



Published in final edited form as:

J Natl Cancer Inst Monogr. 2008 ; (39): 48–51. doi:10.1093/jncimonographs/lgn002.

t(14;18) Translocations and Risk of Follicular Lymphoma

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Abstract

The chromosomal translocation t(14;18)(q32;q21) is characteristic of follicular lymphoma and a frequent abnormality in other types of non-Hodgkin lymphoma (NHL). In healthy individuals, the same translocation may also be found in a small fraction of peripheral blood lymphocytes, the biological significance of which is beginning to be explored. Translocation prevalence and frequency are potential risk factors for developing NHL. Here, we review the published data and describe recent and ongoing work on this promising biomarker. We have a series of studies in four major areas: 1) t(14;18) prevalence and frequency in healthy individuals; 2) maturation of translocation-harboring cells; 3) effect of rituximab treatment on t(14;18) carriage; and 4) predictive and clonotypic relationship between t(14;18) and follicular lymphoma or other NHL. Further studies are warranted to increase understanding of this crucial molecular event in the development of hematopoietic malignancies. Potential applications include determination of elevated risk for lymphoma, early detection of disease, and identification of molecular targets for pre-ventive interventions.

Chromosomal translocations (CTs) that result in the deregulated expression of oncogenes or the generation of novel fusion genes are hallmark mutations of hematopoietic malignancy. An important example is the t(14;18)(q32;q21) which leads to constitutive activation of the *BCL2* oncogene by the enhancers of the immunoglobulin heavy chain locus, *IGH@*. This CT is typically present in tumor cells of follicular lymphoma but can also be found in other non-Hodgkin lymphomas (NHLs).

The number of t(14;18)-positive cells in peripheral blood is also a molecular marker of lymphoma status. High numbers can be detected at the time of diagnosis, decreased numbers of translocated cells in the circulation parallels clinical response, and increases during remission predict imminent relapse (1, 2). Assays for t(14;18) have also been used for quantitative detection of translocation-positive cells before and after autologous bone marrow transplantation (1). Furthermore, mice carrying an *IGH-BCL2* fusion transgene overexpress the antiapoptotic BCL2 protein leading to a three- to fourfold expansion of resting B cells and lymphoid hyperplasia (3). After a median latency of 16 months, about 10% of these transgenic mice develop high-grade diffuse large-cell lymphomas, which

frequently exhibit a rearranged *MYC* (*c-myc*) gene (4). This mouse model suggests that progression from benign follicular hyperplasia to malignant lymphoma requires secondary changes and that the t(14;18) translocation alone is not sufficient to transform a normal B cell into a malignant lymphoma cell.

A small fraction of peripheral blood mononuclear cells (PBMCs) from many healthy individuals also appear to have t(14;18). Limpens et al. (5) first reported detection of t(14;18) in nonneoplastic lymphoid tissues, using nested DNA-PCR; DNA sequencing revealed that the CT junctions occurred in the same breaksite regions as in follicular lymphoma. At their limit of detection (one cell with CT in 10⁵ nonaffected cells), more than half of the individuals tested were PCR positive. This landmark observation has been validated by at least 14 studies of peripheral blood from healthy individuals, which found a median t(14;18) prevalence of 53% (6). However, the median frequency of cells bearing t(14;18) in PBMC of normal individuals is more than three orders of magnitude lower than for individuals with follicular lymphoma before treatment (1 per 200000 cells vs 1 per 100 cells, respectively) (Figure 1).

Potentially, t(14;18) could provide a molecular marker that might be predictive for the development of follicular lymphoma. Several reports have associated t(14;18) with conditions related to risk of this NHL. Although not definitive, current studies have shown that the prevalence of t(14;18) is positively correlated with age (7, 8), heavy smoking (9), HCV infection (10), and pesticide exposure (11), all of which are also associated with increased risk for NHL. t(14;18) has also been reported to increase with sunlight exposure (12), but the epidemiological implications are uncertain because exposure to sunlight has not been associated with increased risk for (13) or mortality from (14) NHL.

While t(14;18) prevalence and frequency have been associated with other NHL risk factors, t(14;18) has not been directly investigated as a risk factor for NHL. Obstacles have included a lack of appropriate prediagnostic biospecimens, assays that require relatively large amounts of sample, and uncertainties regarding interpretation as a marker of heightened susceptibility vs an early marker for disease. To address these concerns, we have undertaken a series of studies to characterize these cells and relate them to risk of NHL:

Determinants of t(14;18) Prevalence and Frequency in Healthy Individuals

Given the low frequency of t(14;18) in lymphocytes of healthy individuals, assay sensitivity is an important factor in these studies. We have developed a highly sensitive real-time quantitative PCR (qPCR) to detect translocation sequences (15). The number of cells per PCR assay is quantitated by determining, in parallel, the level of a reference gene (*KRAS*). Amplification products of all PCR-positive samples are sized by agarose gel electrophoresis to identify samples with more than one unique sequence indicative of independent translocation events and hence multiple clones. Sequencing analysis in an earlier study (15) confirmed that all PCR products detected on the agarose gel are specific translocation fragments.

