for 63 gene panel sequencing. At diagnosis, we identified a total of 30 comutations in addition to *NPM1* in a total of 11 different genes in 11/11 patients (*DNMT3A* [n = 8], *TET2* [8], *SRSF2* [3], *IDH1* [2]), *KRAS* [2], *PTPN11* [2], *ASXL1* [1], *FLT3*-TKD [1], *IDH2* [1], *NRAS* [1], and *STAG2* [1]). At relapse, we identified a total of 32 comutations in 12 different genes in 10/11 patients with a median of 3 mutations per patient (range, 0-5). An overview of mutated genes detected in each patient at diagnosis vs relapse is given in Figure 3A. The color code depicts lost and gained

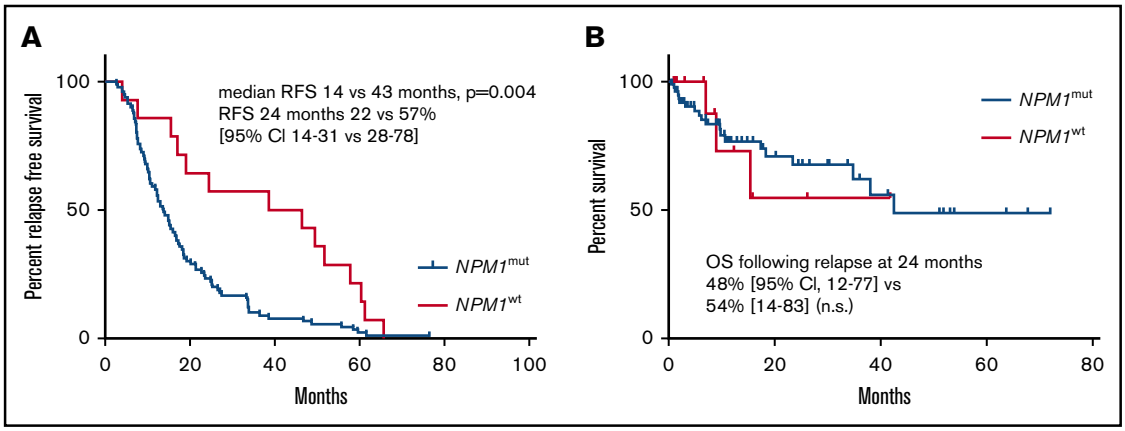
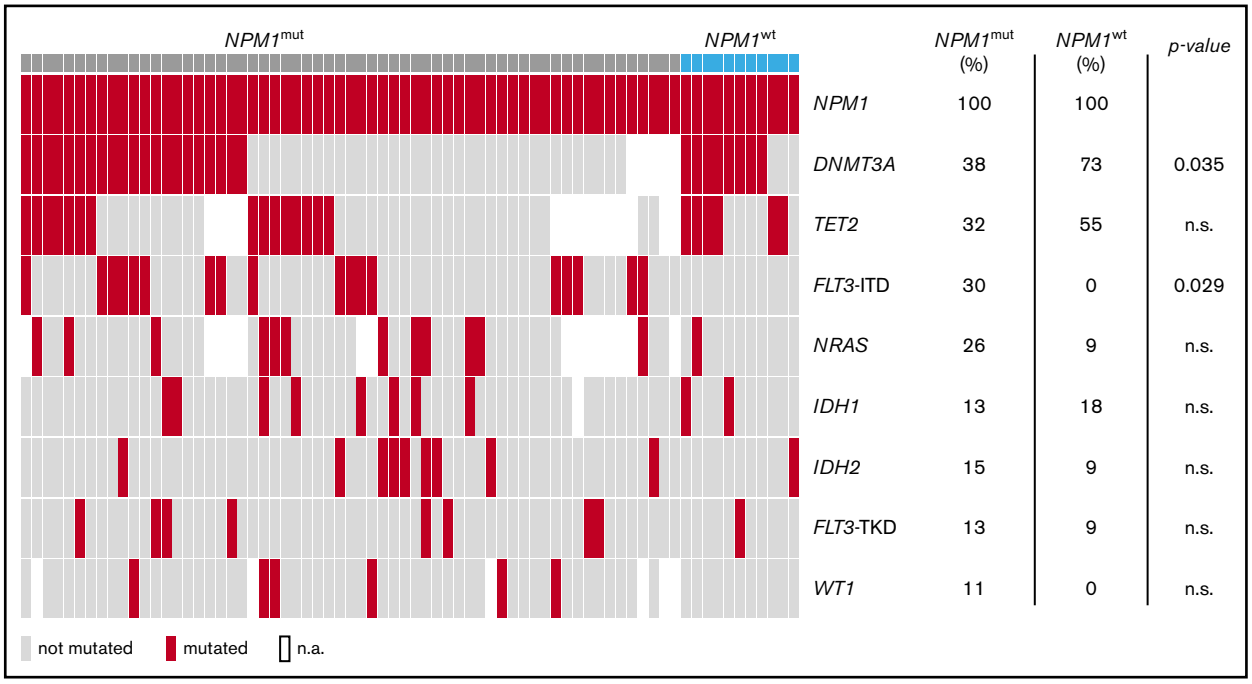


