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Response

Free light chain assay and stringent complete remission in multiple myeloma: more questions than answers

We appreciated the comments by Kröger et al on our article and we have read with interest their experience with free light chain (FLC) measurements in 52 patients with multiple myeloma (MM) in complete remission (CR) after allogeneic stem cell transplantation (Allo-SCT; n = 45), autologous stem cell transplantation (ASCT; n = 3) or conventional bortezomib- or lenalidomide-based therapy (n = 4). The high rate of stringent CR (sCR), with 51 of 52 (98%) of the patients achieving a normal FLC κ/λ ratio, is surprising. Moreover, none of their 13 patients with oligoclonal bands had an abnormal FLC κ/λ ratio, which contrasts with our results.¹

The differences between the 2 studies may be explained by the nature of the treatments and the timing of light chain measurement. In the series by Kröger et al, 87% of the patients had achieved CR after Allo-SCT, whereas in our study 76.5% were in CR after ASCT. It is very likely that the immune reconstitution could be different and more delayed after the allogeneic procedure. Of note, in Kröger's study, the free light chains were measured in patients who had been in stable CR for a minimum of only 3 months and at least 6 weeks from the first negative immunofixation electrophoresis. On the contrary, in our series, median duration of the CR was 5 years (range, 1-23.2 years), and median time of oligoclonal band appearance after allogeneic or autologous SCT was 6 months (range, 2-53 months). It is conceivable, therefore, that in those patients with long-lasting responses after high-dose therapy/SCT the robust immune reconstitution would result in the presence of oligoclonal bands and an abnormal FLC ratio, due to a kappa light chain overproduction. In fact, there is a general thought that these oligoclonal bands are transient.² However, in our 14 patients the duration of oligoclonal bands ranged from 0.7 to 9.4 years and persisted in all of them except in the only one who relapsed, in keeping with a recent report by Mark et al.³

There are still many important unsolved issues concerning FLC measurements and sCR in MM. (1) At what time could the FLC assay be more informative (early or after a certain duration of the CR)? (2) Does the frequency of sCR vary depending on the type of treatment? (3) Is the meaning of sCR different after conventional chemotherapy, ASCT, or Allo-SCT? (4) What is the significance of an abnormal FLC ratio in patients with oligoclonal bands? (5) Is sCR of real prognostic value and, if so, in what population of patients?

Finally, we agree completely with Kröger et al that, with the current therapeutic approaches for MM as well as the availability of novel laboratory technologies, the achievement of serologic CR should no longer be the ultimate endpoint. The recent results on the prognostic impact of minimal residual disease studies by multiparameter flow cytometry⁴ and by molecular quantitative reverse transcription-polymerase chain reaction⁵ are important steps for-

ward that can be crucial to determining for how long beyond serologic CR additional treatment is needed.

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To the editor:

Role of GATA-1s in early hematopoiesis and differences between alternative splicing in human and murine GATA-1

We read with interest the description by Hoeller et al of an exon 2 GATA-1 mutation leading to severe transient myeloproliferative disease (TMD),¹ in which the authors speculated that the severe phenotype might reflect loss of both full-length (FL) GATA-1 and the shorter isoform, GATA-1s, due to the position of the mutation in codon 2. Although almost all GATA-1 mutations in children with Down syndrome are in exon 2²⁻⁴ and lead to loss of GATA-1FL, exon 2 mutations are also predicted to leave GATA-1s protein production unaffected,⁴ by translation of an alternatively spliced mRNA comprising exons 1/3/4/5/6.² In humans, alternative splicing producing an exon 1/2/3/4/5/6 mRNA for GATA-1FL and exon 1/3/4/5/6 splice variant for GATA-1s has been demonstrated in adult bone marrow CD34⁺ cells.² Using exon 1 and 3 primers, we have also found that both variants are consistently expressed in all normal cord blood and second-trimester fetal liver and bone marrow cells we have tested (n = 12; Figure 1A). It seems likely, therefore, that exon 2 mutations would still allow expression of GATA-1s mRNA, which may be difficult to detect at the protein level due to the relative insensitivity of commercial GATA-1 antibodies in immunohistochemical reactions. Interestingly, and by contrast, although mice produce the 2 Gata-1 isoforms by alterna-

tive translation of a single mRNA,⁵ Gata-1s transcripts have not been reported in murine tissues and, in our experience, exon 1 and 3 primers consistently amplify only the FL transcript in mice (Figure 1B) despite evidence of Gata-1s protein production (Figure 1C). Further investigation of the functional consequences of different GATA-1 mutations may shed further light on the enigmatic role of GATA-1s in early hematopoiesis and differences between alternative splicing in human and murine GATA-1.

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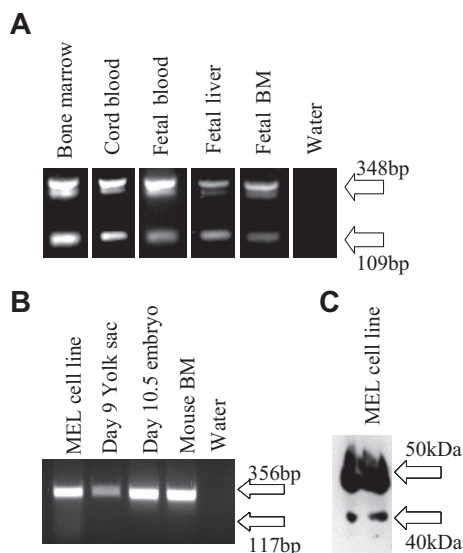


Figure 1. GATA-1 mRNA alternative splicing in human and murine tissues. (A) Reverse transcription–polymerase chain reaction (RT-PCR) of cDNA from human bone marrow, cord blood, and second-trimester (15 weeks) fetal blood, liver, and bone marrow, the expected size of the exon 1/2/3 splice variant (348 bp) and an exon 1/3 splice variant (109 bp) are marked. (B) RT-PCR of cDNA from murine hematopoietic tissues and the erythroleukemia cell line MEL. The expected size of an exon 1/2/3 splice variant (356 bp) and an exon 1/3 splice variant (117 bp) are marked. (C) Western blot of a MEL nuclear extract using M20 GATA-1 antibody (Santa Cruz Biotechnology), the predicted size of GATA-1FL is 47 kDa and GATA-1s 40 kDa.

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Contribution: C.H. performed the research and wrote the paper; O.T. performed the research and analyzed the data; and B.G., I.R., and G.G. designed the research and analyzed the data.

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