Using these techniques, we have demonstrated both the presence of multiple unique sequences in the same individual as well as the persistence of clonotypic sequences for up to 6 years without apparent development of lymphoma (16). In another study comparing peripheral blood lymphocytes from healthy German and Japanese individuals, 52% of the German sample but only 16% of the Japanese sample had detectable t(14;18) (17). Follicular lymphoma is the second most common type of NHL in the United States and Europe but accounts for less than 10% of all NHL among Japanese. The finding that the relatively low incidence of follicular lymphoma in the Japanese population is paralleled by a low background rate of this translocation may be further evidence that circulating t(14;18) is a precursor lesion for lymphoma (18).

It is noteworthy that the presence or absence of t(14;18) is not predictive of the presence of other translocations that have been detected in peripheral blood, such as the *MYC*-activating t(8;14) characteristic of Burkitt's lymphoma. For example, individuals infected with human immunovirus have an increased prevalence of circulating t(8;14) (19) but not of t(14;18) (20). Intriguingly, these results recapitulate the normal risk of follicular lymphoma and the increased risk of Burkitt's lymphoma, respectively, in patients with acquired immunodeficiency syndrome. There was also no association of t(14;18) frequency with low-level exposure to radiation, a clastogen but generally not associated with risk of NHL (21).

Immunophenotype of t(14;18) in Healthy Individuals

The t(14;18) translocation in B cell neoplasms is thought to result from an illegitimate variable diversity joining (VDJ) recombination (22), either during the initial VDJ recombination at the pro-B cell stage or during receptor editing. The aim of this project was to determine whether t(14;18) in healthy individuals results from similar processes and whether the abnormality interferes with subsequent differentiation of the affected cells.

Our first objective was to establish whether t(14;18)-positive cells in healthy individuals reside in a specific B-cell subset, with a working hypothesis that these cells would share phenotypic features with follicular lymphoma. We used magnetic (MACS) and fluorescence activated cell sorting (FACS) to fractionate lymphocytopheresis samples from healthy volunteers with high t(14;18) frequency. These experiments confirmed that t(14;18) frequency was highest in the IgM memory B-cell subset (IgM⁺ / IgG⁺ CD10^{-/+} CD19⁺ CD27⁺) that resembles follicular lymphoma. Like follicular lymphoma, t(14;18)-positive cells have undergone class-switch recombination of the nonproductive translocated IgH allele, lending further support for their common origin (23).

With this knowledge, we were then able to analyze sorted samples of this lymphocyte subset to increase the sensitivity of t(14;18) detection by real-time PCR. Our working hypothesis was that healthy individuals negative by conventional techniques in fact carry the translocation at a lower frequency. Indeed, we consistently detected t(14;18) in this B-cell subset, even for subjects whose unfractionated PBMCs were t(14;18) negative. This work provides evidence that similar processes lead to translocations in healthy individuals and lymphomagenesis (24).

Effect of Rituximab in IgM Neuropathy

If t(14;18) frequency represents a biomarker for lymphoma risk, an intervention to eradicate these cells may be warranted for individuals at high risk of NHL. One possible agent is rituximab, a monoclonal chimeric antibody (human constant region and murine variable sequences) against the B-cell surface molecule CD20. Rituximab has been used to treat malignancies and other disorders of B-cell function, including follicular lymphoma, idiopathic thrombocytopenic purpura, rheumatoid arthritis, systemic lupus erythematosus, and hemolytic anemia (25–27). The standard dose of four weekly infusions of 375 mg/m² leads to a complete depletion of peripheral blood B cells for about 6 months, without a significant drop of immunoglobulin levels or an increase of infectious complications (28).

In studies of minimal residual disease following treatment of lymphoma, we have observed that t(14;18)-positive cell clones unrelated to the lymphoma also disappear with rituximab therapy (G. Dölken, unpublished data). This fortuitous observation stimulated us to assess t(14;18) frequency following rituximab treatment of IgM-neuropathy, a nonmalignant condition associated with autoantibody to peripheral nerve myelin (29). In collaboration with a clinical trial for this condition, we are attempting to determine 1) durability of t(14;18) eradication during recovery of circulating B cells; 2) kinetics and maturation of circulating t(14;18)-positive cells that reappear in specific lymphocyte subpopulations following treatment; and 3) clonal relationship of t(14;18)-positive cells pre- and posttherapy.

