Origin and migration of follicular lymphoma cells

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Follicular lymphoma (FL) was described for the first time by Brill and Symmers in 1925. The primary cytogenetic lesion, the t(14;18) was identified in 1982, and the breakpoint at *BCL2* in 1985. Based on observations that the t(14;18) originates from an erroneous recombination event in precursor B cells,[1] a model evolved in which the tumor develops linearly from such a precursor cell (Figure 1A). Other cytogenetic events frequently accompany the t(14;18), and various authors have attempted to distinguish different subgroups of FL with differences in biological behavior, risk of transformation to an aggressive lymphoma, prognosis and overall survival on the basis of these additional events.[2,3] Apart from these cytogenetic abnormalities not further discussed here, it is evident that FL represents a germinal center lymphoma with high expression of activation-induced deaminase (AID) and in consequence a pattern of ongoing somatic hypermutations of immunoglobulin (IG) loci.[4,5] This is not necessarily a continuous process since tumor cells may leave and (re)enter a germinal center and, in consequence, may periodically acquire novel somatic hypermutations of the *IG* genes. Indeed, by analysis of individual tumor cells or by molecular cloning of *IGH* rearrangements from a pool of tumor cells, it became evident that within each individual lymphoma not all tumor cells share the same somatic hypermutations. This implies subclonal evolution of the lymphoma, each clone being detectable by a unique fingerprint. Thus sampling of multiple (subsequent) lymph nodes of a FL patient might show different fingerprints of these mutations and this type of analysis may provide us with a "genealogical tree" of the individual lymphoma (Figure 1B).

The prototypic FL is a histologically low grade (grade 1 or 2) and clinically indolent lymphoma and affected patients have a median overall survival of approximately 7 years or longer. Of note the great majority of patients present with disseminated disease at the time of diagnosis implying an equally long or even longer period of subclinical disease. This is in line with the observations that approximately half of all healthy adult individuals harbor one or more B-cell clones with a t(14;18), only very few of these clones developing into clinically relevant disease. At present these cells are called "follicular lymphoma-like cells" or FLLC.[6-8] In fact occasional cells carrying these t(14;18) can already be identified in hyperplastic tonsils from children.[9] Besides, so-called follicular lymphoma *in situ* (FLIS) lesions can be identified in less than 3% of all reactive lymph nodes.[10] In these lymph nodes, some germinal centers are focally involved by FL cells as detected by immunohistochemistry, polymerase chain reaction analysis and *in situ* hybridization for the t(14;18).

Interestingly and in contrast to what was expected, these FLLC and likely also FLIS lesions represent expansions of low-affinity IgM(D) expressing (post-germinal) memory B cells that have accumulated high loads of somatic hypermutation.[4,7,8] These relatively recent observations led to models (Figure 1C) in which a full-blown FL might have developed from a t(14;18) carrying precursor B cell that migrated to the blood stream, and subsequently encountered the AID-induced somatic hypermutation machinery in germinal centers, giving rise to long-living FLLC or FLIS cells. According to such a model, additional genetic events such as mutations in the *CREBBP* gene[11-13] (upregulating BCL6) might consolidate the germinal center status of the lymphoma, allowing the cells to accumulate more genetic damage necessary to ultimately switch to a clinically relevant FL. Another, not mutually exclusive, observation is that many FL cells acquire a specific B-cell receptor, containing N-glycosylation motifs. These motifs can interact with stromal elements in the microenvironment, thereby promoting survival and/or proliferation of tumor cells as well.[14-16]

Most essentially, since FLLC and FLIS cells, like fully neoplastic FL cells, may also undergo multiple rounds of somatic hypermutation upon re-entry into germinal centers, this implies that somatic hypermutations as observed in FL are not necessarily produced in the malignant FL cells themselves but can also be generated earlier. This model with FLLC and/or FLIS as an intermediate step in lymphomagenesis (Figure 1C) is very difficult to prove *in vivo*, with only extremely rare observations supporting it (see below).

The publication by Wartenberg *et al.* in this issue of the journal[17] complements these observations. These authors studied the somatic hypermutation pattern of tumor cells in lymph nodes and bone marrow from three FL patients. In one patient a synchronous biopsy of the lymph node and bone marrow was investigated, in the two other patients two and five metachronous biopsies with a maximal interval of 3 years were taken. Using a novel mathematical approach the authors designed both compartment (lymph node/bone marrow) specific pedigrees, as well as a more global pedigree for the entire patient. Based on these calculations, they propose that FL cells start expanding within lymph nodes but may migrate early to the bone marrow and may stay there for long periods, likely years, in a relatively quiescent or dormant state. From the bone marrow these relatively less mutated "founder" FL cells may again invade the lymph nodes at relapse, giving support for bidirectional instead of unidirectional migration (Figure 1D). These data are in line with a previous publication[18] also showing that bone marrow lymphoma cells may represent relatively early subclones. However, using a more conventional algorithm, those authors were not able to determine the exact direction of the migration of cells (migration from or to the bone marrow or vice versa).

