## In rare acute myeloid leukemia patients harboring both *RUNX1* and *NPM1* mutations, *RUNX1* mutations are unusual in structure and present in the germline

It has become clear that there are distinct interrelationships between recurring genetic mutations in acute myeloid leukemia (AML). Whereas certain mutations frequently co-exist in AML, others are mutually exclusive. Mutations in *NPM1* and *RUNX1*, two of the most commonly mutated genes in AML, are almost entirely mutually exclusive. In six recent studies that evaluated the frequency of both *NPM1* and *RUNX1* mutations in AML,[1-6] these two mutations co-existed in just 0.6% (13 of 2348) of the cases. In the two studies conducted by our group[1,4] which included large cohorts of patients with primary AML and a normal karyotype (n=392)[4] and patients with sole trisomy 8 (n=80),[1] *NPM1* and *RUNX1* mutations co-existed in 0.8% (4 of 472) of the patients. The goal of the study reported herein was to more closely examine these few AML patients carrying both mutations in order to better understand the relationship between *NPM1* and *RUNX1* mutations in AML.

Clinical and molecular characteristics of the 4 patients with co-existing *RUNX1* and *NPM1* mutations are provided in Table 1. Patients provided written informed consent, and study protocols were in accordance with the Declaration of Helsinki. All *NPM1* mutations were type A, consisting of duplication of TCTG at position c.860_863 (NCBI Accession NM_002520.5). In contrast, each of the 4 patients had a different *RUNX1* mutation (numbering based on the *RUNX1c* isoform; NCBI Accession NM_001754.4). Patient 1 harbored c.952T>G, resulting in a missense change, p.S318A, in the first amino acid of the transactivation domain (TAD). Patient 2 harbored c.1098_1103delCGGCAT, resulting in an in-frame deletion of two amino acids, p.I366_G367del, in the TAD. Patient 3 harbored c.620G>A, resulting in a missense change, p.R207Q, just downstream of the Runt domain (RD), the domain responsible for DNA binding and heterodimerization with the beta subunit of core binding factor. Patient 4 harbored c.155T>A, resulting in a missense change, p.M52K, upstream of the RD. All *RUNX1* mutations (4 of 4) in *NPM1*-mutated patients were in-frame and located outside of the RD (Figure 1A). In contrast, only 4.5% (4 of 88) of the *RUNX1* mutations in *NPM1*-wild-type patients were in-frame and located outside of the RD ($P$<0.001).

To determine whether *RUNX1* and *NPM1* mutations were somatically acquired in AML patients harboring both mutations, pre-treatment germline material was screened in those *RUNX1*-mutated/*NPM1*-mutated patients for whom buccal cells were available (UPN 1-3). Surprisingly, in all 3 cases, the same *RUNX1* mutation identified in leukemic blasts was also present in the germline (Figure 1B). In contrast, *NPM1* mutations were not detected in the germline (Figure 1C), confirming that germline material was not contaminated with leukemic cells. To determine the specificity of this finding to *NPM1*-mutated cases, we next tested the frequency of germline *RUNX1* mutations in *NPM1*-wild-type patients known to have *RUNX1* mutations in their leukemic blasts. Of the *RUNX1*-mutated/*NPM1*-wild-type patients with buccal cells available (n=56), none harbored germline *RUNX1* mutations ($P$<0.001). Thus, in our cohort, AML patients with co-existing *RUNX1* and *NPM1* mutations were not found to acquire both of these mutations somatically; rather, our data suggest that *RUNX1* mutations pre-existed and *NPM1* mutations were a later, acquired event.