Figure 1. AML relapse with *NPM1*^{mut} occurs earlier than relapse with *NPM1*^{wt}. (A) RFS of patients with *NPM1*^{mut} relapse and *NPM1*^{wt} relapse. (B) OS following relapse of patients with *NPM1*^{mut} relapse and *NPM1*^{wt} relapse. 95% CI, 95% confidence interval; OS, overall survival; RFS, relapse-free survival.

mutations at relapse. Twelve mutations in 8 genes (*TET2*, *PTPN11*, *DNMT3A*, *FLT3*-TKD, *KRAS*, *NRAS*, *SRSF2,* and *STAG2*) were lost at relapse and 14 mutations in 9 genes (*TP53*, *RUNX1*, *IDH1*, *IDH2*, *NF1*, *NRAS*, *SRSF2*, *TET2*, and *WT1)* were gained at relapse. We used digital error correction to reassess these mutations in the diagnostic sample. With a detection limit of up to 0.2% VAF (1/500 molecules), none of the newly gained mutations was detectable at diagnosis. Interestingly, 18 mutations in 7 genes persisted over time, with a median of 2 persistent mutations per patient (range, 0-4; Figure 3B): at relapse, we observed persistent mutations in *DNMT3A*, *TET2*, *IDH1*, *SRSF2*, *ASXL1*, *IDH2*, and *KRAS* (Figure 3C). *DNMT3A,* the most prevalent mutation, was lost in only 1 patient (8/11 at diagnosis, 7/11 at relapse), whereas *TET2* mutations persisted in 3 patients, *IDH1* and *SRSF2* mutations in 2 patients (Figure 3C, right) and *ASXL1*, *IDH2*, and *KRAS* mutations in 1 patient. When persistent, the VAF of mutated genes was generally higher at relapse than at diagnosis; however, because of low numbers, no statistical significance was observed for single genes (Figure 3D and Figure 4C). Interestingly, the VAF at diagnosis was lowest for *ASXL1*, *IDH2*, and *KRAS*.

The high number of persistent mutations raised the possibility of the presence of clonal hematopoiesis as a basis for both initial AML and subsequent relapse. We addressed this point by analyzing remission samples of patients with *NPM1*^{wt} relapse. For 9/11



	NPM1^{mut} (%)	*NPM1*^{wt} (%)	*p-value*
NPM1	100	100	
DNMT3A	38	73	0.035
TET2	32	55	n.s.
FLT3-ITD	30	0	0.029
NRAS	26	9	n.s.
IDH1	13	18	n.s.
IDH2	15	9	n.s.
FLT3-TKD	13	9	n.s.
WT1	11	0	n.s.

Figure 2. *DNMT3A* mutations at diagnosis are associated with *NPM1*^{wt} relapse. Cooccurring mutations with *NPM1* at diagnosis of all patients. For 61/90 patients with *NPM1*^{mut} relapse and for 11/14 patients with *NPM1*^{wt} relapse, the diagnostic sample was available for 63 gene panel sequencing. Shown are mutations in genes affected in >10% of cases. *DNMT3A* mutations are significantly more frequent in patients that relapse with *NPM1*^{wt} disease. *FLT3*-ITD mutations are significantly more frequent in patients that relapse with *NPM1*^{mut} disease. n.a., not available.

