Quantitative PCR Detection of t(14;18) *bcl*-2/JH Fusion Sequences in Follicular Lymphoma Patients

Comparison of Peripheral Blood and Bone Marrow Aspirate Samples

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In patients with follicular lymphoma (FL), it is unresolved whether peripheral blood (PB) can replace bone marrow (BM) aspirate samples for detection of *bcl*-2/JH fusion sequences that result from the t(14;18)(q32;q21). We compare here the results of quantitative polymerase chain reaction (q-PCR) analysis for *bcl*-2/JH involving the major breakpoint cluster region (mbr) on paired PB and BM aspirate samples from 60 consecutive FL patients. There was a significant correlation between the level of *bcl*-2/JH fusion sequence obtained from PB and BM aspirate samples ($r = 0.886$), with 82% of samples showing less than one log of difference. Patients who had histological evidence of FL involving concurrent BM biopsy specimens had moderate to high levels of *bcl*-2/JH in both PB and BM aspirate samples, allowing unequivocal determination of translocation status (median *bcl*-2/JH to cyclophilin level was 8.014%). In contrast, patients with no detectable FL in their BM biopsy specimens often showed low levels of *bcl*-2/JH in both PB and BM aspirate samples (*bcl*-2/JH to cyclophilin median level = 0.006%), in a range similar to background levels that could be detected in patients without FL ($n = 15$, median *bcl*-2 mbr/JH to cyclophilin level = 0.002%). We conclude that PB can be used in place of BM aspirate samples to test for the presence of *bcl*-2 mbr/JH fusion sequence in FL patients and that either PB or BM aspirate testing yields a rough approximation of the degree of BM involvement by FL. However, in patients with minimal levels of *bcl*-2/JH in PB or BM aspirate samples, confirmation of this result by testing the primary tumor is recommended to confirm the presence of an identical *bcl*-2/JH fusion sequence and exclude false-positive results. (*J Mol Diagn* 2004, 6:396–400)

Most cases of follicular lymphoma (FL) carry the t(14;18)(q32;q21), which places the *bcl*-2 gene at 18q21 adjacent to enhancer sequences of the immunoglobulin heavy chain (IgH) locus at 14q32, resulting in overexpression of *bcl*-2.^{1–3} Using conventional cytogenetics, the t(14;18) can be detected in approximately 85% of FL cases,⁴ with the majority of translocations occurring at either the major breakpoint cluster region (mbr) or the minor breakpoint cluster region (mcr). Using polymerase chain reaction (PCR) analysis, *bcl*-2/JH involving the mbr and mcr regions can be detected in 60 to 70% and 8 to 15% of FL cases, respectively.^{5,6}

The failure to detect the t(14;18) in 10 to 25% of FL cases can be due to use of other oncogenic pathways (ie, *bcl*-2-negative FL), methodological issues, or the presence of the t(14;18) involving breakpoints outside the mbr and mcr.⁷ The sensitivity of detection of the t(14;18) by any methodology, including PCR, also varies according to the extent of involvement by FL in the tested sample. While most FL patients have tumor cells present in bone marrow (BM) and peripheral blood (PB), the degree of involvement by lymphoma at these sites can often be low. Furthermore, BM aspirate samples frequently under-represent the extent of involvement because paratrabecular FL cells have a tendency to not be aspirated. Furthermore, the t(14;18) can be detected by PCR with primers specific for the mbr or mcr in patients without FL. These false-positive cases are attributed to *bcl*-2 mbr/JH fusion sequences that arise from benign lymphocytes, or preneoplastic lymphocytes that carry occult translocations.^{8–12}

When PCR detection of the t(14;18) is used to monitor tumor burden, relapse, or minimal residual disease in FL patients,^{12–15} the choice of samples to best avoid false-positive and false-negative results becomes critical. One study has shown that analysis of PB specimens is less predictive of relapse than analysis of BM aspirate specimens.¹³ By contrast, other studies have demonstrated that PB and BM aspirate samples usually yield similar results.¹⁴ The recent development of t(14;18) assays us-

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ing real-time quantitative polymerase chain reaction (q-PCR) methods,^{15–22} followed by fluorescent-based high-resolution capillary electrophoresis,²³ has allowed for more accurate quantitation and size determination of *bcl-2/JH* fusion sequences. However, the detection sensitivity for *bcl-2/JH* in BM aspirate and PB samples using q-PCR methods has not been systematically addressed.

