High-Dose Therapy With Autologous Bone Marrow Support as Consolidation of Remission in Follicular Lymphoma: Long-Term Clinical and Molecular Follow-Up


Purpose: To evaluate the long-term results of high-dose therapy (HDT) in follicular lymphoma, with specific emphasis on the prognostic significance of polymerase chain reaction (PCR)–detectable Bcl-2/IgH rearrangements.

Patients and Methods: Between June 1985 and October 1995, 99 patients with follicular lymphoma received HDT as consolidation of second or subsequent remission. Bone marrow was treated in vitro with anti-B-cell antibodies and complement.

Results: Sixty-five patients remained alive, 49 treatment-failure free, with a median follow-up of 5.5 years (range, 1.5 to 12.5 years). Four “early” and 10 “late” deaths occurred from treatment-related causes; seven of the latter were due to secondary myelodysplasia (s-MDS) or secondary acute myeloblastic leukemia. Overall, 12 (12%) of the 99 patients developed s-MDS or acute myeloblastic leukemia. Kaplan-Meier estimates of freedom from recurrence (FFR) and survival rates at 5 years were 63% (95% confidence interval [CI], 52% to 72%) and 69% (95% CI, 58% to 78%), respectively. For all 99 patients, in multivariate analysis, absence of the Bcl-2/IgH rearrangement at the time of diagnosis (hazards ratio [HR], 0.39; P = .04) and three or fewer treatment episodes before HDT (HR, 0.03; P = .001) were significant prognostic factors for improved survival. For patients bearing Bcl-2/IgH rearrangements, in univariate and multivariate analyses, absence of a PCR-detectable Bcl-2/IgH rearrangement during follow-up was associated with a significantly lower risk of recurrence (adjusted HR, 0.13; P < .001) and death (HR, 0.25; P = .02), whereas the PCR status of the reinfused bone marrow did not correlate with outcome.

Conclusion: Prolonged FFR can be achieved in patients with follicular lymphoma after HDT, but as yet there is no survival advantage compared with conventional treatment. These results confirm that elimination of cells bearing the Bcl-2/IgH rearrangement is highly desirable and should be attempted. The incidence of s-MDS is of increasing concern in this setting.


HIGH-DOSE THERAPY (HDT) with autologous hematopoietic progenitor-cell support has been widely adopted as a potentially curative modality for patients with recurrent aggressive non-Hodgkin’s lymphoma. However, its use in follicular lymphoma has been viewed with caution and remains experimental. This reluctance can be mainly attributed to the relatively long natural history of the disease and the inability of conventional intensive treatment to alter its natural course any more than less intensive treatment. HDT with autologous hematopoietic progenitor-cell support has been evaluated in patients with follicular lymphoma, especially those with recurrent disease.

Comparison with a historical control group treated at St. Bartholomew’s Hospital (SBH) strongly suggests that this treatment prolongs remission duration.

A major concern with autologous hematopoietic progenitor-cell support in follicular lymphoma is the risk of reinfusing occult tumor cells present in the collection. Such cells may contribute to recurrence. The observation that the t(14;18) translocation occurs in the majority of cases of follicular lymphoma, along with the applicability of the polymerase chain reaction (PCR), has introduced the concept of molecular remission. Thus, the presence or absence of cells bearing the Bcl-2/IgH rearrangement at the molecular level may serve as a surrogate marker of disease activity and hence be of prognostic significance in this disease.

In view of the propensity of follicular lymphoma to involve the bone marrow, the stem-cell collection has been manipulated in vitro to reduce the risk of reinfusing lymphoma cells. The use of anti-B-cell antibodies and complement lysis at SBH followed the original observation that this was feasible at the Dana-Farber Cancer Institute (DFCI), Boston. Subsequent data, derived from treatment of a
patient population similar to our study population, showed that at the DFCI, the procedure was effective in “purging” the bone marrow collection in 50% of patients, using a nested PCR technique for detecting cells bearing the Bcl-2/IgH rearrangement. This conferred an advantage in terms of freedom from recurrence (FFR). In addition, it was found that persistence of a PCR-negative state during follow-up after HDT correlated with prolonged remission. The same strategy has been followed at SBH, although, until recently, with less success. Johnson et al. and Pappa et al. were unable, first with single and later with multiple antibodies and complement, to perform the purging procedure as effectively and hence were unable to show its benefit, the overall FFR pattern being identical to that at the DFCI. Close scrutiny of the PCR methods suggested that the discrepancy probably lay in a difference in the sensitivity of the techniques being used. In light of the apparent clinical relevance of the test as used in Boston, the technique was altered at SBH.

