the error frequency rate for each reference laboratory because of the limited sample size; but, of the five incorrect diagnoses, they occurred from three of the four labs that were used, as shown in Table 1, item 2.

The purpose of this letter is to sound an alarm! In the past, we had quick turnaround and an opportunity to review slides in the presence of our pathologists. Patients and family received reports quickly and we were able to react promptly with treatment decisions. Now, there is a considerable delay and many times we do not have the chance to review slides. Errors are being made when pathologists lack clinical input.

To the Editor:

Although massive doses of oral cobalamin seem to work well in a research setting with compliant, motivated patients, the study by Kuzminski et al1 is misleading in important ways. The question surrounding oral therapy has always concerned its effectiveness in patients with defective absorption. Unfortunately, that is not who was tested by the investigators. Almost certainly, 12 of their 18 patients treated orally had normal absorption of cobalamin from pills. Patients with dietary cobalamin deficiency, atrophic gastritis, or acid-suppressive therapy have inadequate intrinsic factor-mediated absorption, with the latter two unable to absorb only food-bound cobalamin. All 12 patients, and certainly those with a presumed dietary basis for deficiency, would probably have responded even to 5 µg pills and, thus, provide no useful information.

Moreover, the higher serum cobalamin levels and lower homocysteine and methylmalonic acid levels at the end of 4 months in the orally treated patients seem explainable more by the dose schedule than by the route. The response curves for the first month of therapy, when injections were administered weekly or more often, showed equal or better response. The response curves for the first month of therapy, when injections were administered weekly or more often, show equal or better response in the patients treated by injection. Only later, when injection frequency was reduced to once monthly, did the response to oral treatment seem to overtake that of parenteral treatment.

In fact, the 4-month serum values at the end of the study are themselves misleading. By then, the parenterally treated patients had been left untreated for 30 days (the last day of injection was day 90), whereas the orally treated patients continued taking their pills up to the day of final testing.

The study only proved that patients, most of whom had unimpeded absorption of cobalamin in pill form, will respond to large doses taken daily. Great caution must be exercised in extrapolating this to patients with defective intrinsic factor-mediated absorption, and especially so in the real world, where life-long compliant ingestion of huge doses every day is unlikely.

Ralph Carmel
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REFERENCE

Response

Dr Carmel fails to appreciate the well-established fact, which is discussed and referenced extensively in our report1 and elsewhere,2 that oral cobalamin at a dose of ≥1 mg/d is effective therapy for all causes of cobalamin deficiency, because approximately 1% of oral cobalamin is absorbed by diffusion regardless of the functional status of the gastrointestinal tract. For example, in a comprehensive Swedish study3 published 30 years ago, 64 patients with well-documented cobalamin deficiency (including 55 with lack of intrinsic factor due to pernicious anemia, 4 with ileal resection, and 1 with total gastrectomy) all had sustained neurologic and hematologic remissions when treated with oral cobalamin at 1 mg/d and followed for up to 5 years (61 of 64 for ≥3 years). In addition, all 64 patients maintained normal serum cobalamin levels. Interestingly, studies of this kind have not been performed for various parenteral regimens, although we are not aware of evidence that incomplete neurologic and hematologic remissions occur with the most commonly used parenteral regimen, in which 1 mg of cobalamin is administered intramuscularly on a monthly basis.

Our study was also performed in patients with well-documented cobalamin deficiency4 and is the first randomized, controlled trial of oral cobalamin (2 mg/d) versus parenteral cobalamin (1 mg administered intramuscularly on 7 occasions in the first month and once monthly thereafter). As expected, excellent and indistinguishable neurologic and hematologic responses were observed in both groups and all (18/18) evaluable patients in the oral group had normal serum cobalamin levels at 1, 2, and 4 months. Serum cobalamin levels were normal in all (15/15) evaluable patients in the parenteral group at 1 month, but 3 of 15 and 4 of 14 evaluable patients had low values at 2 and 4 months, respectively. Elevated levels of serum methylmalonic acid and total homocysteine, which are the most sensitive indicators of cobalamin status,4,5 decreased markedly in both groups, and the mean values were not significantly different at 1 month. However, at 4 months, the mean values for serum methylmalonic acid and homocysteine were lower in the oral group, and the difference was statistically significant for methylmalonic acid. These differences may become even greater at treatment times greater than 4 months (both regimens must be continued for life in almost every patient) and may be very important clinically, because serum total homocysteine is an independent graded risk factor without threshold for all forms of vascular disease6 and appears to be a particularly strong predictor of cardiovascular mortality.7 Metabolic control could be improved in the maintenance portion of the parenteral regimen by administering weekly or biweekly injections, but this would be burdensome.

Dr Carmel also fails to appreciate the fact, which is also discussed and referenced extensively in our report1 and elsewhere,2 that compliance is a comparable problem for both oral and parenteral cobalamin regimens, because most cobalamin injections today are administered in
nonmedical settings. Compliance is easily assessed in patients receiving oral therapy, because compliant patients will have normal serum cobalamin levels. This is not true with respect to the parenteral regimen, where approximately one quarter or more of compliant patients will still have low serum cobalamin levels.