Here, we compare the levels of *bcl-2 mbr/JH* fusion sequences detected by q-PCR in concurrent PB and BM aspirate samples from 60 patients with FL known to have *bcl-2/JH*. For patients with moderate to high levels of *bcl-2/JH*, the results of q-PCR analysis of PB and BM aspirate samples correlates well, and thus PB can be used instead of BM aspirate material for this purpose. Most of the patients with high levels of *bcl-2/JH* also had histological involvement of the BM biopsy specimen by FL, and thus PB levels are an indication of systemic tumor dissemination. However, for patients with low levels of *bcl-2/JH*, most of whom do not have histological evidence of BM involvement by FL, the t(14;18) may or may not be derived from neoplastic cells, and thus confirmation by analysis of the primary tumor is suggested.

Materials and Methods

Study Group

Quantitative PCR analysis for the t(14;18) involving the mbr and mcr is routinely performed on PB and/or BM aspirate samples of all FL patients at time of presentation to our institution. In this study, we focused on 60 FL consecutive patients accessioned between 1999 to 2003 who had *bcl-2 mbr/JH* fusion sequences and who had PCR data from paired PB and BM aspirate samples. To establish the range of detection of *bcl-2/JH* levels that occur among patients without FL, we also included PB and BM aspirate samples from 15 patients diagnosed with other B-cell lymphoproliferative disorders who had detectable *bcl-2/JH* levels.

The primary diagnosis of FL was established by excisional biopsy of lymph nodes in 59 cases and by endoscopic biopsy of a colon polyp in one case. Using the World Health Organization classification system for histological grading, 20 cases were grade 1, 29 cases were grade 2, and 7 were grade 3. The remaining four patients had diffuse large B-cell lymphoma arising in association with an underlying FL. Three patients had discordant disease with large B-cell lymphoma of follicle center cell origin in the tissue biopsy specimens and low-grade FL in BM biopsy specimen. PB and BM aspirate samples represented initial pretreatment samples in 53 cases, and recurrent or residual disease in seven cases. The extent of FL involvement in BM was established by review of hematoxylin-eosin-stained sections of the decalcified, formalin-fixed trephine biopsy specimens. Based on the area involved by lymphoid aggregates and the overall BM cellularity, the percentage of BM involvement by FL was assessed semi-quantitatively, as 0 to 10%, 10 to 20%, etc. For the purpose of statistical analysis, the midpoint of the range (eg, 5%, 15%, etc) was used.

DNA Isolation and q-PCR

High molecular weight genomic DNA from PB and BM aspirate samples was extracted using the ABI 341 nucleic acid purification system (Applied Biosystems, Foster City, CA) or Autopure LS (Gentra Systems, Inc., Minneapolis, MN). Genomic DNA from frozen tissue was isolated using conventional phenol-extraction methods. The concentration of the DNA was determined by absorbance at 260 nm using a Beckman DU-7400 spectrophotometer (Beckman, Fullerton, CA).