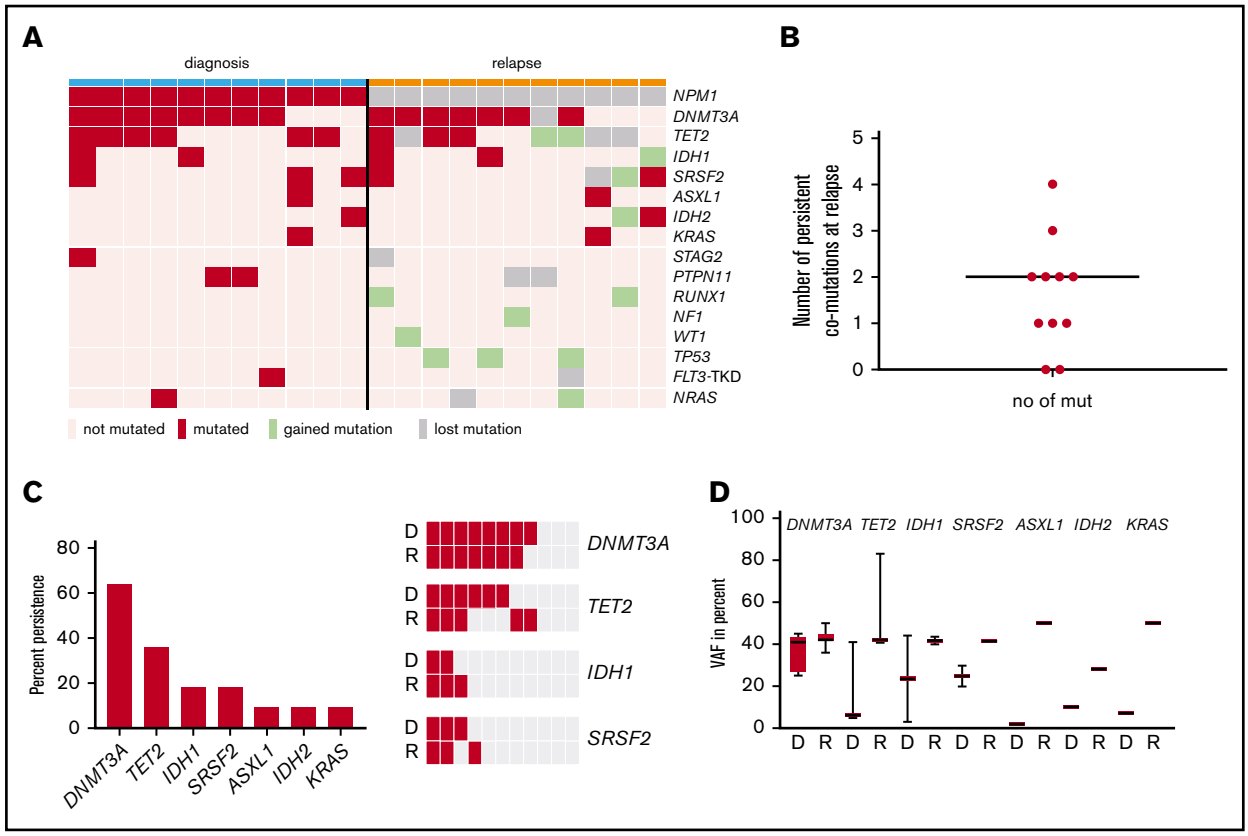


Figure 3. *NPM1*^{wt} relapse is associated with persistent mutations. (A) For 11/14 patients with *NPM1*^{wt} relapse, paired samples at diagnosis and relapse were available for sequencing. Shown are genes mutated at least once in the analyzed samples. Genes with >1 mutation/patient are shown once. (B) Number of persistent mutations at relapse; median, 2 (range, 0-4). (C) Left: mutations in 7 genes detected at diagnosis were found to persist at relapse. Fraction of persistent mutations in relation to all 11 patients is shown. Right: The 4 most frequently mutated genes at D and R are shown as heat maps to visualize persistence of mutations. *DNMT3A* is the most prevalent mutation and also the most stable mutation over time. (D) The variant allele frequency of the indicated genes is given at D and R. D, diagnosis; R, relapse.