This analysis, involving patients with a minimum follow-up twice that of the median duration of second remission of follicular lymphoma when treated conventionally, was therefore conducted to confirm the clinical outcome of patients treated with cyclophosphamide and total-body irradiation (TBI) with autologous bone marrow support as consolidation of second or subsequent remission. Particular emphasis was placed on documenting the efficacy of in vitro treatment of the collected bone marrow using PCR and on demonstrating the relevance of molecular remission during follow-up. Data are presented on 99 patients, including the original 64 previously reported.

**PATIENTS AND METHODS**

**Patients**

The study population consisted of 99 patients with follicular lymphoma (median age, 45 years) in second or subsequent complete remission (CR) or good partial remission (GPR) at the time of HDT, treated at SBH between June 1985 and October 1995. The diagnosis was made according to the Kiel classification, and disease was staged using the Ann Arbor Conference classification. Eligibility criteria for HDT have been previously reported. Clinical characteristics at the time of HDT are listed in Table 1. Sixty-seven patients were in second remission, whereas 32 were in third or subsequent remission. Thirty-eight patients were in CR and 61 patients in GPR (13 of the latter with residual morphologically detectable disease in the bone marrow).

**Historical Control Group**

For purposes of comparison with patients who received high-dose treatment in second remission, a historical control group was created, consisting of patients less than 60 years of age with follicular lymphoma (and no history of transformation) treated at SBH before the introduction of HDT and who received chlorambucil therapy or cyclophosphamide, vincristine, and prednisolone therapy as first-line treatment and at the time of first recurrence.

**Bone Marrow Collection and In Vitro Treatment**

At least 1 L of bone marrow was obtained under general anesthesia. In the first 74 patients, the mononuclear cell (MNC) fraction was treated with anti-CD20 monoclonal antibody (mAb) (provided by Coulter Immunology, Hialeah, FL) and baby rabbit complement (Pel-Freez Clinical Systems, Brown Deer, WI), as previously described. In the remaining 25 patients, the MNC fraction was treated before cryopreservation with four anti–B-cell mAbs (anti-CD10, anti-CD19, anti-CD20, and anti-B5) and baby rabbit complement. The MNC fraction was incubated with saturating concentrations of the four mAbs at room temperature for 30 minutes and then incubated with complement at 37°C for 30 minutes. The cells were then pelleted by centrifugation and washed three times, and the whole procedure was repeated twice. Finally, the cells were resuspended in autologous serum and cryopreserved in dimethyl sulfoxide (Sigma, St Louis, MO).

**HDT and Follow-Up**

HDT comprised cyclophosphamide 60 mg/kg × 2 days and fractionated TBI 2 Gy bid × 3 days. Within 24 hours of the last dose of radiation, the bone marrow was thawed and reinfused, as previously described. After discharge, patients were seen in regular follow-up, monthly for the first 3 months and quarterly thereafter. Surveillance computed tomographic (CT) scans were performed annually, whereas unilateral bone marrow aspirates and trephine biopsies were performed 3 months from the date of reinfusion, at 1 year, and annually thereafter.

**Patient Material**

Lymph node biopsy specimens or bone marrow samples with morphologically detectable infiltration were used to determine which patients had PCR-amplifiable Bcl-2/IgH rearrangements before HDT. Aliquots of at least 10^6 cells were taken from the collection before and after in vitro treatment, frozen, and stored until required for PCR analysis. For the follow-up studies, annual surveillance bone marrow
samples were stored, as well as lymph node and/or bone marrow obtained at the time of overt recurrence.