Following similar experiments as performed on healthy donors, cell sorting and t(14;18) assays will be performed on samples from IgM neuropathy patients treated with either rituximab or placebo. First, we will examine the different B-cell subsets of pretreatment samples and sequence the translocation fragments as specific molecular markers for individual t(14;18)-positive cell clones. Second, t(14;18) levels 1–2 months after rituximab or placebo will be studied for the maximal effect of antibody treatment. Third, samples from 1 year after rituximab treatment or placebo will be assayed in the same way as the baseline samples to assess rituximab-induced changes in absolute numbers and relative frequency of different B cell subsets as well as the re-appearance kinetics of t(14;18)-positive cells in specific subsets. The results in patients who received placebo will indicate whether t(14;18)-positive clones remain stable or whether and to what extent clones re-emerge or disappear spontaneously. Immunophenotype information will provide an important clue to the stage of B cell development at which this translocation is generated (naïve = pregerminal center vs memory = postgerminal center cells), and analyses of the clonotypic translocation sequences will indicate whether t(14;18)-positive clones before and after rituximab treatment are identical or different. Reappearance of the same clones might indicate rituximab resistance of t(14;18)-positive cells in individuals without lymphoma, potentially hampering prophylactic use for high risk individuals. On the other hand, if t(14;18)-positive cells do not reappear when B cell levels return to normal, rituximab might be a feasible treatment to decrease risk for NHL.

Translocations and NHL in the PLCO and EPIC Cohorts

In upcoming work, we plan to investigate whether the prevalence or frequency of t(14;18) is associated with risk of subsequent follicular lymphoma in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) and the European Prospective Investigation into Cancer and Nutrition (EPIC). These large general population cohort studies have serial blood samples leading up to cancer onset, enabling serial t(14;18) measurements to examine temporal changes as well as absolute frequencies as risk factors predicting lymphoma development. Access to tumor tissue would enable comparison of translocation sequence in the lymphoma to that found in prediagnostic peripheral blood. These experiments will reveal whether the CT-bearing cells are clonally related and hence the timing of this mutation relative to tumor onset. We also aim to identify and characterize additional mutations of the precursor cells that subsequently evolve into malignancy because t(14;18) alone is clearly not sufficient for malignant transformation.

The buffy coats collected in epidemiological studies pose additional challenges for t(14;18) analyses. First, because granulocytes are the predominant white cell in blood, roughly three times as much buffy coat DNA has to be analyzed to achieve sensitivity comparable to isolated peripheral blood mononuclear cells. Analyses of 15 μg buffy coat DNA (equivalent to 2.25×10^6 white cells or roughly 0.75×10^6 mononuclear cells) are required for detection at the levels previously reported for healthy individuals. Because a maximum of 1 μg DNA can be tested per PCR assay, each sample requires 15 rather than 5 separate reactions. Second, inhibitory substances (probably traces of hemoglobin) in DNA extracted from buffy coat can decrease the sensitivity of the PCR assays. This possibility must be directly investigated by quality control experiments, and exceptional maneuvers to improve DNA purity must be tailored for the specific characteristics of each variation of buffy coat preparation (Figure 2).

An integral component of our approach is the analysis of paired blood and tumor specimens. Unlike blood assays, tumor analysis does not require large amounts of material, so a single histological section is frequently sufficient. Tumor microdissected from surrounding normal tissue is tested for the presence and sequence of t(14;18). The sequences may then be compared with any t(14;18) sequences in peripheral blood to determine clonal relationship and evolution. In examining risk factors for subsequent NHL, stronger associations should be present for the subgroup with t(14;18)-positive tumors. It is important to note that only half of follicular lymphomas are positive on the t(14;18) assay, which detects major breakpoint region (MBR) but not minor cluster region or other *BCL2* translocations. Peripheral blood PCR is uninformative regarding relapse in patients whose tumors are not detectable. Similarly, an epidemiological study that ignores tumor status could provide a misleading null result.

In summary, there is much to learn about the evolution of t(14;18) and its relationship to NHL. Chromosomal translocations are a critical molecular event in the development of hematopoietic malignancies. A better understanding of the special biology of t(14;18)-positive cells in healthy individuals could provide insight into their potential role as tumor precursors that may, in some instances, become clonogenic founders of NHL. Potential

applications include determination of elevated risk, early detection of disease, and identification of molecular targets for preventive interventions.

Acknowledgments

The authors thank Robert J. Biggar, MD, for helpful comments and suggestions on an earlier draft of the manuscript.