patients analyzed at diagnosis and relapse, 2 remission samples/patient were available for sequencing. The progression of individual mutations is shown in Figure 4A. In molecular remission, we detected persistence of comutations (*DNMT3A* [n = 6], *TET2* [5], *IDH1* [3], *SRSF2* [3], *IDH2* [2], and *STAG2* [1]) in 8/9 patients (Figure 4B). Only 1 patient showed a loss of all comutations (*DNMT3A* and *PTPN11*) at CMR and at relapse. Four patients acquired additional comutations in remission (*TP53* [n = 5], *TET2* [1], and *RUNX1* [1]; Figure 4A). The median VAF of persistent mutations was significantly reduced in the first CMR sample and increased significantly in the second CMR sample and at relapse (Figure 4C).

A recent report showed that a high allelic *NPM1* burden was associated with inferior outcome in de novo AML[8]; therefore, we analyzed the allelic burden of *NPM1* mutations. *NPM1* was sequenced by next-generation sequencing in 13 patients in the cohort with *NPM1*^{mut} relapse and in 4 patients with *NPM1*^{wt} relapse. We did not observe a significant difference of *NPM1* VAF in the 2 subgroups (Figure 5A). Moreover, we analyzed the VAF of *DNMT3A*, *TET2*, and *FLT3*-TKD at diagnosis and relapse and did not find a significant difference in the 2 subgroups (Figure 5A).

As a control, we analyzed the persistence of the most common comutations in the cohort with *NPM1*^{mut} relapse. Fifteen of 22 patients that had a *DNMT3A* mutation at diagnosis were evaluable for *DNMT3A* mutations at CMR: 11 of those 15 patients had a persistent *DNMT3A* mutation in CMR (73%). At diagnosis, the frequency of R882 mutations were comparable in the cohorts with *NPM1*^{mut} and *NPM1*^{wt} relapse: 55% vs 67%, respectively (NS; Figure 5B). We also analyzed the frequency of persistent R882 mutations in CMR: 45% in the *NPM1*^{mut} and 67% in the *NPM1*^{wt} cohort had a persistent R882 mutation (NS; Figure 5C). Moreover, at CMR, 56% of patients had a persistent TET2 mutation (n = 9 patients with initial positivity and analyzed CMR sample) and 60% had a persistent IDH1 (n = 5) mutation and 14% a persistent IDH2 (n = 7) mutation. No patient had a detectable persistent NRAS (n = 7), FLT3-ITD (n = 28), or FLT3-TKD (n = 9) mutation at CMR.

Discussion

We identified 14% of *NPM1*^{mut} AML patients who achieved CMR following intensive therapy and relapsed with an *NPM1*^{wt} AML. To our knowledge, this is the largest cohort of *NPM1*^{wt} AML relapse that has been studied.

In our analysis, *NPM1*^{mut} relapse occurred significantly earlier than *NPM1*^{wt} relapse. These findings are in line with a previous study reporting on 10% of *NPM1*^{wt} relapse with a longer latency.[3] Interestingly, in our cohort, patients that relapsed with *NPM1*^{wt}

