relapsed since the original report. One patient relapsed at 2 years and survives at 8.2 years in remission after a second transplant and another relapsed and died at 3 years. Since the original report, 2 patients in this group have died without relapse from complications of chronic GVHD at 3 and 7 years after transplant.

Figure 1 shows the Kaplan-Meier probabilities of survival (0.51 at 11 years for each regimen, $P = \text{not significant [NS]}$) and the cumulative incidences of relapse. These were 0.39 for the lower and 0.14 for the higher dose regimen ($P = .06$), compared with 0.35 and 0.12 ($P = .06$), respectively, in the original report. Figure 2 shows the Kaplan-Meier probabilities of event-free survival (0.41 and 0.49, respectively; at 11 years for the lower and higher TBI dose; $P = \text{NS}$) and the cumulative incidences of nonrelapse mortality (0.19 for the lower dose of TBI and 0.38 for the higher dose; $P = .05$). Table 1 describes the current status of survivors in each study group.

We believe that publication of the updated outcomes in this study is important. The difference in relapse incidence is unchanged from that originally reported, suggesting that it was not a consequence of delay in relapse events but of more effective elimination of the malignant clone in patients receiving the higher dose of TBI. Also, Fig 2 clearly demonstrates that the increased nonrelapse mortality associated with the higher dose of TBI is limited to the first 6 months after transplant.

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**Table 1. Current Status of Survivors**

<table>
<thead>
<tr>
<th>Study Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.0 Gy TBI (N = 34)</td>
</tr>
<tr>
<td>15.75 Gy TBI (N = 37)</td>
</tr>
<tr>
<td>Alive—No history of relapse</td>
</tr>
<tr>
<td>No history of chronic GVHD</td>
</tr>
<tr>
<td>Karnofsky score = 100%</td>
</tr>
<tr>
<td>Karnofsky score less than 100%</td>
</tr>
<tr>
<td>History of chronic GVHD</td>
</tr>
<tr>
<td>Off all medications. Karnofsky</td>
</tr>
<tr>
<td>Karnofsky score less than 100%</td>
</tr>
<tr>
<td>Alive with history of relapse</td>
</tr>
<tr>
<td>Total no. of survivors</td>
</tr>
</tbody>
</table>

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**REFERENCE**


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The t(8;13) Atypical Myeloproliferative Disorder: Further Analysis of the ZNF198 Gene and Lack of Evidence for Multiple Genes Disrupted on Chromosome 13

To The Editor:

An atypical myeloproliferative disorder has been described that is associated with T-cell leukemia/lymphoma and peripheral blood eosinophilia.1 All these cases are associated with a translocation between 8p11 and 13q11-12 or two rare variant translocations involving 8p11 and 9q32.2-4 or 8p11 and 6q27.5 Recently, we localized the breakpoint on chromosome 8p11 to a region close to the FGFR1 gene.6 Further analysis has shown that the 8p11 translocation breakpoint involved in the t(8;13)(p11;q11-12) and the variant translocations t(8;9)(p11;32) and t(6;8)(q27;p11) disrupts the FGFR1 gene.7 Subsequently, the rearranged gene, named ZNF1988 or RAMP9 on chromosome 13, was cloned by 5' RACE from primers located within the FGFR1 cDNA. In the 5 cases analyzed by these investigators, the translocation break-
points cluster within intron 8 of the FGFR1 gene and result in the fusion of the FGFR1 tyrosine kinase domain to the zinc finger domains of the gene located in 13q11.9. This fusion is thought to result in constitutive activation of the tyrosine kinase domain by oligomerization of the fusion protein. Examination of the cloned ZNF198 and RAMP cDNAs shows a number of discrepancies between the two sequences (Fig 1). The ZNF198 cDNA product is 3,478 bp in length.8 Conceptual translation of the 2,271-bp open reading frame (ORF) predicts a protein of 757 amino acids consisting of a domain containing two zinc fingers of the atypical type Cys-X2-Cys-X9-12-Cys-X7-Cys-X13-17-Cys-X13-17-Cys-X7-Cys, two proline repeats, and a carboxy terminal acidic domain. The RAMP cDNA product derived by Smedley et al12 is 2,553 bp long, with an ORF of 699 amino acids. This predicted protein contains three extra N-terminal zinc fingers, but lacks the acidic activation domain and terminates 48 amino acids after the second proline repeat. Our reconstruction of the ZNF198/RAMP gene has confirmed the presence of six sequencing errors in the RAMP sequence that lead to the introduction of a stop codon, resulting in a truncation of the ORF. The 5′ end of the sequence of the ZNF198 cDNA, described as the 5′ untranslated region, appears to represent an unspliced intron from the ZNF198 gene. These errors have probably occurred during the cloning of the products of the 3′ and 5′ RACE reactions, respectively. A recent report by Popovici et al10 shows that the complete ZNF198/RAMP cDNA sequence, which they have named FIM, is, therefore, 5,008 bp long, which is in agreement with the predicted mRNA transcript size detected by Northern blot analysis.8,10 Thus, the gene encodes a protein of 1379 amino acids consisting of an N-terminal domain containing five zinc fingers, two proline repeats, and a carboxy terminal acidic activation domain (Fig 1). The translocation, therefore, fuses the N-terminal zinc finger domain to the FGFR1 tyrosine kinase domain, resulting in a protein of 1309 amino acids with a predicted molecular mass of 146 kD.