All specimens including the control group were analyzed by TaqMan *bcl-2/JH* q-PCR coupled with fluorescent-based high-resolution capillary electrophoresis and GeneScan DNA fragment analysis (Applied Biosystems). The PCR reaction used 1 μ g of genomic DNA and was described previously.²³ All reactions were performed in duplicate. DNA obtained from the HL60 cell line was used as a negative control. Serial dilutions from 10^{-1} to 10^{-5} -fold of a t(14;18)-positive follicular large cell lymphoma cell line,²⁴ were used to generate standard curves. Amplification of a 93-bp sequence from the cyclophilin gene was performed simultaneously in all cases to normalize t(14;18) values to amount of input DNA.²⁵ Following amplification, GeneScan analysis was conducted by capillary electrophoresis in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). This method eliminates the need for gel electrophoresis and provides an accurate measurement of the size of the amplicon. By combining q-PCR with GeneScan analysis, we are able to eliminate the risk of error due to co-amplification of non-target DNA.^{23,26}

Statistical Analysis

Threshold cycle values of two replicates from each sample were averaged and the absolute *bcl-2 mbr/JH* and cyclophilin (Cy) levels were calculated by mapping the threshold cycle for each patient sample on a standard curve of known dilution of DNA from a positive control cell line. The *bcl-2 mbr/JH* to Cy ratio was then calculated to a normalized *bcl-2/JH* level. Because of the expected variance of PCR efficiency over different target concentrations, $\log_{10}$ (normalized *bcl-2/JH*) ratios were also computed to obtain a mean log difference between PB and BM aspirate samples.

We calculated correlation coefficients using a pairwise comparison of *bcl-2/JH* levels in PB and BM aspirate samples. We similarly calculated correlation coefficients for *bcl-2/JH* levels in BM aspirate specimens with histological extent of FL involving the BM biopsy specimen. R-squared values were taken graphically and used to calculate correlation coefficients (r).

Results

GeneScan analysis showed identically sized *bcl-2 mbr/JH* fusion sequences in paired PB and BM aspirate samples from all 60 patients with FL. The normalized PB and BM fusion levels were highly correlated in most pa-

Figure 1. Correlation between normalized *bcl-2* mbr/JH levels in paired bone marrow aspirate and peripheral blood samples. **A:** Levels among patients in which concurrent BM core biopsy was involved by lymphoma. **B:** Levels among patients in which bone marrow biopsy was not histologically involved.

tients regardless of whether or not there was histological evidence of FL in the concurrent BM biopsy specimen (Figure 1). Following censure of the three most divergent samples because one sample of the pair appeared to have technical failure of the assay, a highly significant correlation coefficient ($r = 0.887$) was noted between the two sample types. The normalized *bcl-2*/JH level was higher in BM aspirate than in PB in 67% of cases and was higher in PB than BM aspirate in the remaining patients.

There was a highly significant correlation between the extent of FL seen in the BM biopsy specimen and the normalized *bcl-2*/JH level detected in concurrent BM aspirate samples ($r = 0.71$, Figure 2). A less significant correlation was observed between PB *bcl-2*/JH level and extent of FL in the BM biopsy specimen ($r = 0.55$).

Figure 2. Correlation between extent of follicular lymphoma present in bone marrow biopsy and level of normalized *bcl-2* mbr/JH product in BM aspirate.

Figure 3. Comparing levels of *bcl-2*/JH product in bone marrow aspirate and peripheral samples in the 60 patients with mbr+ follicular lymphoma. **A:** Comparison of BM and PB *bcl-2*/JH levels seen in patients who had lymphoma detected in concurrent BM core biopsy. **B:** Comparison of BM and PB *bcl-2*/JH levels in patients who did not have lymphoma detected in concurrent BM core biopsy.

Among the 33 (55%) patients who had histological evidence of FL in the concurrent BM biopsy specimen, the median ratio of *bcl-2*/JH to cyclophilin was 8.01% in BM aspirate and 0.83% in PB samples. As shown in Figure 3A, 27 of 33 (82%) FL patients with histologically involved BM biopsy specimens had *bcl-2*/JH levels in PB and BM aspirate samples that were within one log difference; the mean log difference was 0.628. The levels of normalized *bcl-2*/JH in PB were not highly correlated with level of PB lymphocytosis ($r = 0.362$).