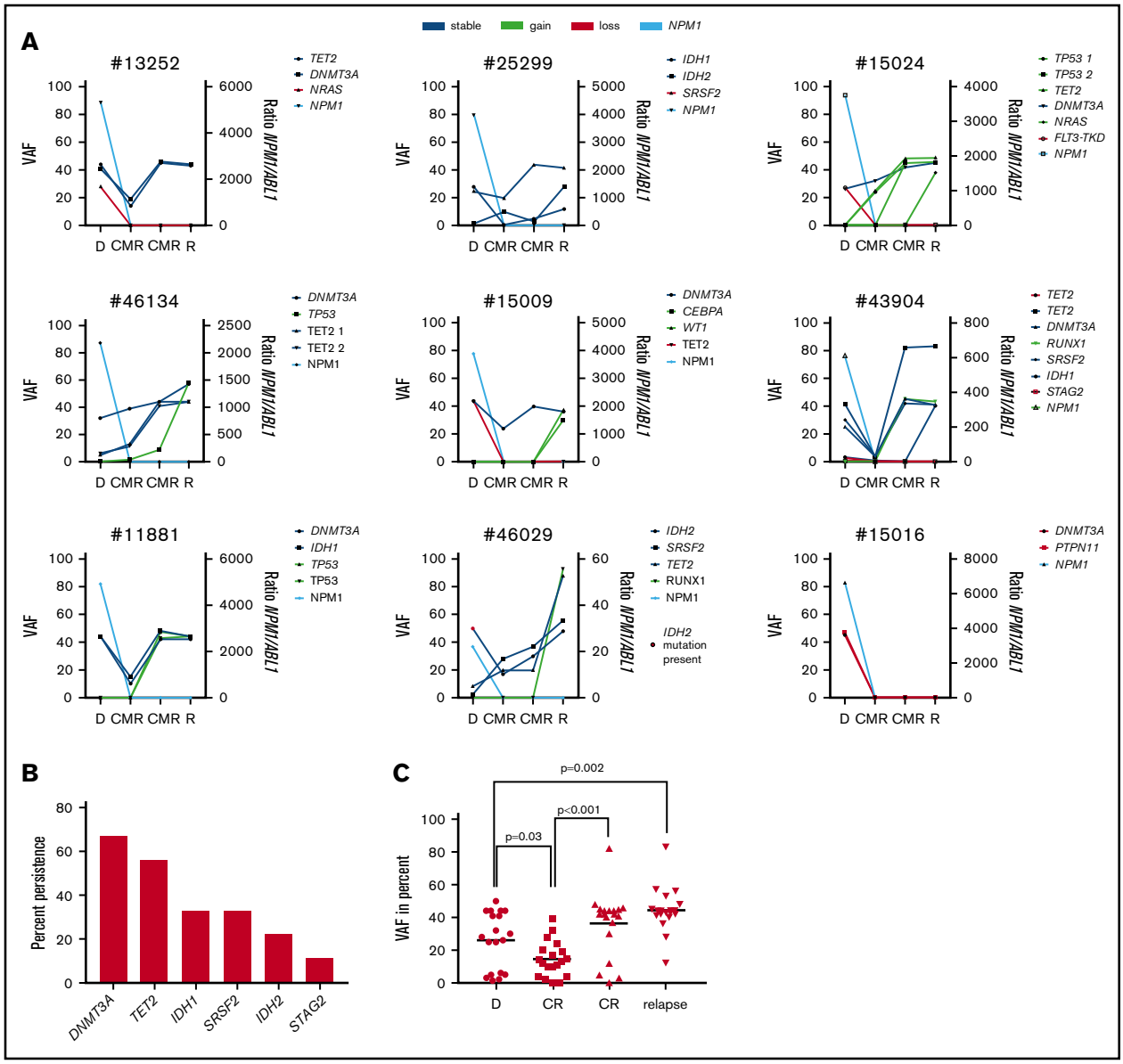


Figure 4. Premalignant clonal hematopoiesis underlies *NPM1*^{wt} relapse. (A) For 9/11 patients, 2 remission samples/patient were available for sequencing analysis. Eights of 9 patients showed persistent mutations in CMR and 4 patients acquired additional mutations before the onset of relapse. (B) Mutations in 6 genes detected at diagnosis were found to persist in remission. Shown is the fraction of persistent mutations in relation to all 9 patients. (C) VAF of persisting mutations at diagnosis, CMR, and relapse. Black line, median; CR, complete remission.

disease had distinct clinical and molecular features at diagnosis, with significantly lower WBC counts and higher platelet counts, a higher frequency of *DNMT3A* mutations, and a lower frequency of *FLT3*-ITD mutations. All patients were treated with induction and consolidation therapy and there was no difference in treatment intensity in the 2 subgroups.

