expression. Analysis of CIR for patients treated with versus without ATRA (26 patients with ATRA-ICE *vs.* 22 patients with ICE) showed a distinct difference for the subgroup with high or low Ski expression (Figures 1A and 1B). These 48 patients were also analyzed for relapse free survival (RFS). There was a trend towards longer RFS in patients with low Ski expression randomized to ATRA therapy. Of 8 patients with RFS over 1,000 days, 7 were in the ATRA-ICE, and one in the ICE group. However, the Gray test for the low Ski group was not significant (p=0.11). Interestingly, the cells of the 5 long-term RFS patients randomized to ATRA had very low Ski levels compared with other AML patients (relative Ski expression of those 5 patients: 0.03; 0.059; 0.098; 0.12; 0.158; compared with range 0.023-4.39 in all 132 AML samples (median 0.36)). Of the 5 patients with longest RFS, 4 were in the low and one in the high Ski group (Figure 2). These data suggest that patients with very low Ski expression levels might be a special subgroup responsive to ATRA treatment.

To summarize, there was a trend to prolonged RFS for patients in CR with low Ski expression treated with additional ATRA compared with those who received ICE treatment only. Interestingly, long term survivors (>1,000 days) treated with ATRA had very low Ski expression levels. In AML, low Ski expression seems to mark a subgroup responsive to ATRA when given in combination with ICE during induction chemotherapy. This should be addressed in future trials.

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NOTCH2 mutations in marginal zone lymphoma

The Notch family of proteins are highly conserved cell surface receptor proteins that are important for cell fate decisions and cell differentiation of various tissues of the body.[1] Ligand activation initiates a cascade of proteolytic events that translocates the intracellular part of the Notch protein to the nucleus where it activates different target genes. Some main targets are known.[1] However, much of the intricacies of the Notch signaling pathway still need to be discovered. It has been known for a while that Notch1 has a critical role in T versus B cell fate development from a common progenitor in the thymus[2-4] and is involved in leukemogenesis. Activating NOTCH1 mutations were recently found in more than 50% of T-ALL patients.[5] The mutations affect two main regions, the extracellular heterodimerization domain (HD) and the C-terminal transcriptional activation domain (TAD) or PEST domain.[5] Notch2 on the other hand promotes the selective development of B1 B lymphocytes[6] and is predominantly expressed in mature B cells.[7] Furthermore, several studies have shown that Notch2 is indispensable for the development of marginal zone B cells.[8-10] As a first step to investigate the involvement of Notch2 in B-cell lymphoma development, we studied oncogenic NOTCH2 mutations in several types of B-cell malignancies. The samples studied comprised 41 cases of marginal zone lymphoma (MZL) and 28 cases of other B-cell lymphoma types. The MZL were diagnosed as splenic (28 cases), nodal (3 cases) or extranodal MALT-type (10 cases) and were obtained from the Department of Pathology, The Norwegian Radium Hospital and from the Department of Pathology, University Hospital of Leuven, Belgium. All other lymphoma types were obtained from the Department of Pathology, The Norwegian Radium Hospital. In a selection of the lymphoma patients, tissues not involved by lymphoma were also studied. Additionally, two marginal zone cell lines, SSK41 and Karpas 1718, were studied. The procedures followed in the present study were in accordance with institutional ethical standards.

The detection of potentially oncogenic mutations in

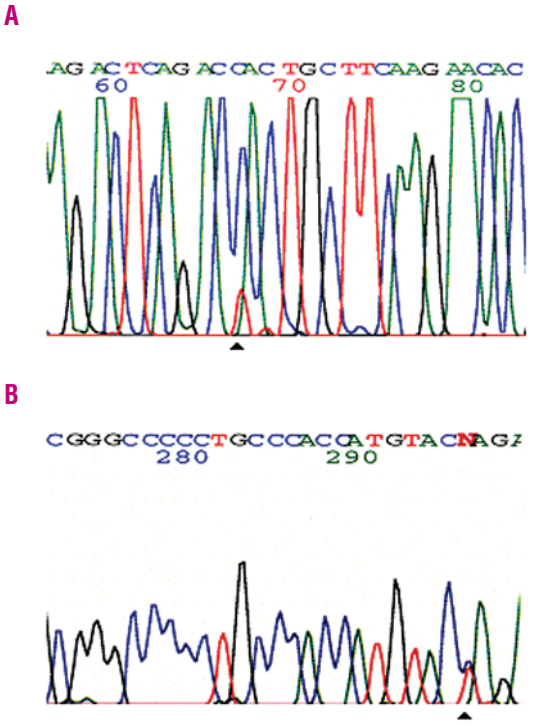

Figure 1. Chromatograms of NOTCH2 mutations identified in MZL samples by direct DNA sequencing. The arrows indicate the identified mutation. (A) Case 1. (B) Case 2.

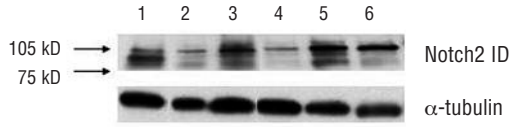

Figure 2. Immunoblot analysis of B-cell lymphoma cases. Lane 1: Case 2 with Stop codon in exon 34, Lanes 2 and 3: MZL case without mutation, Lanes 4 and 5: MCL control cases, Lane 6: DLBCL control case. Notch2 ID: Notch2 intracellular domain.

exons 26, 27 and 34 of *NOTCH2* was performed by direct sequencing from both sites of PCR products from isolated cDNA from frozen tissue by using the Big Dye terminator sequencing kit and Genetic Analyzer 3100 (Applied Biosystems, Wieterstadt, Germany). The sequences obtained were compared with wild-type NOTCH2 (NM_024408.2 or AL359752.11).