Among the 27 (45%) FL patients who had no histological evidence of FL in the concurrent BM biopsy specimen, the median normalized levels of *bcl-2*/JH were 0.00615% in BM aspirate and 0.0095% in PB samples, with a high correlation between the values from the two sample types ($r = 0.93$). Twenty-five (93%) of these patients had normalized *bcl-2*/JH levels in BM and PB that were within one log difference (Figure 3B). The mean log difference between PB and BM aspirate levels in this group was 0.35. Twenty-five of 27 (59.3%) patients had higher normalized levels in PB than in BM aspirate. Sixteen FL patients had normalized *bcl-2*/JH levels that were 0.015% or less, representing 59% of FL patients with negative BM biopsy specimens and 27% of all FL patients studied. In the three patients with the lowest normalized *bcl-2*/JH levels, PCR and GeneScan analysis of the primary tumor revealed a *bcl-2*/JH fusion sequence of identical size to that detected in the PB and BM aspirate samples. These results demonstrate that the low levels of *bcl-2*/JH detected

Table 1. Comparison of *bcl-2* mbr/JH Levels Detected in PB and BM Samples

Category	No. of cases	BM mbr/Cy median	BM mbr/Cy range	PB mbr/Cy median	PB mbr/Cy range
Follicular lymphoma with involved BM	33	8.014%	0.027–93.3%	0.83%	0.002–66.9%
Follicular lymphoma with negative BM	27	0.006%	0.003–1.667%	0.01%	0.0001–1.692%
B-LPD other than FL	15	0.003%	0.001–0.012%	0.003%	0.002–0.014%

Abbreviations: BM, bone marrow biopsy specimen; PB, peripheral blood; mbr, *bcl-2*/JH translocation detected using mbr primers; Cy, cyclophilin A (normalizing DNA sequence); B-LPD, B-cell lymphoproliferative disorders; FL, follicular lymphoma.

in PB and BM aspirate samples of these three patients were derived from the neoplastic clone.

For comparison, we studied 15 paired PB and BM aspirate samples from patients with B-cell lymphoproliferative disorders other than FL who also had low levels of *bcl-2* mbr/JH detected. In these patients, the level of *bcl-2*/JH ranged from 0.0002 to 0.014 in PB and 0.0001 to 0.012 in BM aspirate samples (Table 1). In three cases, the *bcl-2*/JH fusion sequences were detected only in the BM or the PB samples. The diagnoses for these 15 cases were chronic lymphocytic leukemia ($n = 7$), extranodal marginal zone B-cell lymphoma ($n = 4$), anaplastic large cell lymphoma ($n = 1$), marginal zone B-cell lymphoma ($n = 1$), mantle cell lymphoma ($n = 1$), and acute lymphoblastic leukemia ($n = 1$). Because of the low levels of product and the absence of FL based on the results of tissue biopsy and flow cytometric characterization of the PB and BM samples, we attribute these *bcl-2*/JH fusion products to background non-neoplastic or preneoplastic lymphocytes carrying an occult t(14;18).

Discussion

This study was designed to address whether levels of *bcl-2* mbr/JH fusion sequence detected by q-PCR in PB and BM aspirate samples obtained from FL patients are similar, thereby obviating the need for BM examination solely for this purpose. We included 60 consecutive FL patients with the t(14;18) involving mbr who had q-PCR analysis performed on paired PB and BM aspirate samples. Overall, there was a strong correlation between levels detected in PB and BM aspirate samples, however, because we had a limited number of cases with extensive BM involvement, the absolute correlation between BM aspirate and PB samples may be somewhat overstated. In cases with moderate to high levels of *bcl-2*/JH, both PB and BM aspirate samples usually had high levels of product but in 33% of cases, the normalized *bcl-2*/JH levels were higher in PB than in parallel BM aspirate samples. These results closely approximate those reported previously by Summers and colleagues²¹ and suggest that PB can be used in place of BM aspirate samples for determination of translocation status.