In fact these novel observations fit well in a model in which the bone marrow provides a niche for the neoplastic cells, allowing them to survive and repopulate the body again, even after chemotherapy. Indeed a bone marrow-specific niche has already been proposed by several groups.[18-20] In consequence it could even be that clonally related and bone marrow-resident FLLC, instead of fully malignant FL cells, cause a relapse of lymphoma (Figure 1E).

The type of mutation analysis of the *IG* genes may have its limitations since recent deep sequencing studies on FL, not only addressing the *IG* genes but also multiple other genes, targets or not of AID, showed that minor subclones with mutations, not detectable by conventional cloning techniques, may already be present very early, and may persist at different frequencies in different subclones.[12] A very elegant example was recently published by Weigert *et al.*[21] These authors investigated two follicular lymphomas that developed 7 years after allogeneic stem cell transplantation, both clinically evident in the donor and recipient. Both lymphomas were clonally related and harbored both common and different mutations of the *IG* and other genes. Moreover, clonally related FLLC cells could be detected in the original graft as well. Most importantly, deep sequencing of both lymphomas showed many similar mutations, however, often at very different frequencies. This suggests that most mutations in FL are already present in FLLC far before onset of the clinically detectable lymphoma, but are differently selected for.
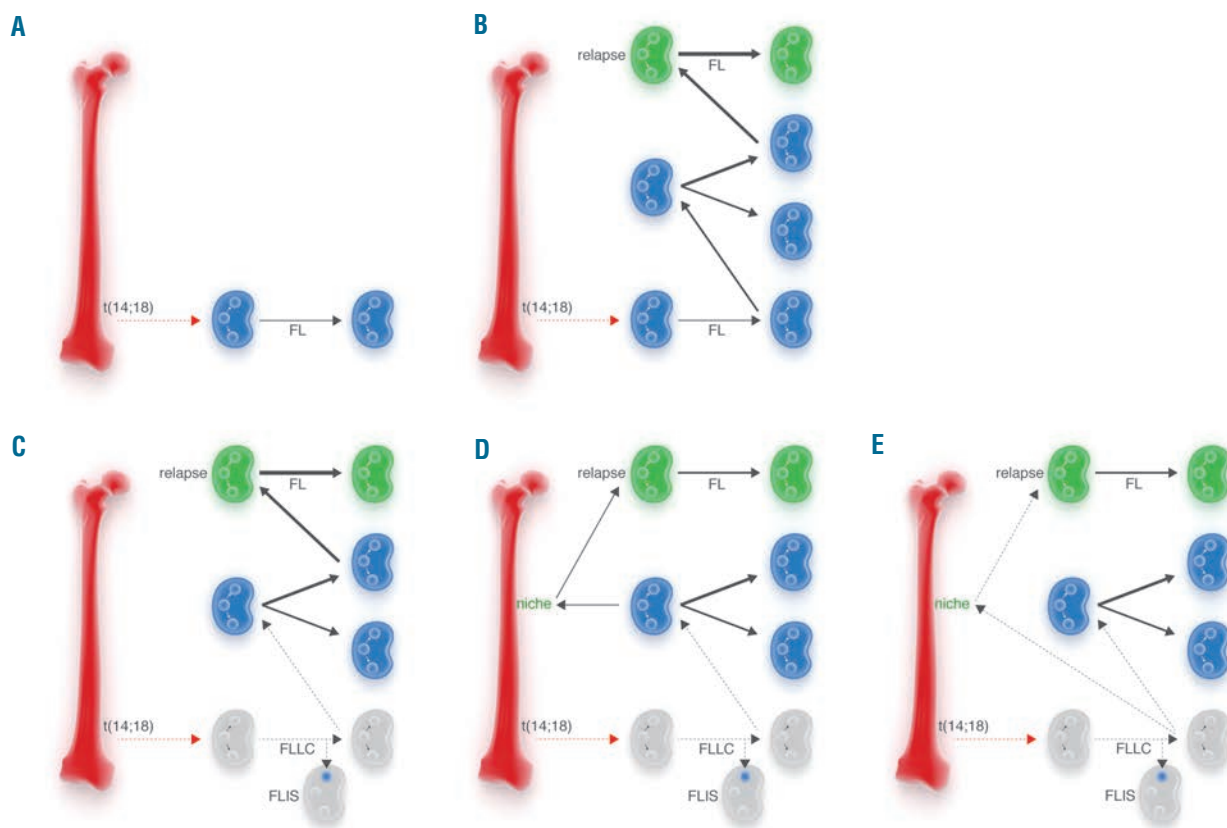
Figure 1. Models to explain the pattern of somatic hypermutations of the *IGH* locus in follicular lymphoma. Dotted arrows: premalignant t(14;18)-carrying B cells circulating in the body, as pre-germinal center B cells without accumulation of somatic hypermutation (red dotted arrow) but mainly as post-germinal center memory B cells (black dotted arrows). Straight black arrows: malignant t(14;18) carrying B cells circulating in the body, as post-germinal center memory B cells. The thickness of the lines indicates the load of somatic hypermutation in the B cells. Gray lymph nodes: normal lymph nodes that nevertheless may host t(14;18) positive "follicular lymphoma-like cells" (FLLC) as passengers, allowing them to expand and accumulate somatic mutations. Gray lymph nodes with single blue germinal center: idem, but containing FLIS. Blue lymph nodes: lymph nodes with follicular lymphoma. Green lymph nodes: lymph nodes with follicular lymphoma at relapse. (A) Model showing the original concept that the t(14;18) arises as an error in the bone marrow. Subsequently this cell is expanded in germinal centers of lymph nodes and other lymphoid organs, giving rise to follicular lymphoma after acquisition of additional genetic hits. (B) Model to explain the observed heterogeneity in mutational load in FL. This model suggests a stepwise accumulation of mutations with time, dependent on the frequency of re-entry in germinal centers and, thus the exposure to activation-induced deaminase (AID). (C) In this model new observations on premalignant FLLC that circulate in many healthy individuals but represent post-germinal center memory B cells are incorporated. Theoretically, some of these cells may colonize individual germinal centers and give rise to a "so-called follicular lymphoma *in situ*" (FLIS). Like malignant FL cells, these cells have already accumulated somatic mutations. Likely, in few individuals FLLC acquire additional oncogenic hits such as *CREBBP* mutations giving rise to a follicular lymphoma. (D) This model incorporates the observations published by Wartenberg *et al.*[17] These authors suggest that relatively fewer mutated FL cells may find a niche in the bone marrow. These cells may be relatively quiescent and in consequence escape the effect of chemotherapy, and may repopulate the body after therapy ("founder cells"). This might explain why there is no linear accumulative load of somatic mutations in FL patients. In fact in some patients, biopsies at relapse show fewer mutations than in the original diagnostic biopsy. (E) Model similar to that shown in (D). However, this model shows the theoretical possibility that also premalignant FLLC migrate back to the bone marrow and colonize the bone marrow niches, giving rise to late relapses.

