To the editor:

Mutation analysis of the transcription factor PU.1 in younger adults (16 to 60 years) with acute myeloid leukemia: a study of the AML Study Group Ulm (AMLSG ULM)

The transcription factor PU.1 plays an important role in the differentiation of myelopoiesis by regulating the expression of a number of myeloid target genes. Recently, Mueller et al. identified 10 mutant alleles of the PU.1 gene in 9 of 126 acute myeloid leukemia (AML) patients. However, these findings could not be confirmed in 2 subsequent studies. Vegenes et al. analyzed 381 samples of hematopoietic and solid malignancies, including 60 cases of de novo AML and 60 cases of myelodysplastic syndromes (MDSs), and were not able to identify PU.1 coding region mutations. Similarly, Lamandin et al. failed to detect PU.1 gene mutations in 77 primary AML samples.

We performed PU.1 mutation analysis in 112 AML patients (normal karyotype, n = 93; various chromosome abnormalities, n = 19) entered into the AML HD93 treatment trial of the AML Study Group Ulm. Mutation screening was performed by direct sequencing of genomic DNA after polymerase chain reaction (PCR) amplification of each single exon using intronic primer pairs. We detected a biallelic mutation (A>C transversion; K216Q) in the DNA-binding domain in one patient. Since nonhematopoietic tissue was not available in this patient we analyzed bone marrow cells at the time of complete remission and identified the identical sequence pattern. We conclude that this point mutation was constitutional rather than disease-associated. However, one could not exclude that the mutation represented residual disease. In a second patient, a silent mutation in exon 5 (G>A transversion; L203L) was detected.

Our results are at variance to those of Mueller et al. but they confirm the data published by Vegenes et al. and Lamandin et al. who did not identify a significant number of PU.1 mutations in AML and MDS. There are 2 issues that may explain these controversial findings: the methodology applied and patient selection. In contrast to Mueller et al, who used direct sequencing of cDNA in 99 of 126 AML samples, Vegenes et al. screened by PCR single-strand conformation polymorphism (SSCP) of genomic DNA by confirmatory sequencing, and Lamandin et al. and our group used direct sequencing of genomic DNA. Specifically, the use of genomic DNA could miss large deletions, of which 2 were detected by Mueller et al. The second issue relates to patient selection: in the studies published by Mueller et al., mutations predominantly occurred in undifferentiated (M0) or in monocytic (M4/M5) AML. However, we and Lamandin et al. examined a large number of such cases. Notably, in analogy to the patient cohort described by Mueller et al., we also included therapy-related AML. Finally, there may be ethnic differences since Mueller et al. looked mainly at Japanese AML patients. To further address this question, analyses of larger patient cohorts from different ethnic groups may give conclusive results.

We conclude that inactivating PU.1 mutations are not a common mechanism in the pathogenesis of AML in our study group. This does not exclude other mechanisms (down-regulation and/or inhibition of its activity) leading to loss of PU.1 function.

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Supported by grant 01G99981 from the Bundesministerium für Bildung und Forschung ("Kompetenzzentrum Akute und chronische Leukämien") and by grant P766 of the Medical Faculty of the University of Ulm, Germany.

References

Response:

Frequency of PU.1 mutations in acute myeloid leukemia

We appreciate the information from Döhner et al., in which their group was unable to identify PU.1 mutations as a common finding in their patient samples with acute myeloid leukemia (AML). However, they have identified at least 2 base pair changes in 2 different patients that could potentially represent mutations. It would be of interest to test the functional effect of the K216Q mutant, since, as they point out, the presence of this mutation in a remission sample could represent residual disease. Secondly, could the second silent mutation in exon 5 potentially result in a splicing change, creating a new splice acceptor site (GG>A)? This possibility should also be tested before concluding that this is a silent mutation.