We considered the possibility that these germline *RUNX1* mutations may have been inherited and caused a predisposition to the development of AML (familial platelet disorder with associated myeloid malignancy: Online Mendelian Inheritance in Man #601399). To address this issue, we sought to identify whether any of these patients or their family members had a history of hematologic problems, including myelodysplastic syndrome and/or AML. Direct contact with patients and/or families was not possible since all patients are now deceased and had not consented to their families being contacted at the time of study enrollment. Thus, medical and family histories taken at the time of diagnosis were obtained from chart review. This limited analysis revealed that none of the germline *RUNX1*-mutated patients had a known history of bleeding or thrombocytopenia pre-dating their diagnosis of AML, or a family history of hematologic problems. However, since the recorded family histories included information on first-degree relatives only, and *RUNX1* mutation-associated syndromic disease is variably expressed,[11] this analysis was insufficient for formal evaluation of familial phenotype. To attempt to address the disease potential of the *RUNX1* mutations discovered here, we subjected them to MutationTaster analysis, which is a free, web-based application for the evaluation of disease-causing potential of DNA sequence variations.[12] This analysis predicted that c.952T>G and c.1098_1103delCGGCAT were likely to be harmless polymorphisms; in contrast, c.620G>A and c.155T>A were predicted to be disease-predisposing. Supporting the likelihood that c.620G>A plays a causal role in AML pathogenesis is the fact that it was somatically acquired in another patient within our cytogenetically normal AML cohort.[4] Despite these predictions, the extent to which the

Table 1. Clinical and molecular characteristics of primary AML patients with co-existing *NPM1* and *RUNX1* mutations.

| UPN | Age[a] | Gender | Karyotype[a] | CALGB treatment protocol number[b] | CR | DFS (months) | OS (months) | Specific *RUNX1* mutation | Additional mutations[c] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 37 | F | Normal | 9621[8] | Yes | 3.9 | 6.2 | c.952T>G | *FLT3*-ITD, *DNMT3A* (*non-R882*), *WT1, TET2* |
| 2 | 52 | M | Normal | 19808[10] | No | – | 5.3 | c. 1098_1103delCGGCAT | *FLT3*-ITD, *DNMT3A (R882)* |
| 3 | 81 | M | Sole Trisomy 8 | 10201[9] | Yes | 58.0 | 63.6 | c.620G>A | *IDH2 (R140), ASXL1* |
| 4 | 70 | M | Normal | 8525[7] | Yes | 5.7 | 6.6 | c.155T>A | *DNMT3A (non-R882)* |

*UPN: unique patient number; F: female; M: male; CALGB: Cancer and Leukemia Group B; CR: complete remission; DFS: disease-free survival; OS: overall survival;* FLT3-*ITD: internal tandem duplication of the* FLT3 *gene.* [a]*At diagnosis;* [b]*Protocol number is followed by a superscript reference number;* [c]*Leukemic blasts from these patients were screened for mutations in the following genes:* RUNX1, NPM1, DNMT3A, WT1, TET2, IDH1, IDH2, ASXL1, CEBPA, *and for* FLT3-*ITD,* FLT3 *tyrosine kinase domain mutations* (FLT3-*TKD*)*, and* MLL *partial tandem duplication* (MLL-*PTD*)*.*