At diagnosis, *DNMT3A* mutations were the most frequently observed comutations across all patients and significantly more frequent in patients who later relapsed with *NPM1*^{wt}. *DNMT3A* was assessed in only 70% of all patients at diagnosis (54% in the *NPM1*^{mut} relapse; 100% in the *NMP1*^{wt} relapse cohort), making this observation less reliable. However, *DNMT3A* mutations most frequently persisted in CMR, and the *DNMT3A* mutation was lost at relapse in only 1/8 patients. Interestingly, DNMT3A was also the most frequent mutation in the *NPM1*^{mut} relapse cohort at diagnosis and persisted frequently during remission in this group. Mutated *DNMT3A* at diagnosis has been described in AML and is only moderately associated with outcome.[9] The persistence of *DNMT3A* mutations in remission has also been addressed by several groups, and was not associated with clinical outcome.[10-12] However, the landmark analysis that defined clonal hematopoiesis reported *DNMT3A* as the most frequently mutated gene.[13,14] These analyses finally laid the basis for the definition of clonal hematopoiesis of indeterminate potential (CHIP).[15] A recent report investigated persistent mutations at remission of AML patients and showed that DTA mutations (mutations in *DNMT3A*, *TET2*, and

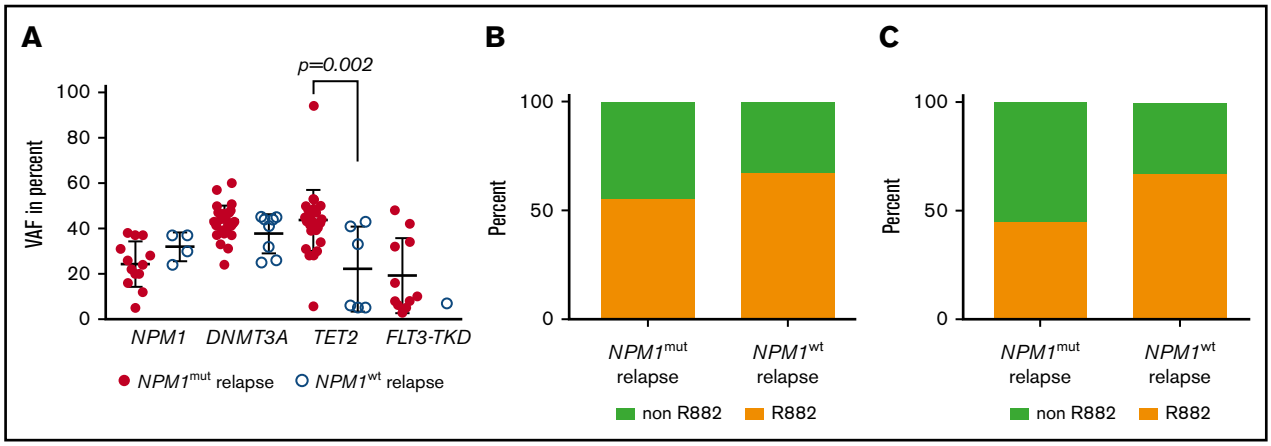


Figure 5. *NPM1*^{mut} allelic burden and *DNMT3A* mutation type is not associated with *NPM1*^{wt} relapse. (A) The variant allele fraction of *NPM1, DNMT3A, TET2*, and *FLT3*-TKD mutations at diagnosis of the indicated cohort. (B) The fraction of *DNMT3A* R882 mutations at diagnosis for the indicated cohort (n = 22 *NPM1*^{mut} relapse, n = 9 *NPM1*^{wt} relapse, NS). (C) At CMR, 11/15 patients in the cohort with *NPM1*^{mut} relapse showed a persistent *DNMT3A* mutation and 6/9 in the cohort with *NPM1*^{wt} relapse. Shown is the fraction of persistent *DNMT3A* R882 mutations in the respective cohort (NS).