As we noted previously, we have reviewed all of our own primary sequencing data and confirmed the results that were reported in our original article. As reported in the article, sequencing results from samples containing mutations were independently repeated 3 to 6 times, including repetitions of the polymerase chain reaction and sequencing with alternative primers. In 3 patients we had available both cDNA and genomic DNA at diagnosis, and the mutation was detectable from both sources.

Since our publication, we have screened an additional 113 patients from North America and European origin by direct genomic sequencing. We detected one additional PU.1 mutation in

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a patient with t(8;21) AML (283G->T, in which bp 1 represents the start of the longest open reading frame, resulting in the amino acid change V95F; 4 of 7 subclones demonstrated this mutant allele). It is difficult to understand why screening cDNA versus genomic samples should make a difference, unless some type of mechanism such as RNA editing is taking place. Therefore, we think it likely that ethnic differences in the patient populations could account for some of the differences between our results. Additional studies analyzing larger numbers of patients from different ethnic groups may be needed to assess the true frequency of such mutations. At the same time, as has been described in the case of CCAAT/enhancer binding protein α, other mechanisms affecting PU.1, such as down-regulation and/or inhibition of activity, may also implicate loss of PU.1 function in other cases of AML that do not harbor mutations. Indeed, we have recently described how the AML1/ETO fusion product produced by the t(8;21) translocation can inhibit PU.1 function.

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References

To the editor:

Treating patients of myelodysplastic syndrome with antithymocytic globulin—should we be more selective?

We read with interest the article by Steensma et al1 on use of antithymocytic globulin (ATG) for the management of myelodysplastic syndrome (MDS). This study showed a very poor response to ATG therapy with substantial toxicity in all of the 8 MDS patients and suggests that clinicians be more selective in offering ATG therapy for patients with MDS.

A majority of the studies on immunosuppressive therapy with ATG/antilymphocyte globulin or cyclosporine in MDS have consistently shown a poor response in the subset of MDS patients with refractory anemia with excess blasts (RAEB) and refractory anemia with excess blasts in transformation (RAEBT).2,3,4 Even the study5 that the authors cite as the inspiration for their study on immunosuppressive therapy in MDS patients showed a poor response in the subset of MDS patients with RAEB (minimal response in 2 of 6 patients with RAEB). It is therefore surprising that such a study was undertaken in which the majority of the patients (6 of 8) were with RAEB. This also explains the poor response to ATG therapy. Moreover, use of an immunosuppressive agent in cases of RAEB without any evidence of immune disturbance may not be justified, since in theory the removal of a significant component of immune surveillance by ATG may lead to an accelerated progression to acute myeloid leukemia.

Various immunologic abnormalities are seen in patients with MDS,5,6 but only 10% of these patients have clinical autoimmune disorders such as skin vasculitis, rheumatic disease, autoimmune hemolytic anemia, and others, and a significant number of these patients have abnormal immunologic laboratory tests.5,6 The exact role of these abnormal immune reactions in the etiopathogenesis of MDS is not known, however they may aggravate the cytopenias in these patients. MDS is a heterogeneous group of disorders of hematopoietic stem cells that results in inhibition of normal hematopoiesis and contributes to the development of hematologic malignancies. This development occurs in a stepwise manner beginning with refractory anemia (RA), RAEB, RAEBT, and, finally, full-blown acute leukemia. A study suggested7 that immunologic abnormalities are seen more frequently in patients with RA compared with other subtypes of MDS. Based on these observations, immunosuppressive therapies may be considered rationally in these subsets of MDS patients. It will be interesting to see if a selected subset of MDS patients, who have partially responded to some sort of immunomodulatory therapy such as cyclosporine, danazol, or steroids, or those who have clinical or laboratory evidence of certain autoimmune phenomena, treated with ATG fare well with this therapy. As ATG therapy is neither cheap nor devoid of substantial risk of various complications, this modality of therapy for MDS is unlikely to find widespread use unless the type of study mentioned above for selected subsets with MDS shows substantial benefits.

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References


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