**PCR Methods**

All follow-up PCR analyses were performed on bone marrow samples, which permit better detection, compared with peripheral blood, of residual Bcl-2/IgH rearrangement–bearing cells at the molecular level.23 Genomic DNA was extracted according to established procedures, and the risk of cross-contamination of PCR reactions was minimized using standard precautions. Initially, baseline lymph node or bone marrow specimens were amplified and sequenced before analysis of follow-up samples from the same patient. For each sample, three separate nested oligonucleotide amplifications were performed at both the major break-point region (mbr) and minor cluster region of the Bcl-2/IgH fusion gene. To increase the efficiency of the amplification, apart from using a JH consensus primer, primers complementary to regions 3’ to the JH4, JH5, and JH6 exons was also included in every PCR assay, as previously described.16

Once the patient-specific break point had been determined, the appropriate primer was used for the analysis of subsequent serial samples. For samples analyzed before June 1993, a “touchdown” nested PCR technique was used,16 to analyze up to 6 μg of DNA, with a sensitivity of approximately 1 in 10⁶. In all subsequent samples, a modified two-step nested PCR technique was used, to analyze up to 1.5 μg of DNA, with a sensitivity of between 1 in 5 × 10⁶ and 1 in 5 × 10⁵, as determined from a dilution of the mbr control cell line DOHH2 in normal lymphocytes obtained from the peripheral blood of healthy volunteers. The DHL-16 cell line (kindly provided by J.G. Gribben, DFCI) was used as a positive control for the minor cluster region. A negative control with normal DNA and a reagent control were also included in every PCR assay to check reliability. In brief, 500 ng of DNA was amplified in a total volume of 50 μL of PCR reaction mixture consisting of 1 × Taq buffer (50 mmol/L KCl, 10 mmol/L Tris-HCI pH 9, and 0.1% [wt/vol] gelatin), 2.5 mmol/L MgCl₂, 1 μmol/L of each primer, 0.2 mmol/L diethylnitrophenyl thiophosphate, and 1 unit of Taq polymerase. The amplification procedure was as follows: after an initial 10-minute denaturation period, 40 cycles were performed with denaturation at 95°C for 45 seconds, annealing at 56°C for 25 seconds, and extension at 72°C for 30 seconds. A 5-μL aliquot of the reaction was then reamplified for 30 cycles (with the same cycling conditions) using internal primers. Finally, 7.5 μL of the PCR product was subjected to electrophoresis in a 2% agarose gel and visualized by ethidium bromide staining and ultraviolet illumination.

Sequencing of PCR products obtained up to June 1993 was performed as previously described.16 For all subsequent products that were sequenced, purification was performed using the GeneClean II Kit (BIO 101, Vista, CA) and the MERmaid Kit (BIO 101) according to manufacturer instructions. DNA was directly sequenced with a primer internal to the inner mbr 5’ primer using the ABI PRISM DyeDeoxy Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA) on an ABI DNA Sequencer 373A (PE Applied Biosystems). Data editing was performed using DNAstar (Madison, WI) software.

**Definitions**

Survival time was considered the time between reinfusion of bone marrow and death.

A patient was considered to be in CR before HDT if he or she was in normal health, with no abnormal physical findings; normal CT scans of the chest, abdomen, and pelvis; and no morphologic nor phenotypic evidence of infiltration in bilateral iliac crest bone marrow aspirates and biopsy specimens.

<table>
<thead>
<tr>
<th>Table 2. Patient Outcome</th>
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<tr>
<td>Status</td>
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<tr>
<td>Alive</td>
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<tr>
<td>In remission</td>
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<tr>
<td>With disease</td>
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<tr>
<td>Dead</td>
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<tr>
<td>&quot;Early&quot; treatment-related death</td>
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<td>&quot;Late&quot; death</td>
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<td>Death from lymphoma</td>
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*Seven from s-MDS (three with disease), two from secondary cancer, one from suicide, and one from pulmonary fibrosis.

A patient was considered to have achieved GPR if he or she was in normal health, with clinical or radiologic evidence of residual lymphadenopathy (lymph nodes up to 2 cm in diameter) at no more than three sites, or equivocal CT scan abnormalities and/or bone marrow infiltration of up to 20% in a bone marrow biopsy specimen.