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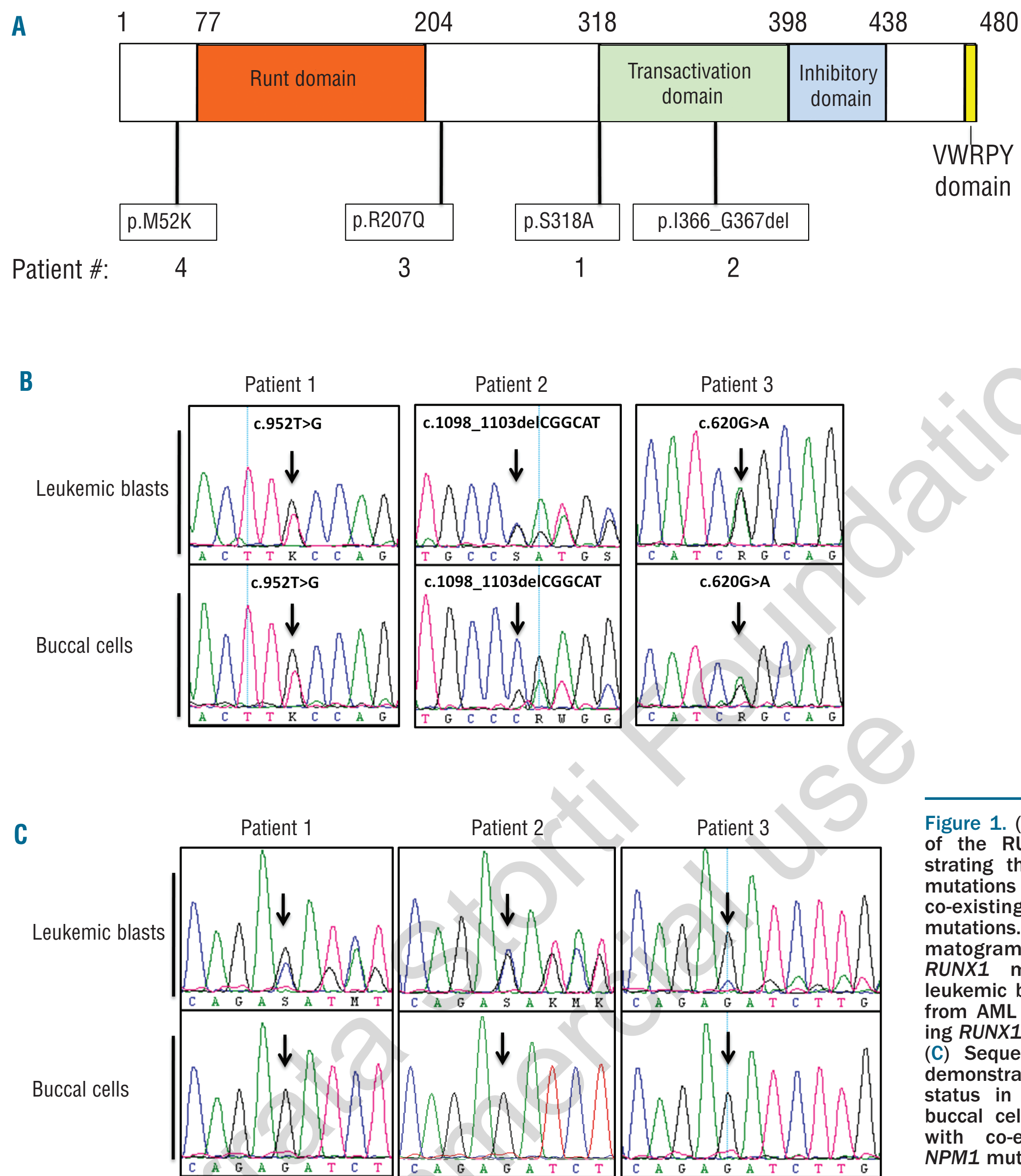


**Figure 1.** (A) Schematic diagram of the RUNX1 protein demonstrating the location of *RUNX1* mutations in AML patients with co-existing *RUNX1* and *NPM1* mutations. (B) Sequencing chromatograms demonstrating *RUNX1* mutational status in leukemic blasts and buccal cells from AML patients with co-existing *RUNX1* and *NPM1* mutations. (C) Sequencing chromatograms demonstrating *NPM1* mutational status in leukemic blasts and buccal cells from AML patients with co-existing *RUNX1* and *NPM1* mutations.

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germline *RUNX1* mutations discovered here contributed to the pathogenesis of AML remains unclear. Although several previous studies have highlighted the near mutual exclusivity of *RUNX1* and *NPM1* mutations,[1-6] to our knowledge, no prior study has closely examined the rare AML cases harboring both mutations. We found that the *RUNX1* mutations in these cases are structurally unusual relative to the majority of those found in AML; namely, they are in-frame, located outside the RD, and present in the germline. The functional significance of these types of *RUNX1* mutations has not been established[13] and future studies should be aimed at determining whether they are innocent polymorphisms or disease-predisposing alleles. The complete exclusivity between *RUNX1* mutations known to be disease-predisposing and NPM1 mutations in our AML cohort suggests that they may have redundant roles in promoting leukemogenesis. This role may be one of causing expansion of the myeloid precursor cell compartment, as recently demonstrated in mouse models expressing either *RUNX1*[14] or *NPM1*[15] mutations in primitive hematopoietic progenitor cells. In summary, our work suggests that *NPM1* mutations do not co-exist with classical *RUNX1* mutations (i.e. those *RUNX1* mutations known to be disease-predisposing) in primary AML. In rare patients harboring both *NPM1* and *RUNX1* mutations, *NPM1* mutations seem to be acquired in the context of pre-existing *RUNX1* mutations of unclear functional significance. Thus, our work adds to the growing body of knowledge defining key mutational interrelationships underlying the pathogenesis of AML.

