Circulating CD19+ Blood Cell Levels in Myeloma

To the Editor:

Cells of B-cell lineage that represent a component of the malignant population in patients with myeloma have been detected through the analysis of several features, including gene rearrangement patterns for both heavy and light chain and the DNA content of these blood B cells.1,4 The nature of these circulating B cells and their ability to repopulate or seed distant sites with clonal cells has important clinical relevance. A recent report in Blood by Bersagel et al.7 has provided additional information about both the qualitative and quantitative aspects of the circulating CD19+ B cells in myeloma. Their analysis reaffirmed that circulating CD19+ B cells have clonotypic rearrangement patterns similar to the marrow plasma cells. Importantly, they also noted a major increase in the numbers of circulating CD19+ B cells of myeloma patients. Because the quantitative level of malignant B cells may be a critical component of the clinical status of each patient, we report the CD19 levels seen in a large patient cohort (N = 534) entered on the ECOG trial 9486.

In this ECOG phase 3 trial, previously untreated patients entered on protocol were required to have peripheral blood submitted for blood lymphocyte phenotype analysis immediately before the initiation of therapy. We were thus able to generate baseline blood lymphocyte data on the numbers of T cells, B cells, and natural killer cells on these patients at diagnosis.

In brief, the anticoagulated blood specimens were subjected to ficoll-hypaque centrifugation without monocyte depletion and then incubated with appropriate concentrations of anti-CD19 reagent (Becton Dickinson, San Jose, CA). Fluorescent levels of CD19 binding was detected by analysis of at least 10,000 cells using multiparameter flow cytometry (FACStar system) and subsequent data analysis through a Simulset version 2.3.3 and Lysis version 1.0.2 consort (Becton Dickinson). Blood levels of CD19+ cells were then calculated using the percentage of CD19+ cells multiplied by the total white blood cell count multiplied by the percentage of lymphocytes. Table 1 summarizes our data on the patient cohort in comparison with an age-matched, control population (N = 24).

Our data indicate that, at diagnosis, there is a marked heterogeneity in levels of CD19+ B cells in myeloma patients. Notably, there is a wide range in circulating CD19+ cells compared with normal controls. Similar to the results reported by Bersagel et al.,7 we confirm that there are some myeloma patients with significantly increased levels of circulating CD19+ cells. Specifically, there are 21% or 112 of these patients with absolute CD19+ cells greater than 2 standard deviations above the control mean. We also note that there are myeloma patients with very low levels of circulating CD19+ B cells when compared with normal controls. In contrast to Bersagel et al.,7 who reported a mean circulating CD19+ value nearly 5 times normal controls, we find that the mean of the 534 patients we analyzed is not significantly different from our normal control values (Table 1). Certainly, the nature of circulating B cells in myeloma is complex. We suggest that there is considerable heterogeneity in the levels of circulating CD19+ B cells and propose that future analysis of myeloma patients, especially in the context of large clinical trials, examine the nature and quantity of putative circulating clonal B cells. This is the subject of ongoing investigations of the ECOG myeloma clinical trials laboratory study group.

Table 1. Circulating CD19+ B Cells in Myeloma

<table>
<thead>
<tr>
<th>Patients at Diagnosis</th>
<th>Myeloma (n = 534)</th>
<th>Controls (n = 24)</th>
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<tbody>
<tr>
<td></td>
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<td>75th percentile</td>
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REFERENCES
The report by Kay et al indicates considerable heterogeneity in the proportion and absolute numbers of circulating CD19+ B lymphocytes in myeloma patients. This confirms our own work showing a broad range of circulating B-cell numbers in myeloma blood. However, Kay et al report mean values of B cells that are approximately half of those found in our study. We suggest that this reflects their use of the monoclonal antibody (MoAb) Leu12 (CD19) as their detecting MoAb. In our report, we emphasized that, in our hands, Leu 12 (Becton Dickinson, San Jose, CA) did not reliably detect CD19+ B cells in myeloma blood and that the use of either B4 (Coulter, Hialeah, FL) or FMC63 (Serotec Canada, Ltd, Toronto, Canada) was essential. Our results with Leu12 were in fact comparable to those of Kay et al, which led us to use only B4 or FMC63 to detect circulating B cells in myeloma. In addition, reliable detection of CD19 B cells was optimized when the detecting conjugate was CD19-fluorescein isothiocyanate (FITC) rather than CD19-phycoerythrin. Using either B4 or FMC63 MoAb (with no gates on forward or side scatter) in untreated myeloma patients, we find 28% CD19+ B cells or $440 \times 10^9$/L blood. We found considerable heterogeneity, with values ranging from 1% to 3% up to occasional values as high as 80% of mononuclear cells; but, as we reported, even for those patients with very low numbers, the circulating B cells had a highly abnormal phenotype and included cells with clonotypic IgH CDR3 rearrangements and DNA aneuploidy.

As we have suggested in the past and as Kay et al suggest in their letter, it would be valuable to include a quantitative and qualitative analysis of circulating B cells in new clinical trials. However, we would emphasize that, for optimum results, such studies should utilise either B4-FITC or FMC63-FITC at an optimized concentration for detection of CD19 expression by circulating lymphocytes in myeloma.

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Circulating CD19+ blood cell levels in myeloma. ECOG Myeloma Clinical Trials Laboratory Study Group [letter]

NE Kay, MM Oken, N Bone, RA Kyle, P Greipp, B Van Ness and T Leong