Fluorescence in situ hybridization (FISH) analysis of metaphase spreads from patients carrying the t(8;13) translocation with YACs from different regions of chromosome 13 suggests that multiple, distinct breakpoints are involved in the t(8;13) translocation.11,12 We have recently shown that YAC 943E4, which is used for FISH analysis of the 13q11 breakpoint in three cases of this leukemia, contains DNA from both the telomeric region of the 13q11-q12 YAC contig13 and the pericentromeric region close to D13S183E (Still et al, manuscript in preparation). This results in the detection of signal on both derivative chromosomes, when this YAC is used. Interestingly the patient described by Smedley et al12 was determined to have a breakpoint in 13q12, defined by markers D13S267 and D13S220. The breakpoint in this patient was localized by these investigators within the ZNF198/RAMP FIM gene.9, which is located on the 967B1 and 911H8 YACs located in 13q1113 (Still et al, manuscript in preparation), suggesting that this patient may carry a complex translocation. To determine whether the breakpoints in two recently identified patients, RB and SR,5,14 are similar to those previously described, we have performed reverse transcription-polymerase chain reaction (RT-PCR) using RNA isolated from somatic cell hybrid RBF12 and a bone marrow aspirate sample from patient SR.15 As shown in Fig 2, identical PCR products are obtained when primers are used to detect the ZNF198/FGFR1 fusion cDNA in RBF1 (lane 1) and SR (lane 2). This demonstrates that the breakpoints occur in the same introns of the FGFR1 gene and of the ZNF198 gene, as already described by Xiao et al,8 and therefore the fusion cDNA generated in patient RB and SR are identical to the 7 other patients already characterized.5-10 Interestingly, we were also able to detect the reciprocal FGFR1/ZNF198 fusion product in patient SR, albeit at a lower level than the ZNF198/FGFR1 product (Fig 2, lane 5). This demonstrates that all 9 cases molecularly characterized to date generate the same ZNF198/FGFR1 fusion protein and that there is only a single gene in 13q11 disrupted by the t(8;13) translocation.
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Single-Tube Polymerase Chain Reaction for Rapid Diagnosis of the Inversion Hotspot of Mutation in Hemophilia A

To The Editor:

Hemophilia A is the most common X-linked coagulation disorder, with an incidence of about one in 5,000 males. About half the families with severe disease have a large genomic inversion of the factor VIII gene that separates the first 22 exons from the final 4 exons. This inversion results from a hotspot of recombination between a 9.5-kb gene that separates the first 22 exons from the final 4 exons. To design PCR primers, additional sequences flanking the inversion hotspot were required. Fifty basepairs of available sequence flanking the extragenic homolog were required. Female carriers produce PQ, PB AQ, and AB (10 kb) are produced in hemizygous wild-type males. Males with hemophilia due to the inversion produce PB (11 kb) and AQ segments (11 kb) along with the 10-kb AB segment from the nonrecombined extragenic homolog. Female carriers produce PQ, PB + AQ, and AB segments. In all cases, an AB segment serves as an internal control because at least one copy of int22h2 or int22h3 remains intact. The three segment sizes are readily separated on a 0.6% agarose gel. High yield and reproducible amplification depended on three unusual modifications to standard long-distance PCR protocols: (1) high concentrations of dimethylsulfoxide (DMSO) additive; (2) substantially increased amounts of Taq and Pwo DNA polymerases; and (3) 50% deaza-dGTP.

To design PCR primers, additional sequences flanking the int22h homologs were required. Fifty basepairs of available sequence flanking the S’ end of int22h1 was extended by sequencing a 6-kb PCR fragment that spans the distance between exon 22 and the S’ end of int22h1. Additional sequences flanking int22h2 and int22h3 were obtained by a method of inverse PCR.5 A blinded analysis was performed with 40 blood samples provided by hemophilia centers in the United States. Genomic DNA was isolated from whole blood by phenol extraction.5 Thirteen samples were hemizygous for the inversion, 6 carried the inversion, and 21 were wild-type, as determined by standard Southern blot analysis. The PCR analysis was performed successfully in less than 1 day, with complete concordance with the Southern blot results. The PCR was successful
The t(8;13) Atypical Myeloproliferative Disorder: Further Analysis of the ZNF198 Gene and Lack of Evidence for Multiple Genes Disrupted on Chromosome 13

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