ASXL1) in remission were not associated with an increased risk of relapse. Interestingly, those mutations are all associated with CHIP or age-related clonal hematopoiesis.[16] The biologic role of DTA mutations in leukemogenesis is still under investigation. Preclinical work shows that early purified stem cells in *NPM1* mutated AML wild-type for *NPM1* carry *DNMT3A* mutations and display an increased repopulating activity.[17] What is more, Single-nucleotide polymorphism array analysis identified that *DNMT3A* was part of the most commonly focally deleted region in AML, underscoring its role in leukemogenesis.[18] Because persistence of somatic mutations per se is associated with relapse,[19,20] persistent clonal hematopoiesis in remission might increase repopulating activity sufficient to trigger a treatment related disease or relapse with a preexisting leukemic clone.

Although CHIP does not define a disease entity, there is growing evidence that certain mutations confer a high risk to develop hematologic malignancies.[21] The biologic role of mutant *DNMT3A* or other mutations in *NPM1*^{mut} AML might therefore cause a clonal hematopoiesis that renders progenitor cells susceptible to transformation. In line with this notion is our observation of persistent mutations in remission and relapse that identify clonal hematopoiesis in the majority of patients. The rising VAF and acquisition of mutations in 4 patients (ie, *TP53*; Figure 2A) in CMR prove clonal evolution and raise the possibility that chemotherapy could have induced these novel mutations. Moreover, the loss of *NPM1* mutation at relapse shows that *NPM1* is not the sole driver in *NPM1*-mutated AML. This is supported by various preclinical models in which transgenic animals and knock-in animals develop a myeloproliferative phenotype or full AML only with a long latency.[22-24] In this context, other typical driver mutations such as *FLT3* or *NRAS* were not observed in remission in either cohort. Taken together, this could indicate that those driver mutations are second hits required for *NPM1*-driven leukemogenesis. Supportive preclinical evidence shows that only compound mice carrying

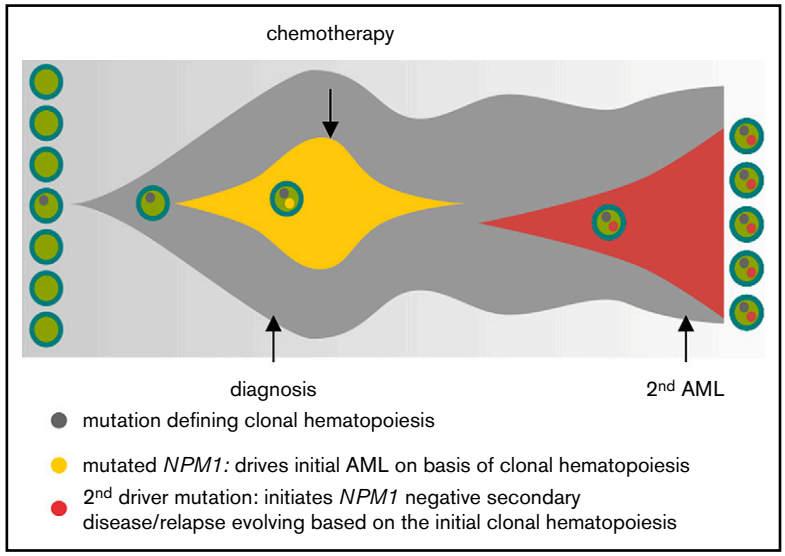


Figure 6. Model for clonal hematopoiesis driving relapse or secondary leukemia. The first leukemia is cured by chemotherapy based on the underlying clonal hematopoiesis; a second driver mutation than leads to overt relapse.