**Statistical Methods**

FFR duration was considered the time between reinfusion of bone marrow and recurrence, deaths in remission being censored in this analysis. Survival and FFR curves were estimated using the product-limit method of Kaplan and Meier.27 To identify prognostic variables for remission duration and survival, the following factors were examined using the log-rank test28 and the Cox proportional hazards regression model29: interval from diagnosis to HDT, number of prior chemotherapy regimens, remission status (CR or GPR) at the time of HDT, remission number at the time of HDT (second or third or more), presence of marrow involvement at the time of collection, detection of a Bcl-2/IgH rearrangement at the time of diagnosis, presence of Bcl-2/IgH rearrangement–bearing cells in the bone marrow collection after in vitro treatment, and detection of Bcl-2/IgH rearrangement–bearing cells in follow-up bone marrow samples. The association between post-HDT molecular monitoring and FFR and survival were evaluated using the Cox time-dependent regression model,30 which tested whether the patient was at increased risk of recurrence or death in the period when PCR positivity was detected. In the analyses undertaken, deaths due to secondary malignancies were also taken in account, to determine any effect they could have on the results. Analyses of categorical data was performed using Fisher’s exact test.

**RESULTS**

**Treatment Outcome**

The results of treatment are listed in Table 2. There were four “early” treatment-related deaths (resulting from nonengraftment, cerebral hemorrhage, Candida septicemia, and aspergillosis) and 10 “late” deaths from posttreatment-related causes. Twelve patients developed secondary myelodysplasia (s-MDS) or secondary acute myeloblastic leukemia; seven died, four without evidence of recurrent lymphoma at 3, 4, 5, and 6 years and three with concurrent lymphoma at 4, 5.5, and 8.5 years. Five patients with s-MDS remain alive, four without evidence of lymphoma. Three patients died from pulmonary fibrosis, carcinoma of the small bowel, and squamous cell...
carcinoma of the skin at 4, 6, and 57 months, respectively. One patient committed suicide 1 year after HDT.

Thirty-eight patients (including four with s-MDS) developed recurrent lymphoma. In 29 patients, the recurrence was overt, whereas in nine it was detected through an annual surveillance bone marrow trephine biopsy, on a CT scan, or both. Thirty patients presented with recurrence at previous sites of disease; at the time of recurrence, 11 patients were found to have evidence of transformation to diffuse large B-cell histology. Sixteen of the 38 patients remained alive at a median of 4.5 years after recurrence.

Forty-nine patients remained in continuous remission, with a median follow-up of 5.5 years (range, 1.5 to 12.5 years). Kaplan-Meier estimates of FFR and survival rates at 5 years were 63% (95% confidence interval [CI], 52% to 72%) and 69% (95% CI, 58% to 78%), respectively (Fig 1). In comparison with the historical control group, the FFR curve for the 67 patients treated in second remission was significantly better and diverged further with longer follow-up ($P < .001$, log-rank test) (Fig 2). There was no significant difference in survival ($P = .3$, log-rank test). There was also no significant difference in survival from the time of original diagnosis or in time from diagnosis to recurrence ($P = .11$ and $P = .13$, respectively; log-rank test) (data not shown).

**Efficacy of In Vitro Treatment**

PCR-detectable Bcl-2/IgH rearrangements in lymph node or bone marrow samples were found in 72 (73%) of the
original 98 patients before HDT (in one patient, PCR status was not recorded). Samples before and after in vitro treatment of the bone marrow collection were available for analysis from 61 of 72 patients. Samples in eight of these 61 patients were found to be PCR negative before and after in vitro treatment. All eight patients had no morphologic evidence of lymphomatous infiltration in bilateral restaging trephine biopsy specimens before HDT. Therefore, 53 samples from the collection testing positive with PCR at the time of HDT were analyzed after purging for the purpose of detection of residual Bcl-2/IgH rearrangement–bearing cells. Ten (25%) of the 40 collections treated with one mAb and four (31%) of the 13 treated with four mAbs became PCR negative after in vitro treatment. The use of additional mAbs did not therefore improve the efficacy of the in vitro purging procedure \((P = .73)\). Examination for any correlation between efficacy of in vitro treatment and the presence of morphologic infiltration in the bone marrow before HDT revealed that none of the seven patients with bone marrow infiltration tested PCR negative after in vitro treatment, compared with 14 (30%) of the 46 patients with no bone marrow disease, but this difference was not significant \((P = .17)\).