Samples from 16 (27%) FL patients in this study had low *bcl-2* mbr/JH levels detected in PB and BM aspirate samples that were in the range that was occasionally seen in patients without FL, namely 0.015% (*bcl-2*/JH divided by cyclophilin) or less. Assuming equal efficiency of amplifica-

tion for *bcl-2* mbr/JH and cyclophilin DNA targets, this ratio approximates the number of cells bearing a t(14;18) compared to the overall number of cells analyzed. Thus, there is a threshold of approximately 1 *bcl-2* mbr/JH-bearing cell in 3000 cells that defines a lower limit below which the fusion sequence detected could be derived from occult translocations in non-neoplastic or preneoplastic cells (ie, "background levels").^{8–12} In this circumstance, concurrent analysis of the primary tumor to confirm the true translocation status is likely required for definitive interpretation of the significance of a low/borderline *bcl-2*/JH level in PB or BM aspirate samples.¹²

This analysis is supported by previous work by Tsimberidou et al²² who reported low/background levels of *bcl-2* mbr/JH fusion sequences in PB specimens obtained from 22% of non-FL donors, with a frequency as high as 40% when a 10-fold excess of genomic DNA (10 μ g) was used for amplification. They also reported that 2 to 3% of non-FL samples had *bcl-2*/JH levels that overlapped with levels that can be detected in FL samples. Previous research indicates that t(14;18) translocations are spontaneously generated in lymphocytes of healthy individuals, and that such cells only rarely progress to overt lymphoma.^{9,12} Furthermore, age appears to have a significant influence on the likelihood of translocation detection, with older individuals more frequently having positive PB samples, and also having a much higher fusion product levels.⁸

The correlation between *bcl-2*/JH levels detected in BM aspirate samples and the approximate extent of FL involving the concurrent BM biopsy specimen also suggest that q-PCR may be used as an alternate method for determining the presence and general extent of BM involvement by FL. As expected, the concordance between morphological findings and q-PCR was less significant at low *bcl-2*/JH values where histological involvement may be difficult to detect. Although there was a trend toward a correlation, *bcl-2*/JH levels in PB samples were less useful for predicting the extent of lymphoma involvement in BM biopsy specimens, indicating that the absence of *bcl-2*/JH positivity by PCR in PB samples cannot be used to absolutely predict the absence of BM involvement by FL.

At our institution, patients with FL commonly are first diagnosed by tissue biopsy elsewhere, and fresh tissue or paraffin blocks of the primary tumor are often not available for molecular testing. In these cases, in terms of patient comfort and expense, a PB sample is clearly

preferred over BM aspiration for determination of translocation status. We believe this study demonstrates that real-time q-PCR combined with capillary electrophoresis and amplicon sizing using GeneScan analysis can reliably allow analysis of *bcl-2* mbr/JH status in PB samples in lieu of BM aspiration. This approach is highly sensitive when compared with non-quantitative PCR. By accurate size comparison of the fusion sequences between two sites, GeneScan analysis also allows one to eliminate false-positive results due to non-lymphoma-associated translocations.^{23,26} Furthermore, the correlation between the results obtained from PB and BM aspirate samples at all levels of disease (ranging from very high to very low levels of *bcl-2*/JH), suggests that PB analysis is likely to be as informative as BM aspirate analysis for monitoring tumor burden in FL patients over time.

However, for newly diagnosed cases of FL with low *bcl-2*/JH levels in PB or BM aspirate samples, confirmation of the *bcl-2*/JH amplicon size by testing of the primary tumor is justified. The data in this study also suggest that high levels of *bcl-2*/JH detected in PB or BM aspirate samples can be used as a substitute for testing of the primary neoplasm. However, as already stated, relatively low levels of *bcl-2*/JH detected in PB and BM aspirate samples may not be derived from neoplastic cells, necessitating a repeat analysis on a tissue biopsy, or obtaining the original biopsy specimen, if available, for *bcl-2*/JH analysis.