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## References

1. Becker H, Maharry K, Radmacher MD, Margeson D, Mrózek K, Whitman SP, et al. Sole trisomy 8 in patients (pts) with de novo acute myeloid leukemia (AML) is associated with age-independent poor outcome that is modified by molecular markers and with unique gene- and microRNA (miR)-signatures: a Cancer and Leukemia Group B (CALGB) study. Blood. 2010; 116(21):255.(Abstract 577).
2. Gaidzik VI, Bullinger L, Schlenk RF, Zimmermann AS, Röck J, Paschka P, et al. RUNX1 mutations in acute myeloid leukemia: results from a comprehensive genetic and clinical analysis from the AML Study Group. J Clin Oncol. 2011;29(10):1364-72.
3. Greif PA, Konstandin NP, Metzeler KH, Herold T, Pasalic Z, Ksienzyk B, et al. RUNX1 mutations in cytogenetically normal acute myeloid leukemia are associated with a poor prognosis and up-regulation of lymphoid genes. Haematologica. 2012;97(12):1909-15.
4. Mendler JH, Maharry K, Radmacher MD, Mrózek K, Becker H, Metzeler KH, et al. RUNX1 mutations are associated with poor outcome in younger and older patients with cytogenetically normal acute myeloid leukemia and with distinct gene and microRNA expression signatures. J Clin Oncol. 2012;30(25):3109-18.
5. Schnittger S, Dicker F, Kern W, Wendland N, Sundermann J, Alpermann T, et al. RUNX1 mutations are frequent in de novo AML with noncomplex karyotype and confer an unfavorable prognosis. Blood. 2011;117(8):2348-57.
6. Tang J-L, Hou H-A, Chen C-Y, Liu C-Y, Chou W-C, Tseng M-H, et al. AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. Blood. 2009;114(26):5352-61.
7. Mayer RJ, Davis RB, Schiffer CA, Berg DT, Powell BL, Schulman P, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. N Engl J Med. 1994;331(14):896-903.
8. Kolitz JE, George SL, Dodge RK, Hurd DD, Powell BL, Allen SL, et al. Dose escalation studies of cytarabine, daunorubicin, and etoposide with and without multidrug resistance modulation with PSC-833 in untreated adults with acute myeloid leukemia younger than 60 years: final induction results of Cancer and Leukemia Group B study 9621. J Clin Oncol. 2004;22(21):4290-301.
9. Marcucci G, Moser B, Blum W, Stock W, Wetzler M, Kolitz JE, et al. A phase III randomized trial of intensive induction and consolidation chemotherapy ± oblimersen, a pro-apoptotic Bcl-2 antisense oligonucleotide in untreated acute myeloid leukemia patients >60 years old. J Clin Oncol. 2007;25:(18):(Suppl)7012.
10. Kolitz JE, George SL, Marcucci G, Vij R, Powell BL, Allen SL, et al. P-glycoprotein inhibition using valspodar (PSC-833) does not improve outcomes for patients younger than age 60 years with newly diagnosed acute myeloid leukemia: Cancer and Leukemia Group B study 19808. Blood. 2010;116(9):1413-21.
11. Owen CJ, Toze CL, Koochin A, Forrest DL, Smith CA, Stevens JM, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. Blood. 2008;112(12):4639-45.
12. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods. 2010;7(8):575-6.
13. Mangan JK, Speck NA. RUNX1 mutations in clonal myeloid disorders: from conventional cytogenetics to next generation sequencing, a story 40 years in the making. Crit Rev Oncog. 2011;16(1-2):77-91.
14. Matsuura S, Komeno Y, Stevenson KE, Biggs JR, Lam K, Tang T, et al. Expression of the runt homology domain of RUNX1 disrupts homeostasis of hematopoietic stem cells and induces progression to myelodysplastic syndrome. Blood. 2012;120(19):4028-37.
15. Chou S-H, Ko B-S, Chiou J-S, Hsu Y-C, Tsai M-H, Chiu Y-C, et al. A knock-in npm1 mutation in mice results in myeloproliferation and implies a perturbation in hematopoietic microenvironment. PLoS One. 2012;7(11):e49769.

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