**Outcome in Relation to the Presence of a Bcl-2/IgH Rearrangement During Follow-Up**

Sixty-eight of the original 72 patients with amplifiable Bcl-2 rearrangements survived more than 4 months and were assessable for molecular follow-up after HDT. Fifty-three of the 68 patients had at least one follow-up bone marrow sample available with sufficient DNA for PCR analysis (Fig 3). In the case of 23 patients, all follow-up samples were consistently PCR negative. Only four (17%) of these 23 patients developed recurrent lymphoma (Fig 3A), in contrast to 12 (92%) of 13 patients whose follow-up samples tested PCR positive (Fig 3B). In the remaining 17 patients, PCR analysis of serial samples at different time points revealed both positive and negative results (ie, mixed). Six (35%) of these 17 patients relapsed (Fig 3C). In the case of 12 (71%) of the patients with mixed molecular responses, PCR positivity was found in the first serial sample analyzed. Conversion to a consistently PCR-negative state was observed in the case of six of 12 patients, 1 to 2 years after HDT. None of these six patients developed recurrence.

**Prognostic Factors for All 99 Patients**

The absence of a Bcl-2 rearrangement at the time of diagnosis was marginally associated with a survival benefit (HR, 0.46; 95% CI 0.2 to 1.1; \(P = .09\)) (Fig 4). The precise position of the translocation did not have any impact on this finding; the results were the same, irrespective of whether the translocation was at the major breakpoint or minor cluster region. Of all other factors considered, only the total number of treatment episodes before HDT was found to be of marginal prognostic significance, with patients receiving three or fewer treatment episodes surviving longer (HR, 0.46; 95% CI, 0.2 to 1.1; \(P = .06\)). Adjusting for other factors by means of multivariate modeling produced significant HRs of 0.39 (95% CI, 0.16 to 0.95; \(P = .04\)) for the effect of undetectable Bcl-2/IgH rearrangements and 0.30 (95% CI, 0.15 to 0.59; \(P = .001\)) for the effect of three or fewer treatment episodes. Censoring for deaths due to secondary malignancies had no influence on these results. None of the factors examined were found to be predictive of FFR.

**Prognostic Factors for Patients With Known Bcl-2 Rearrangements**

In a Cox time-dependent regression analysis, the absence of a Bcl-2/IgH rearrangement during follow-up was found to be strongly associated with a reduced risk of recurrence and death. During the period in which patients’ samples were recorded as being PCR negative, the risk of recurrence was reduced to a ratio of 0.13 (95% CI, 0.04 to 0.39; \(P < .001\)) and the risk of death to 0.25 (95% CI, 0.08 to 0.78; \(P = .02\)) (Table 3). The correlation between outcome and efficacy of in vitro treatment of the bone marrow collection was examined. In univariate analysis, the PCR status of the reinfused bone marrow did not have any influence on FFR or survival (Table 4). Adjusting for deaths from secondary malignancies again had no influence on these results. In multivariate analyses, other factors found to be significantly predictive of improved outcome were absence of bone marrow infiltration at the time of diagnosis, three or fewer treatment episodes before HDT, and younger age at the time of HDT (Table 3).

**DISCUSSION**

Our findings confirm previous reports indicating that FFR is longer than expected after less intensive treatment.\(^6\)\(^,\)\(^18\) Although the FFR curve diverged further after the original report\(^6\) \((P = .001)\), there remains no statistical advantage in terms of survival after this treatment. Although such an advantage could emerge later, it might be precluded by the unexpectedly high incidence of therapy-related deaths due to s-MDS.\(^31\)\(^,\)\(^32\) Noted in the previous report\(^6\) were four cases of s-MDS in the first 64 patients, with a median follow-up of 3.5 years. With longer follow-up, secondary malignancies emerged as the second major cause of treatment failure, after recurrence. Nine of the 11 late deaths in this study were directly attributable to this cause. With a median
follow-up of 5.5 years, 12 (12%) of the 99 patients (eight in continuous remission) developed s-MDS, a median of 4 years after HDT. The incidence (12%) is similar to that suggested in a report of a study at the University of Nebraska (an estimated cumulative risk of 13% at 5 years for patients with non-Hodgkin’s lymphoma who were ≥ 40 years old at the time of HDT and who received a TBI-containing regimen).31
In our study, recurrence remained the major cause of treatment failure, with 38 patients (38%) developing recurrent lymphoma a median of 16 months after HDT. Recurrences were observed as late as 8 years after HDT, reflecting the relatively long clinical course of this illness. The majority of patients (79%) presented with recurrence at previous sites of disease, an observation in accordance with that reported by Freedman et al\textsuperscript{33} for patients treated using a similar approach. This pattern of recurrence suggests that such recurrence is due to endogenous lymphoma resistant to myeloablative doses of therapy rather than to reinfusion of lymphoma cells present in the bone marrow collection, although one cannot exclude the possibility that reinfused tumor cells “seek refuge” in previous sites of disease by means of homing receptor mechanisms. The survival patterns after recurrence for 33 of these patients have been reported, the results suggesting that HDT does not compromise outcome in patients in whom it fails, reflecting outcome of the disease when treated conventionally.\textsuperscript{34}

