deletions. We would like to point out that in each hybridization we can detect the germline band(s) of expected size, either from the nonmutated tumors in the marrow sample or the nontranslocated allele, serving as an additional internal control. Importantly, alternative enzyme/probe hybridizations were performed in our study according to recombinant bands already demonstrated with the “original” combinations and were used primarily as confirmation of the screening Southern blots. These alternative combinations were useful in eliminating undetected legitimate (especially downstream and inversion) switches, and possible artifacts that can be introduced by restriction fragment length polymorphisms (RFLPs) even in the original screening hybridizations.

The second criticism of Dr Schmidt relates to our analysis of 1 of 4 examples in Figure 2A (Patient 1). We agree that if a single translocation accounts for the 4.9 kilobase (kb) SphI fragments detected by 5'Sa and 3'Sa, and the 5.2 kb BglII-3'Sa fragment, then a BglII recombinant band should also be detected by 5'Sa. However BglII-3'Sa is clearly germline. From the sequence database, we verified for both Sc I and 2 that a BglI site is present 1.3 kb downstream of the 3′ SphI probe (292 base pair upstream of the 3′ SphI site), and there are no other BglI sites in Sa. From our germline hybridization, upstream BglII sites appear to be located at least 12 kb from the 3′ site. Hence the 5.2 kb BglII fragment detected by 3'Sa is most likely to be caused by an extraneous BglII site on a translocated fragment at the 5′ end, replacing the S'Sa probe sequence. In the absence of other BglII sites in Sa, we agree with Dr Schmidt that based on our findings, the SphI-5'Sa and 3'Sa recombinant fragments, although of the same size, must be independent. However this does not reduce the possibility as we had suggested that the 5.2 kb BglII and the 4.9 kb SphI 3′Sa fragments most likely represent the same illegitimate recombination, although this could only be formally proved by cloning. We have repeatedly demonstrated the SphI-5'Sa fragment and the absence of matching nongermline SphI fragments hybridized by 3′Sa and 5′Sa, excluding downstream or inversion switches. We accept the criticism that the HindIII 5′ Sa recombinant fragments cannot be used as confirmation of the HindIII-5′ Sa fragment, as 5′Sa is located upstream of the HindIII site, a fact that we had specified in the legend (Fig 2AI1) but overlooked in the analysis. Nevertheless Dr Schmidt agrees with us that the 2 kb BglII fragments hybridized by both 5′ Sa and 5′ Sa strongly suggest a recombination event downstream of 5′ Sa. From this analysis we believe that our data remain fully consistent with the presence of illegitimate recombinations in this patient, and our conclusions on the relationship with disease behavior are unchanged. Regarding HindIII RFLPs in Sy, we wish to point out that HindIII digests from all patients were hybridized by both 5′ Sy and 3′ Sy, which would have shown if any of the extraneous bands were due to RFLPs. For the 12 kb and 3.5 kb HindIII-3′ Sy bands (Fig 2A), we can confirm that no such bands were detected by 5′ Sy.

As a result, while cloning provides the ultimate proof of the nature of a recombinant fragment in the IgH genes, we believe that our investigation of illegitimate switch recombinations by the adaptation of an established Southern Blot assay was justified, given the paucity of available tumor material from human myeloma bone marrow. Our exhaustive analysis using the original screening blots for detection of possible illegitimate switches and their verification by probes and enzymes that have previously revealed recombinant bands have provided us with useful and reliable information. Since our paper was published more than a year ago, substantial refinements have been made to molecular cytogenetics, which are likely to be quicker, less labor intensive and more accurate in demonstrating chromosome 14q translocations, and would be superior to Southern hybridization for large-scale patient screening. Finally, we would like to re-emphasize that our conclusions regarding illegitimate switch recombinations were made after careful investigation of each nongermline band. We fully agree that detailed analysis is required in the interpretation of the Southern Blot assay and thank Dr Schmidt for his note of caution.

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To the editor:

Myelodysplastic syndromes: from French-American-British to World Health Organization: a commentary

Nösslinger and coworkers1 are to be complimented on a carefully conducted retrospective survival study of 431 patients with primary myelodysplastic syndromes (MDSs) comparing the original French-American-British (FAB) proposals2 and the recently published World Health Organization (WHO) proposal classifications of MDS.3 However, we are concerned about the authors’ interpretation of the WHO criteria and the resulting impact on their survival studies. The critical changes in the WHO classification from the FAB include the following: (1) lowering the blast percentage for the diagnosis of acute myeloid leukemia (AML) to 20% from 30%, thus eliminating refractory anemia with excess blasts in transformation (RAEB-T); (2) moving dyslastic chronic myelomonocytic leukemia (CMMML) into a proposed new category of myeloid disease with features overlapping myelodysplastic syndromes and myeloproliferative disorders; (3) subdividing RAEB into 2 types: RAEB-1 (5%-9% marrow blasts) and RAEB-2 (10%-19% marrow blasts); and (4) separating refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) into 2 broad categories
based on the presence of multilineage (2 or 3 myeloid cell lines) or unilineage (mainly affecting the erythroid series) dysplasia.