What do the present data mean for our understanding of the behavior of FL and clinical practice? One suggestion made by the current and previous authors, is that the bone marrow may harbor a small pool of less-cycling "founder" cells, either fully malignant (FL) or "pre-malignant" (FLLC), which may be relatively resistant to chemotherapy, explaining the frequent but sometimes very late relapses observed in FL. It would, therefore, be very informative to have a more thorough characterization of the bone marrow resident cells in FL (and possible also other B-cell lymphomas) at the molecular level, although this is a major challenge due to their very low frequency and the fact that the bone marrow compartment is not easily accessible. Extensive mutation analysis of original lymphoma samples and their paired samples taken during late relapses might also be informative.

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Transplantation in follicular lymphoma: not "yes or no" but "whom and when"

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After years of debate, the question as to what is the optimal use of transplantation strategies in indolent lymphoma remains controversial. In the July issue of Haematologica, a study was published by the EBMT Lymphoma Working Party that aims to define indications for hematopoietic stem cell transplantation in follicular lymphoma in Europe.[1]

While autologous stem cell transplantation (ASCT) still offers the possibility of turning follicular lymphoma (FL) into a chronic rather than a life-threatening disease, with a modest impact on Quality of Life, allogeneic hematopoietic stem cell transplantation (allo-HSCT) as a treatment option has curative potential. However, its use has been limited to a selected patient population in which the disease risk outweighs the procedure-related morbidity and mortality. It, therefore, has been applied mainly after failure of autologous SCT. Now the introduction of dose-reduced intensity conditioning (RIC) with or without T-cell depletion has lowered the treatment-related mortality of allo-HSCT. In a recently published retrospective analysis of EBMT Registry data on patients in 2nd or higher treatment line, the survival curves for progression free (PFS) and overall (OS) survival appear to cross in favor of allogeneic SCT beyond the 2nd or the 8th year, respectively, despite an adverse risk profile of the allo-transplanted cohorts.[2] So, in the light of these improvements, could allogeneic SCT become the standard treatment for all eligible patients in relapse?

To answer this, it is important to understand how alter-native treatment options have been developed. Prior to the introduction of new agents in the treatment of lymphoma (namely rituximab), life expectancy was dramatically decreased by the diagnosis of FL, and responses to first-line treatment, and especially to later treatment lines, were frequently moderate and/or short lived. However, the introduction of the first anti-CD20 antibody has turned FL into a chronic disease for many patients. In addition, new treatment options, e.g. inhibitors of the B-cell receptor pathway, have appeared on the horizon and these promise new and potentially better treatment options. As the non-relapse mortality (NRM) of allo-HSCT still remains within the range of 15-25%, these developments make it hard to decide in favor of this type of treatment. So, should we forget about allogeneic transplantation?

Not yet! Although the last 15 years have seen improvements in allogeneic HSCT and in conventional therapy that, ironically, seem to favor opposing trends with a tendency to low-intensity treatments, we must bear in mind that patients continue to die of this disease. The younger the patients are, the higher the likelihood is they will lose many years of life. Consequently, the question has to be not "if", but "whom, how and when" to apply transplantation strategies in FL.

But how can we identify the right patient? There is an unfortunate lack of prospective randomized trials and comprehensive retrospective studies. Furthermore, primary treatment of FL is more diversified than in other