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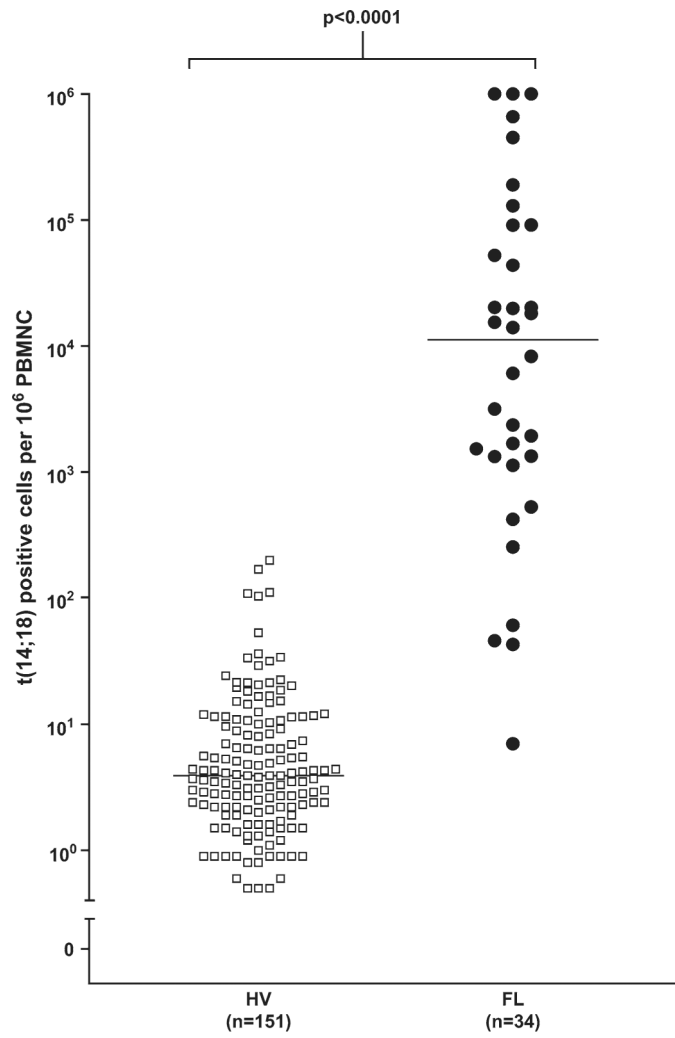


Figure 1. Comparison of t(14;18) frequencies between t(14;18)-positive healthy volunteers (HV) and patients with t(14;18)-positive follicular lymphoma (FL) before therapy.

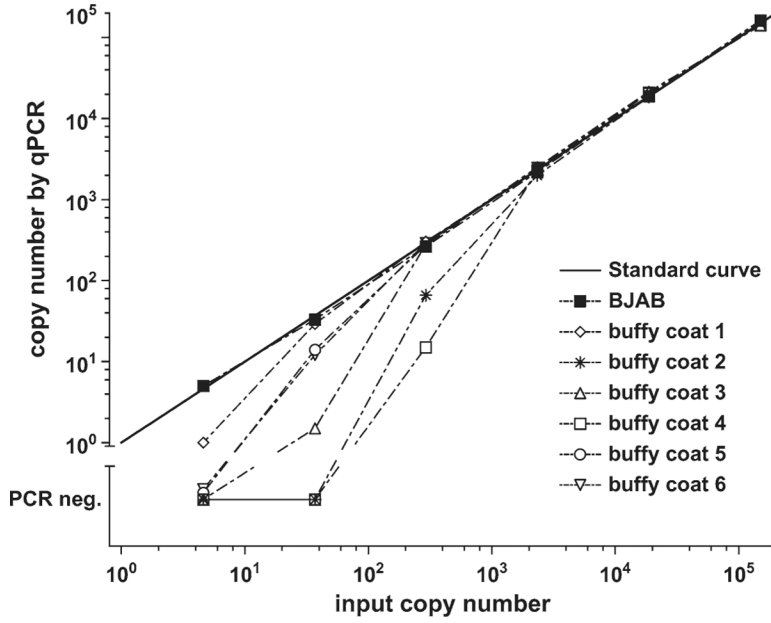


Figure 2. The graph shows t(14;18) measurements in serial dilutions of a t(14;18)-positive cell line (SU-DHL 6) in a t(14;18)-negative cell line DNA (BJAB) and six buffy coat-derived DNAs (buffy coat 1–6) in comparison to dilutions in water (standard). All buffy coat samples show significant inhibition in the five copy dilution with polymerase chain reaction (PCR)–negative results in 5/6 samples. In dilutions containing 40 t(14;18) copies 5/6 buffy coat DNAs caused a significant PCR inhibition with quantitative PCR (qPCR) results less than predicted based on standard curve and negative PCR results in 2/6 samples. Dilutions with BJAB cell line DNA gave qPCR results similar to the standard curve at all tested copy numbers.