Acute myeloid leukemia (AML) is a myeloid malignancy that arises spontaneously or that may evolve from myelodysplastic syndrome (MDS). AML is characterized by somatic cytogenetic and molecular mutations associated with distinct clinical outcomes. In patients with normal cytogenetics, genetic techniques have been used to discover novel mutations.\(^1\),\(^2\) In order to identify new candidate mutations involved in AML disease progression and pathogenesis, we conducted whole exome sequencing (WES) on DNA from a patient with cytogenetically normal-acute myeloid leukemia (CN-AML) evolving from MDS.

A patient in the seventh decade of life with CN-AML evolving from MDS was identified for exome sequencing. The patient initially presented with anemia and thrombocytopenia, and a bone marrow biopsy (BMBx) showed refractory anemia with excess blasts-1 (RAEB-1). Cytopenias worsened and a second BMBx was done 4 months after diagnosis, which also revealed RAEB-1. Six months later, cytopenias worsened and myeloblasts were detected in the peripheral blood. A third BMBx showed AML with 26% blasts. The cytogenetics were normal, and the detected in the peripheral blood. A third BMBx showed AML with 26% blasts. The cytogenetics were normal, and the detected in the peripheral blood. A third BMBx showed AML with 26% blasts. The cytogenetics were normal, and the detected in the peripheral blood. A third BMBx showed AML with 26% blasts. The cytogenetics were normal, and the detected in the peripheral blood. A third BMBx showed AML with 26% blasts. 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R27X mutation in patients with MDS and CMML. Furthermore, next-generation genes on the long arm of chromosome 7 (7q) including the same group disclosed two additional mutations in Blood Cancer Journal & clone comprising heterozygous, this allelic frequency points to the malignant blast percentage (Table 1). Assuming these mutations are in other studies.6,7 exceeding the blast percentage, a finding that has been seen in other studies.5

As all the novel mutations identified in our index patient were present prior to development of AML at the preceding MDS stage, we started by looking for recurrent mutations in the above identified genes in MDS patient samples. We analyzed a set of 29 MDS patients from our tumor bank using hME (Supplementary Table S1). None of the 11 point mutations found in our index patient were found in this heterogeneous group of 29 MDS patients (IDH2 and RUNX1 not assessed and ZNF672 technically unsuccessful). In order to determine if this set of genes was mutated at other locations, we conducted targeted exon sequencing on 8 of the 11 original genes (DAAM2, NF1A, TNS3, GRLF1, LUC7L2, MYBPC2, PGK2 and GH1) by Fluidigm PCR-amplified DNA and Illumina sequencing in a subset of 12 of the above 29 MDS samples, which had a normal karyotype. No novel non-synonymous SNVs were detected in these 12 patients. Using identical methodology, we then analyzed a set of 11 patient samples (106 AML, 4 CMML, 1 biphenotypic leukemia) from the MGH tumor bank initially with hME followed by targeted exon sequencing on a subset of 47 patients with CN-AML (Supplementary Table S1). No recurrent or novel mutations were identified in the genes analyzed.

Our findings provide several insights into next-generation sequencing studies of samples from patients with myeloid malignancies. The first is that relevant sequence data may be obtained from patient samples containing only low levels of myeloblasts. The second is that many somatic SNVs identified using next-generation sequencing are unique to the patient, and of questionable relevance to pathogenesis given they are not recurrently identified at a high frequency in other patient samples. That is, there are likely only several driver mutations and many additional passenger mutations.

Our study also points to the potential role of LUC7L2 as a novel candidate gene in the pathogenesis of myeloid malignancies, as this mutation was acquired coincident with disease progression of the index patient’s disease. LUC7L2 resides on chromosome of 7q34. Indeed, chromosome 7 represents one of the most frequently altered chromosomes in patients with AML and MDS, with a variety of deletions and translocations described.8,9 Indeed, expression and deletion analyses have identified decreased expression of a variety of genes on the long arm of chromosome 7 (7q) including LUC7L2 in patients with MDS and CMML.9 Furthermore, next-generation sequencing of DNA from a patient with secondary AML revealed a R27X mutation in LUC7L2.10 An extension of this study by the same group disclosed two additional mutations in LUC7L2 in a cohort of 99 patients with myeloid malignancies (70 MDS, 7 AML, 22 CMML) (Table 2) (Methodology included in Supplementary Methods). This work was presented at the 2012 American Society of Hematology Meeting.11 These preliminary data also suggest a worse survival for patients with MDS/AML who carry loss of expression of LUC7L2. Therefore, while the frequency of LUC7L2 mutations is unclear given the ongoing nature of this study, these findings raise the possibility that mutations in this gene may be found at a low frequency in patients with MDS and AML.

