More About: B-Cell Non-Hodgkin's Lymphoma: Evidence for the t(14;18) Translocation in All Hematopoietic Cell Lineages

Follicular lymphoma is a mature B-cell lymphoma, characterized by the presence of the t(14;18) translocation that juxtaposes the BCL2 locus on chromosome 18q21 to the immunoglobulin H (IgH) locus on chromosome 14q32, resulting in overexpression of the anti-apoptotic protein Bcl2 *(1)*. In a previous issue of the Journal, Yarkoni et al. *(2)* demonstrated t(14;18) fluorescence *in situ* hybridization (FISH) signals in nearly 100% of fluorescence-activated cell-sorted (FACS) fractionated $CD34^+$ hematopoietic stem cells and (B-cell, T-cell, myeloid, erythroid, and megakaryocytic) committed progenitor cells derived from this lineage in seven of the eight t(14;18)-positive patients with non-Hodgkin's lymphoma analyzed. The authors concluded that the t(14;18) occurs in an early multilineage progenitor stem cell. A subsequent letter by Siebert et al. *(3)* disagreed and stated that it was unlikely that, in the majority of B-cell lymphomas, the t(14;18) translocation occurs in a very early multilineage progenitor cell.

Currently, positive selection of the $CD34^+$ hematopoietic progenitor cell fraction is being evaluated as a strategy to eliminate t(14;18)-bearing cells from reinfused grafts. In our experience at St. Bartholomew's Hospital, $CD34^+$ selection by use of an avidin–biotin immunoadsorption device can eliminate all polymerase chain reaction (PCR)-detectable t(14;18)-bearing cells from 50% of contaminated peripheral blood progenitor cell collections obtained from patients with follicular lymphoma *(4)*. The purity of the $CD34^+$ fraction obtained with this device is highly variable, suggesting that tumor cells escape the positive selection process and continue to contaminate the reinfused CD34 concentrate. To investigate whether the $CD34^+$ compartment in these CD34 concentrates contributes to contamination with t(14;18)-bearing cells, a FISH assay using 40-kilobase cosmids for the IgH (constant) region and BCL2 (major breakpoint region) was combined with immunophenotyping for CD34 (FICTION technique) *(3,5)*. Cells were also subjected to immunohistochemistry to detect Bcl2 protein.

Control studies were performed on lymphocytes from normal individuals, and the cutoff level for a false-positive FISH signal was set at 2% (±0.4% standard deviation). Cytospin preparations made from cryopreserved CD34 concentrates from seven patients with follicular lymphoma who received high-dose chemotherapy and reinfusion of CD34 concentrates were analyzed. Five of these concentrates contained a PCR-detectable t(14;18) translocation. FICTION analysis demonstrated t(14;18) fusion signals above the cutoff level in $CD34^+$ cells from three patients—15%, 4.8%, and 4.6%, respectively. In one patient's sample, the high percentage of t(14;18) colocalization signals in the $CD34^+$ compartment (Fig. 1) as opposed to that in the $CD34^-$ compartment of the CD34 concentrate (15% versus 7%, respectively) (data not shown) lends further support to the hypothesis that the t(14;18) translocation occurs in $CD34^+$ cells. In the remaining four patients, the detection of t(14;18) fusion signals in 2.5%–3% of $CD34^+$ cells was difficult to interpret in view of the false-positive rate. The low

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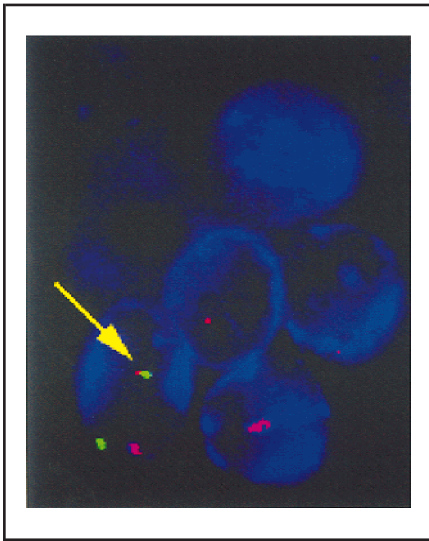

Fig. 1. A CD34+ cell (aminomethyl coumarin acetic acid [AMCA]-anti-CD34, blue signal) with a positive fluorescence *in situ* hybridization signal (red–green signal, arrowhead) from one patient (original magnification ×250).

number of cells expressing Bcl2 (0%–1.5%) suggests that the majority of $CD34^+$ t(14;18)-bearing cells do not have increased expression of Bcl2 protein and may well correspond to detectable "physiological" rearrangements, similar to those detected in normal individuals *(6).*

In partial agreement with results reported by Yarkoni et al. *(2),* our results also demonstrate that the t(14;18) translocation is detected in $CD34^+$ cells. However, we observed positive FISH signals in only a small proportion of cells. This finding favors the hypothesis that the t(14;18) translocation occurs during early B-cell development ($CD34^+19^+$) at the pro-B cell *(7)* rather than in an early multilineage hematopoietic stem cell. This finding also lends support to the hypothesis that nonmalignant t(14;18)-positive $CD34^+$ cells are "regularly" generated in the bone marrow.

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EDITOR'S NOTE

Shai Yarkoni et al. declined to respond to John Apostolidis et al.'s correspondence.

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