In previous published reports from SBH, the “touchdown” nested PCR technique was noted to permit analysis of a greater amount of DNA; whereas in all subsequent samples, a modified two-step nested PCR technique was used to analyze up to 1.5 µg of DNA. Modification of the PCR technique has led to an apparent increase in the efficacy of the in vitro treatment. Within the limitations of the methods, both technical and statistical, the increase from one to four anti–B-cell antibodies did not improve the efficacy of purging (25% and 31%, respectively). Similarly, there was no statistical advantage in receiving “clear” as opposed to “contaminated” bone marrow to support HDT, as suggested by other groups.\textsuperscript{7,14,35} This is despite the fact that the current PCR assay, as used at SBH, is very similar in sensitivity to that used at the DFCI (ie, 1 in \textsuperscript{10}\textsuperscript{5}),\textsuperscript{14} although no direct comparison between the two techniques has been made. The controversy regarding the relative contribution of residual endogenous disease and reinfused tumor cells to recurrence can be resolved only with gene marker studies, as used in other malignancies.\textsuperscript{36,37} More important (in terms of both the use of HDT and the concept of molecular remission), this study has confirmed that recurrence is most unlikely in patients who have "undetectable" Bcl-2/IgH rearrangements at diagnosis.

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<th>Table 3. Multivariate Analysis of Factors Predictive of Improved Outcome for Patients With Bcl-2/IgH Rearrangements</th>
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<tr>
<td><strong>PCR – versus PCR + at follow-up</strong></td>
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<tr>
<td><strong>HFR</strong></td>
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<tr>
<td>Hazards Ratio</td>
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<tr>
<td>0.13</td>
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<tr>
<td>NS</td>
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<tr>
<td>0.42</td>
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<td>1.08</td>
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Abbreviations: BM, bone marrow; NS, not significant; s-MA, secondary malignancy.
able” numbers of cells bearing the Bcl-2/IgH rearrangement in the bone marrow at follow-up. This is entirely in keeping with the results of identical therapy at the DFCI,15,33 is supported by results obtained with conventional intensive therapy as given at the M.D. Anderson Cancer Center,38 and lends support to the concept that molecular remission is a goal worth achieving.

However, the demonstration that patients with early- or advanced-stage follicular lymphoma remain in long-term remission after conventional treatment despite detection of persistent Bcl-2/IgH rearrangement–bearing cells39,40 and similar observations for patients after HDT (eg, patient nos. 3, 13, and 26; Fig 3C) raise certain questions. The nature of these residual Bcl-2/IgH rearrangement–bearing cells remains uncertain. In some patients, these cells may not be “true” clonogenic cells and may represent cells that have acquired only the “first hit” event required for malignant transformation.41 The hypothesis is supported by the observation that Bcl-2/IgH rearrangement– bearing cells can be detected in the peripheral blood of healthy individuals and even more frequently in lymphoid tissues of individuals with reactive follicular hyperplasia.42,43 It is also possible that a proportion of patients with intact immune surveillance mechanisms may be capable of controlling or even eradicating Bcl-2/IgH rearrangement–bearing cells over time, as suggested by the fact that in this study, a proportion of patients whose initial samples were PCR positive at follow-up had no detectable Bcl-2/IgH rearrangement–containing cells in later samples (Fig 3C). Finally, it is arguable that patients in clinical remission with persisting or reappearance Bcl-2/IgH rearrangements are ultimately destined to develop recurrent disease. Ongoing studies, evaluating the use of real-time quantitative PCR, might shed light onto the significance of minimal residual disease and its prognostic value in follicular lymphoma.44