LUC7L2 appears to participate in RNA-splicing though its function is still under evaluation.12 However, its identification as possibly recurrently mutated is compelling given recent observations that other RNA-splicing molecules are mutated in MDS.13–15 That the LUC7L2 mutation was acquired coincident with disease progression and that LUC7L2 mutations have been identified in other patients suggest that further study of this gene in myeloid malignancies is warranted.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS
Conception and design of study, data analysis, data interpretation, reviewed and revised manuscript: HS, AL, MC, DS, BP, JM and EA. Reviewed and revised manuscript: KB and PA. Reviewed and revised manuscript: RS. All authors approved the final version of this manuscript.

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### Table 2. Recurrent mutations in genes identified in an index patient in a cohort of 99 patients with myeloid malignancies analyzed by whole exome sequencing

| Gene     | Diagnosis | Karyotype | SNV                | Amino acid change | Additionally mutated genes* |
|----------|-----------|-----------|--------------------|-------------------|-----------------------------|
| LUC7L2 (n = 3) | MDS     | 45,XY,add(5)(q15)[8], add(10)(q26),-12[8],-17, + mar[8] ,del(p11)[46,XY][11] | 7_139094327_C/A | Q236K | None |
|          | MDS     | 46,XX,add(15)(p11.1), add(22)(p11.2)[3] | 7_139060825_C/T | R27X | None |
|          | MDS     | 46,XX,del(17)(q12.1) | 7_139097301_C/T | R262X | None |
| LYZL4 (n = 1) | CMML   | —         | 3_42448641_G/T | G35C | RUNX1 |
| TN53 (n = 1) | MDS     | —         | 7_47343019_C/G | P996A | None |

*Additionally mutated genes refers only to the 11 other genes identified in the index patient.
REFERENCES

1. Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. Nature 2008; 456: 66–72.

2. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K et al. Recurring mutations found by sequencing an acute myeloid leukaemia genome. N Engl J Med 2009; 361: 1058–1066.

3. Ley TJ, Ding L, Walter MJ, McLellan MD, Lampecht T, Larson DE et al. DNMT3A mutations in acute myeloid leukemia. N Engl J Med 2010; 363: 2424–2433.

4. Greif PA, Eck SH, Konstandin NP, Benet-Pages A, Ksienzyk B, Dufour A et al. Identification of recurring tumor-specific somatic mutations in acute myeloid leukemia by transcriptome sequencing. Leukemia 2011; 25: 821–827.

5. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 2011; 364: 2496–2506.

6. Walter MJ, Shen D, Ding L, Shao J, Koboldt DC, Chen K et al. Clonal architecture of secondary acute myeloid leukemia. N Engl J Med 2012; 366: 1090–1098.

7. Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan M et al. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. Leukemia 2011; 25: 1153–1158.

8. Hussain FT, Nguyen EP, Raza S, Knudson R, Pardanani A, Hanson CA et al. Sole abnormalities of chromosome 7 in myeloid malignancies: spectrum, histopathologic correlates, and prognostic implications. Am J Hematol. 2012; 87: 684–686.

9. Jerez A, Sugimoto Y, Makishima H, Verma A, Jankowska AM, Przychodzen B et al. Loss of heterozygosity in 7q myeloid disorders: clinical associations and genomic pathogenesis. Blood. 2012; 119: 6109–6117.

10. Makishima H, Visconte V, Sakaguchi H, Jankowska AM, Abu Kar S, Jerez A et al. Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. Blood 2012; 119: 3203–3210.

11. Hosono N, Makishima H, Jerez A, Gomez-Segui I, Przychodzen B, Sekeres MA et al. Mutation Screening Associated with Chromosome 7 Abnormalities Using Next Generation Whole Exome Sequencing. ASH Annual Meeting Abstracts 120: 173.

12. Howell VM, Jones JM, Bergren SK, Li L, Billi AC, Avenarius MR et al. Evidence for a direct role of the disease modifier SCNM1 in splicing. Human molecular genetics 2007; 16: 2506–2516.

13. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 2011; 478: 64–69.

14. Papaemmanuil E, Cazzola M, Boultonwood J, Malcovati L, Vyas P, Bowen D et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med 2011; 365: 1384–1395.

15. Narla A, Hurst SN, Ebert BL. Ribosome defects in disorders of erythropoiesis. Int J Hematol 2011; 93: 144–149.

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