NPM1 and *FLT3* or *NPM1* and *NRAS* mutations develop AML with high penetrance and short latency.[25] This is especially interesting because we observed a strong enrichment for *FLT3*-ITD mutations in the cohort of patients that had *NPM1*^{mut} relapse, indicating that 2 driver mutations cause relapse with the initial aggressive clone. In the same line is our observation that high WBC and low platelet counts, both indicative of highly active disease, are associated with *NPM1*^{mut} AML relapse. Two reports describe patients with *NPM1*^{mut} AML that develop *NPM1*^{wt} myelodysplastic or myelodysplastic/myeloproliferative neoplasms following CMR.[26,27] Together with our data, there is growing evidence that clonal hematopoiesis is an underlying phenomenon in this AML entity. Our data expand these findings and suggest that relapse or therapy-related leukemia can arise from preleukemic clones with wild-type *NPM1*. Recent work established that relapse is driven by drug-resistant AML stem cells.[28] If this is the case, mutations present at diagnosis, remission, and relapse should also be present in these stem cells. Leukemia would then arise from a stem cell that drives a premalignant clonal hematopoiesis and give rise to initial leukemia and relapse. Although the *NPM1*^{wt} relapses occurred significantly later than *NPM1*^{mut} relapses, we observed some early relapses in the *NPM1*^{wt} cohort. One of the patients with the earliest relapse (7.7 months, no material available for the patient with a relapse 4.1 months after diagnosis) lost 3 comutations at relapse (*KRAS*, *SRSF2*, *TET2*) and the *NPM1* mutation, which could either be clonal evolution of a preexisting clone or argue for a treatment-related disease. *DNMT3A* mutations are the most common comutations in *NPM1*^{mut} AML and have also been shown to persist in remission.[4,10] Therefore, we analyzed *DNMT3A* mutations in the cohort of patients with *NPM1*^{mut} relapse as control. Persistent *DNMT3A* mutations were detected in 73% of patients in remission. Moreover, *TET2* and *IDH1* mutations persisted frequently in remission; therefore, clonal hematopoiesis might also contribute to *NPM1*^{mut} relapse. Interestingly, *FLT3*-ITD, *FLT3*-TKD, and *NRAS* mutations were not observed in CMR in either the cohort with *NPM1*^{mut} or *NPM1*^{wt} relapse. Although regularly analyzed in remission, the benefit of minimal residual disease based on those markers is therefore questionable in *NPM1*-mutated AML. To analyze the cellular compartment from which the *NPM1* clone arises and is eventually lost at relapse warrants cell sorting and single-cell sequencing analysis in a prospective manner.

Previous work showed that high allelic *NPM1* burden at diagnosis is a negative prognostic factor.[8] In our analysis, *NPM1* allelic burden was not associated with *NPM1*^{wt} or *NPM1*^{mut} relapse. However *NPM1* is not routinely analyzed by next-generation sequencing; therefore, the number of evaluable patients for *NPM1* VAF is relatively low in our analysis. The *DNMT3A* mutation subtype has frequently been queried for an association with prognosis, and a recent meta-analysis showed that *DNMT3A* R882 mutations are significantly associated with reduced relapse-free and overall survival.[29] In our analysis, the R882 mutations were slightly more prevalent in the subgroup with *NPM1*^{wt} relapse; however, this trend was not significant. This could also be due to the low patient number.

Taken together, our data suggest that, in *NPM1*^{mut} AML, the persistence of mutations defines premalignant clonal hematopoiesis and drives a treatment-related leukemia or relapse (Figure 6). Although we show that a proportion of patients with *NPM1*^{mut} AML lose the *NPM1* mutation at relapse, for the majority of patients, minimal residual disease monitoring by *NPM1* quantitative polymerase chain reaction is absolutely mandatory. Although it is not feasible to monitor all *NPM1*$^{+}$ AML patients longitudinally with large sequencing panels, there is increasing evidence that supports the screening for persistent mutations in at least 1 remission sample.

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Authorship

Contribution: A.H. and T.H. designed the study; A.H. interpreted the data and wrote the manuscript; S.J., M.M., F.D., and N.N. did molecular analyses; T.H. was responsible for cytomorphologic analyses; C.H. was responsible for cytogenetic and fluorescence in situ hybridization analyses; W.K. was responsible for immunophenotyping; and all authors read and contributed to the final version of the manuscript.

Conflict-of-interest disclosure: A.H., M.M., F.D., S.J., and N.N. are employed by MLL Munich Leukemia Laboratory. C.H., W.K., and T.H. have equity ownership of MLL Munich Leukemia Laboratory.

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