Dr Carmel refers to 2 mg of cobalamin as a “large,” “huge,” and “massive” amount, but it is important to note that 2 mg of cobalamin readily fits into a single small tablet or capsule. This results in a simple and convenient treatment regimen that is also inexpensive, free of side effects, and cost effective.

In summary, we believe that a number of studies, including ours, have demonstrated that oral cobalamin therapy is safe and effective for all causes of cobalamin deficiency and is the treatment of choice for most patients. Oral cobalamin therapy has been used commonly and successfully in Sweden for many years, and its use is already increasing in the United States.

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REFERENCES


Expression of p53, Bcl-2, and Bax in CD34+ Cells Recovering After Chemotherapy

To the Editor:

We read with interest the report of Wlodarski et al published in a recent issue of Blood concerning the role of p53 in the recovery of hematopoiesis after cytotoxic treatment. The investigators used as an experimental model p53 wild-type and p53 knock-out mice treated with 5-fluorouracil (5-FU). They showed that bone marrow (BM) mononuclear cells recovering in p53 knock-out mice contain a higher number of both primitive and multipotential progenitors than BM cells recovering in p53 wild-type mice. Moreover, p53 knock-out progenitors are responsible for the long-term engraftment of hematopoiesis when transplanted in lethally irradiated recipients. Thus, these data undoubtedly indicate that p53 is involved in the induction of apoptosis of hematopoietic progenitors previously exposed to DNA-damaging drugs.

To our knowledge, no data have yet been reported about the modulation of the tumor-suppressor gene p53 or of its target genes in human hematopoietic cells after in vivo chemotherapy administration. To this purpose, we used flow cytometry to evaluate the expression of p53 and of two apoptosis-related genes, bcl-2 and bax, in CD34+ cells recovering after chemotherapy. We evaluated 5 patients affected by non-Hodgkin’s lymphomas, candidates for peripheral blood (PB) stem cell collection and transplantation. All patients received cyclophosphamide (7 g/m2) and, from day 2 after chemotherapy, granulocyte-colony-stimulating factor (G-CSF; 5 μg/kg). In all patients, BM and PB samples were collected on day 12 after chemotherapy. Data obtained in patients recovering from chemotherapy were then compared with those obtained in CD34+ cells isolated from normal BM and PB. All of the samples were collected after informed consent was obtained.

CD34+ cells were isolated from mononuclear cell fractions by the miniMACS starting kit and the CD34 multiset kit (Miltenyi Biotec Inc, Auburn, CA), according to the manufacturer. The purity of immunoselected CD34+ cells was evaluated as previously reported, and in all cases exceeded 90%. For the measurement of intracellular antigens, immunoselected CD34+ cells were fixed and permeabilized by the Ortho PermeaFix TM solution (Ortho Diagnostic System, Raritan, NJ); cells were then incubated with the following unconjugated monoclonal antibodies (MoAbs): anti–wild-type p53 (Ab1 clone; Calbiochem, Cambridge, MA) and anti–Bcl-2 (Ab2 clone; Calbiochem) or with the anti–Bax rabbit polyclonal antibody (P-19 clone; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour at 4°C. Control samples were incubated in the same experimental conditions with isotype-matched irrelevant unconjugated MoAb or with rabbit preimmune serum. After washing, cells were further incubated with fluorescein isothiocyanate (FITC)-conjugated goat antimouse MoAb (Ortho) or with FITC-conjugated goat antirabbit IgG (Dako A/S, Glostrup, Denmark) for 1 hour at 4°C, at 1:20 and 1:1,000 final dilution, respectively. All samples were analyzed with a FACSscan flow cytometer (Becton Dickinson, Mountain View, CA) equipped with an argon laser emitting at 488 nm. Results were expressed as the mean fluorescence intensity ratio (MFI-R), which is calculated as the ratio between the MFI of test curve and the MFI of control curve.

In addition, CD34+ cell samples were evaluated for the content of apoptotic cells by in situ end-labeling of DNA strand breaks with the TdT-mediated biotin-nick-end-labeling (TUNEL), as previously described. The slides were counterstained with hematoxylin and 100 consecutive cells in 3 or more fields were counted. Three different experiments were performed. The statistical analysis of data obtained was performed using the Mann Whitney test for unpaired data.

Results of flow cytometry analysis are shown in Fig 1. It emerged that p53, undetectable in normal BM and PB CD34+ cells, was expressed in BM but not in PB progenitors recovering after chemotherapy. Moreover, in BM progenitors, the induction of p53 was accompanied by a significant reduction of the expression of Bcl-2 and by the appearance of Bax. On the contrary, in PB CD34+ cells recovering after chemotherapy, no differences in the protein levels of Bcl-2 and Bax were detected when compared with normal PB CD34+ cells. In addition, in normal PB CD34+ progenitors, Bax was expressed at a higher level than in normal BM CD34+ progenitors, but its expression did not further increase after
Oral Cobalamin Therapy in Patients Who Absorb It Normally

Ralph Carmel