References

1. Tsujimoto Y, Cossman J, Jaffe E, Croce CM: Involvement of the *bcl-2* gene in human follicular lymphoma. *Science* 1985, 228:1440–1443
2. Bakhshi A, Jensen JP, Goldman P, Wright JJ, McBride OW, Epstein AL, Korsmeyer SJ: Cloning the chromosomal breakpoint of *t*(14;18) human lymphomas: clustering around JH on chromosome 14 and near a transcriptional unit on 18. *Cell* 1985, 41:899–906
3. Cleary ML, Sklar J: Nucleotide sequence of a *t*(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint cluster region near a transcriptionally active locus on chromosome 18. *Proc Natl Acad Sci USA* 1985, 82:7439–7443
4. Yunis JJ, Frizzera G, Oken MM, McKenna J, Theologides A, Arnesen M: Multiple recurrent genomic defects in follicular lymphoma: a possible model for cancer. *N Engl J Med* 1987, 316:79–84
5. Cotter FE, Price C, Meerabux J, Zucca E, Young BD: Direct sequence analysis of 14q+ and 18q- chromosome junctions at the MBR and MCR revealing clustering within the MBR in follicular lymphoma. *Ann Oncol* 1991, 2 Suppl 2:93–97
6. Horsman DE, Gascoyne RD, Coupland RW, Coldman AJ, Adomat SA: Comparison of cytogenetic analysis, southern analysis, and polymerase chain reaction for the detection of *t*(14; 18) in follicular lymphoma. *Am J Clin Pathol* 1995, 103:472–478
7. Buchonnet G, Lenain P, Ruminy P, Lepretre S, Stamatoullas A, Parmentier F, Jardin F, Duval C, Tilly H, Bastard C: Characterization of BCL2-JH rearrangements in follicular lymphoma: PCR detection of 3' BCL2 breakpoints and evidence of a new cluster. *Leukemia* 2000, 14:1563–1569
8. Liu Y, Hernandez AM, Shibata D, Cortopassi GA: BCL-2 translocation frequency rises with age in humans. *Proc Natl Acad Sci USA* 1994, 91:8910–8914
9. Limpens J, Stad R, Vos C, de Vlaam C, de Jong D, van Ommen GJ, Schuurin E, Kluin PM: Lymphoma-associated translocation *t*(14;18) in blood B cells of normal individuals. *Blood* 1995, 85:2528–2536
10. Dolken G, Illerhaus G, Hirt C, Mertelsmann R: BCL-2/JH rearrangements in circulating B cells of healthy blood donors and patients with nonmalignant diseases. *J Clin Oncol* 1996, 14:1333–1344
11. Aster JC, Kobayashi Y, Shiota M, Mori S, Sklar J: Detection of the *t*(14;18) at similar frequencies in hyperplastic lymphoid tissues from American and Japanese patients. *Am J Pathol* 1992, 141:291–299
12. Summers KE, Goff LK, Wilson AG, Gupta RK, Lister TA, Fitzgibbon J: Frequency of the Bcl-2/IgH rearrangement in normal individuals: implications for the monitoring of disease in patients with follicular lymphoma. *J Clin Oncol* 2001, 19:420–424
13. Gribben JG, Neuberger D, Barber M, Moore J, Pesek KW, Freedman AS, Nadler LM: Detection of residual lymphoma cells by polymerase chain reaction in peripheral blood is significantly less predictive for relapse than detection in bone marrow. *Blood* 1994, 83:3800–3807
14. Leonard BM, Hetu F, Busque L, Gyger M, Belanger R, Perreault C, Roy DC: Lymphoma cell burden in progenitor cell grafts measured by competitive polymerase chain reaction: less than one log difference between bone marrow and peripheral blood sources. *Blood* 1998, 91:331–339