Unfortunately the reference quoted for the WHO classification did not provide sufficient information on the precise criteria described in detail in the recently published WHO manual, and, in addition, the authors do not reference the Germing et al paper, which concerns these new proposals. An additional review of the new WHO classification was published before the final criteria of the precise percent of dysplastic cells and the consideration of merging the dysplastic and proliferative forms of CMML were agreed upon.

The major difficulty we have with the Nößlinger et al study is the adoption of the “50%” criteria for dysplasia in 2 or more cell lines for refractory cytopenia with multilineage dysplasia (RCMD). In the WHO proposals the threshold of 50% dysplasia has been utilized only in identifying AML with multilineage dysplasia but not for the MDS category of RCMD. In fact, in the WHO proposals RCMD is defined as an MDS subgroup with fewer than 5% blasts in the bone marrow, and dysplasia in 10% or more of the cells of 2 or more myeloid lineages (erythroid, granulocytic, and/or megakaryocytic). These criteria were adapted in the study of 1600 patients with MDS by Germing et al, although they did elect to use a 40% threshold for megakaryocytes. Germing et al and others have confirmed the worse prognosis of RCMD compared to RA or RARS. To accurately evaluate the WHO proposals it will be necessary to reassess the “unclassified” group in the Nößlinger et al study utilizing these criteria. It is very likely that the “unclassified” category (MDS-U) would diminish considerably, impacting the survival results.

In addition others have demonstrated that the survival of CMML is dependent on the bone marrow blast percentage and that CMML is much more heterogeneous than other subtypes of MDS. In order to emphasize the prognostic importance of the blast percentage in CMML the WHO classification divides CMML into 2 categories, CMML-1 and CMML-2, depending on the blast count in the peripheral blood and the bone marrow. It does not subdivide CMML according to the white blood cell count. In Table 1 of the Nößlinger et al article, CMML resembles RAEB in the International Prognostic Scoring System (IPSS) distribution. The separation of RAEB into 2 types (5%-9% blasts and 10%-19% blasts) is of importance as the authors demonstrate with a significant difference in IPSS distribution. A similar analysis of their patients with CMML should be performed. Confirmation of the similarities in outcome for RAEB-T and AML in the Nößlinger et al study provides further evidence in support of allowing such patients (20%-30% marrow blasts) to enter AML trials where appropriate.

In summary it is our hope that Nößlinger and colleagues will consider reviewing their data using the recently published WHO criteria. Such an effort would be important, because, although the Nößlinger et al study is interesting, it does not justify any statement about the validity of the WHO classification. We anticipate that a new look at the data of Germing et al would confirm the conclusion that the WHO system does provide improved and relevant guidelines for the classification of patients with MDS.

John M. Bennett, Richard D. Brunning, and James V. Wardiman

To the editor:

Mutations of Chk2 in primary hematopoietic neoplasms

Chk2 is a novel checkpoint kinase isolated as a human homologue of yeast Cds1/Rad53. Recent analyses have revealed that it is among key molecules signaling DNA damage via the ATM protein kinase to p53. Of great interest is the report that germ line mutations of the Chk2 gene are found in a fraction of Li-Fraumeni syndrome (LFS), a hereditary cancer-susceptibility syndrome originally linked with germ line p53 mutations, suggesting that Chk2 is a tumor suppressor gene whose functional deficit will lead to development of human cancers. Given that the p53 and ATM genes are inactive in leukemias and lymphomas, it is intriguing to investigate whether or not somatic mutations of Chk2 are also responsible for leukemias and lymphomas.

To address this point, we screened for mutations of Chk2 in a variety of human hematopoietic neoplasms.

A total of 109 tumor specimens of hematopoietic malignant disorders were examined for mutations of Chk2 using reverse transcriptase–polymerase chain reaction/single strand conformational polymorphism (RT-PCR/SSCP) analysis. Numbers and diagnoses of these patients are listed in Table 1. Two samples showed abnormally migrating bands on RT-PCR/SSCP analysis of the Chk2 transcripts (patient 1375 and patient 154), and the nucleotide alterations were further confirmed by sequencing analysis in both cases (Figure 1 and Table 2).

References

Myelodysplastic syndromes: from French-American-British to World Health Organization: a commentary

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