In the present study, patients who did not demonstrate Bcl-2/IgH rearrangements before HDT had a significant survival advantage compared with patients with such rearrangements. However, patients in whom PCR-detectable Bcl-2/IgH rearrangements were not found represent two populations: individuals with no straightforward t(14;18) translocation and those with the translocation but at a site that cannot readily be amplified by PCR using the methods employed in this study. The prognostic significance of the rearrangement in follicular lymphoma remains controversial,45,46 and further studies are required to elucidate this issue.

In the absence of a less toxic alternative, it seems appropriate to continue to investigate HDT as part of treatment for follicular lymphoma, even though in its present form, it is not curative for at least half of those treated. However, it is imperative to improve the efficacy of the treatment and try to reduce the incidence of s-MDS. Incorporation of low-dose targeted irradiation as part of the high-dose regimen47,48 is currently being evaluated. This might also reduce the incidence of s-MDS.49 More intensive pre-HDT induction is likely to be limited in its application because of potential difficulties in subsequently obtaining stem cells.50 Whether the use of peripheral-blood progenitor cells, variably purged ex vivo, will reduce the recurrence rate remains to be proven.51,52 The concept of using anti-CD20 therapy before the collection of stem cells is clearly attractive; better remissions might be achieved without increased myelotoxicity. There is strong evidence that the blood and bone marrow compartment is at least transiently cleared of cells containing the Bcl-2/IgH rearrangement.53,54 Hence, in vivo purging might be achieved.

The second concern is the emerging high incidence of s-MDS. It is now clear that there is a significant risk of s-MDS after HDT. Questions remain regarding how much this relates to prior chemotherapy, as opposed to the high-dose regimen.55 Without doubt such risk must be taken into account when treatment is being recommended and when clinical trials are being designed.56 The potential benefit will outweigh the risk in some patients.

There are presently several encouraging relatively novel therapies for follicular lymphoma, ranging from new chemotherapeutic regimens57 via HDT to biologic therapies including interferon therapy,58,59 antibody therapy (with or without chemotherapy or irradiation),47,48,60,61 and active immunotherapy.62,63 One can argue that high-dose treatment should be followed by additional therapy (eg, interferon therapy), as is being investigated, or, alternatively, by anti-CD20 therapy. A randomized trial of this approach is also currently in progress. Given that most recurrences after

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### Table 4. Univariate Analysis of the Effect of PCR Status of the Bone Marrow Collection Before and After In Vitro Treatment on FFR and Survival in Patients With Bcl-2/IgH Rearrangements

<table>
<thead>
<tr>
<th>PCR Status</th>
<th>FFR</th>
<th>Survival After HDT</th>
<th>Survival After HDT (censored for s-MA)</th>
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<tr>
<td></td>
<td>No. of Recurrences</td>
<td>Hazards Ratio</td>
<td>95% CI</td>
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<tr>
<td>+</td>
<td>39</td>
<td>16 (baseline)</td>
<td>+</td>
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<tr>
<td>+/-</td>
<td>22</td>
<td>6</td>
<td>+</td>
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high-dose treatment occur in the first 2 years (Fig 2), such additional therapy should probably be given early, after high-dose treatment. The use of quantitative, real-time PCR may shed light on the optimal time point for such therapeutic interventions. Allogeneic bone marrow transplantation is clearly also a form of immunologically mediated therapy. Although not applicable to the majority of patients, nonmyeloablative or mixed chimeric allogeneic transplantation is currently being investigated. With the reduction in treatment-related mortality compared with conventional allogeneic transplantation, such treatment might become applicable to a greater number of patients.

It may take time to demonstrate whether any of these approaches, alone or in combination, are curative, or whether a new algorithm needs to be constructed. Meanwhile, there remains a population of patients with repeatedly responsive follicular lymphoma in whom this therapy should be explored further.

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