15. Olsson K, Gerard CJ, Zehnder J, Jones C, Ramanathan R, Reading C, Hanania EG: Real-time *t*(11;14) and *t*(14;18) PCR assays provide sensitive and quantitative assessments of minimal residual disease (MRD). *Leukemia* 1999, 13:1833–1842
16. Sarris AH, Jiang Y, Tsimberidou AM, Thomaidis A, Rassidakis GZ, Ford RJ, Medeiros LJ, Cabanillas F, McLaughlin P: Quantitative real-time polymerase chain reaction for monitoring minimal residual disease in patients with advanced indolent lymphomas treated with rituximab, fludarabine, mitoxantrone, and dexamethasone. *Semin Oncol* 2002, 29(1 Suppl 2):48–55
17. Luthra R, McBride JA, Cabanillas F, Sarris A: Novel 5' exonuclease-based real-time PCR assay for the detection of *t*(14;18)(q32;q21) in patients with follicular lymphoma. *Am J Pathol* 1998, 153:63–68
18. Estalilla OC, Medeiros LJ, Manning Jr JT, Luthra R: 5'→3' exonuclease-based real-time PCR assays for detecting the *t*(14;18)(q32;q21): a survey of 162 malignant lymphomas and reactive specimens. *Mod Pathol* 2000, 13:661–666
19. Hirt C, Dolken G: Quantitative detection of *t*(14;18)-positive cells in patients with follicular lymphoma before and after autologous bone marrow transplantation. *Bone Marrow Transplant* 2000, 25:419–426
20. Ladetto M, Sametti S, Donovan JW, Ferrero D, Astolfi M, Mitterer M, Ricca I, Drandi D, Corradini P, Coser P, Pileri A, Gribben JG, Tarella C: A validated real-time quantitative PCR approach shows a correlation between tumor burden and successful ex vivo purging in follicular lymphoma patients. *Exp Hematol* 2001, 29:183–193
21. Summers KE, Davies AJ, Matthews J, Jenner MJ, Cornelius V, Amess JA, Norton AJ, Rohatiner AZ, Fitzgibbon J, Lister TA, Goff LK: The relative role of peripheral blood and bone marrow for monitoring molecular evidence of disease in follicular lymphoma by quantitative real-time polymerase chain reaction. *Br J Haematol* 2002, 118:563–566
22. Tsimberidou AM, Jiang Y, Ford RJ, Lichtiger B, Medeiros LJ, McLaughlin P, Cabanillas F, Sarris AH: Quantitative real-time polymerase chain reaction for detection of circulating cells with *t*(14;18) in volunteer blood donors and patients with follicular lymphoma. *Leuk Lymphoma* 2002, 43:1589–1598
23. Sanchez-Vega B, Vega F, Hai S, Medeiros LJ, Luthra R: Real-time *t*(14;18)(q32;q21) PCR assay combined with high resolution capillary electrophoresis: a novel and rapid approach that allows accurate quantitation and size determination of *bcl-2*/JH fusion products. *Mod Pathol* 2002, 15:448–453
24. Sundaresan A, Claypool K, Mehta K, Lopez-Berestein G, Cabanillas F, Ford Jr RJ: Retinoid-mediated inhibition of cell growth with stimulation of apoptosis in aggressive B-cell lymphomas. *Cell Growth Differ* 1997, 8:1071–1082
25. Medhurst AD, Harrison DC, Read SJ, Campbell CA, Robbins MJ, Pangalos MN: The use of TaqMan RT-PCR assays for semi-quantitative analysis of gene expression in CNS tissues and disease models. *J Neurosci Methods* 2000, 98:9–20
26. Luthra R, McBride JA, Hai S, Cabanillas F, Pugh WC: The application of fluorescence-based PCR and PCR-SSCP to monitor the clonal relationship of cells bearing the *t*(14;18)(q32;q21) in sequential biopsy specimens from patients with follicle center cell lymphoma. *Diagn Mol Pathol* 1997, 6:71–77