Interphase fluorescence *in situ* hybridization (FISH) for detection of structural chromosomal abnormalities involving NOTCH2 was carried out on nuclei isolated from frozen tumor tissue of 23 of the MZL cases, 2 of which also showed NOTCH2 mutations. Several probes mapping to different segments of NOTCH2 were used. FISH was performed according to standard methods. For the quantitative detection of NOTCH2 mRNA expression and one of the Notch target gene HES-1 commercial primers and probes (PE Applied Biosystems, Hs00225747 and Hs00172878) were used. An ABI PRISM 7700 instrument (PE Applied Biosystems) was used for the PCR. Relative mRNA concentrations were calculated using the comparative C_T method (PE Applied Biosystems, user bulletin No.2, 1997) with β-glucuronidase (GUS) as an internal control. All samples were compared to the relative mRNA level obtained in the SSK41 cell line. Immunoblot analysis was performed with frozen tissue sections lysed in lysis buffer, proteins were resolved by SDS polyacrylamide gel electrophoresis and hybridized with Notch2 ab (C651.6DbHN, The University of Iowa, IA, USA) and α-tubulin ab (Oncogene, Cambridge, MA, USA). In addition, we have tried to demonstrate Notch2 *in situ* expression by immunohistochemistry. Unfortunately, the available antibody has proven not to be useful for this technique.

We searched for possible oncogenic mutations in the *NOTCH2* gene in 69 cases of B-cell non-Hodgkin's lymphomas and identified 2 cases of MZL with *NOTCH2* mutations. No mutations were detected in the other B-cell lymphomas, although more cases will need to be studied to conclusively exclude mutations in other B-cell lymphoma types (Figure 1). Additionally, no mutations were detected in the 2 marginal zone lymphoma cell lines studied. Case #1, a MZL originating in the stomach, has a point mutation in the heterodimerization domain, located at nucleotide 5168 that results in an amino acid change at position 1638 (H1638Y) in the protein (numbered according to Accession NM_024408). This mutation is located between the two proteolytic sites S1 and S2. Case #2, a splenic MZL, reveals abnormalities close to the PEST sequence. This mutation is a nonsense mutation (Q2341STOP). Immunoblot analysis of this case shows the presence of both the full length intracellular domain of Notch2 and a shorter protein product of Notch2 intracellular domain (Figure 2). The size difference obtained between these two bands was about 15kD, as expected from the location of the STOP codon. These two mutations were not detected in the respective normal tissue from the same patient. We further identified two polymorphisms in *NOTCH2* in 2 of the lymphoma patients, not published in the dbSNP database (*www.ncbi.nlm.nih.gov/SNP/snp_blastByOrg.cgi*.) nor in the literature. These two polymorphisms (K1641T and P2359A) are located in the heterodimerization domain and the PEST domain. Interphase FISH, applied to MZL cases, did not reveal structural chromosomal abnormalities of the *NOTCH2* gene. The peak height for the mutation observed in case #1 (Figure 1A) seems rather low compared to the tumor content of the sample. It is, therefore, possible that the mutation only exists in a subclone. This might indicate that the *NOTCH2* mutation observed in this case is not a primary oncogenic event.

Thus, potentially oncogenic *NOTCH2* mutations were found in 5% of the MZL cases. We identified only single-base substitutions in *NOTCH2*. This is in contrast to the results obtained for NOTCH1 in T-ALL where also deletions and insertions were identified,[5] especially in the PEST domain. Both detected mutations in *NOTCH2* described here are novel. As with NOTCH1 in T-ALL, we found mutations both in the C-terminal heterodimerization domain and in the PEST domain of *NOTCH2*, suggesting that these mutations also may lead to increased Notch2 activity. Notably, one of the mutations introduces a STOP codon. Such mutations are also frequently found in NOTCH1. Of interest, we have previously demonstrated[11] that splenic MZL cases express elevated NOTCH2 mRNA levels compared to other lymphoma cases. This has also been confirmed in the present study, and we have additionally shown high Notch2 mRNA expression in other types of MZL with a mean relative mRNA expression of Notch2 of 117±97 (±SD) in MZL compared

to a mean relative expression of 40±28 (±SD) in other lymphoma cases (p<0.001, Mann-Whitney U-test). Furthermore, we obtained high expression of one of the Notch2 target genes HES1 with a mean relative mRNA expression of Hes1 of 1.1±1.0 (±SD) in MZL compared to a mean relative expression of 0.34±0.30 (±SD) in other lymphoma cases (p<0.001, Mann-Whitney U-test). These data are in agreement with the importance of Notch2 for marginal zone B-cell differentiation.[8-10] Interestingly, the level of NOTCH2 mRNA expression was not higher in the 2 MZL cases with mutations compared to MZL lymphoma cases without mutations. In conclusion, we identified potentially activating mutations of NOTCH2 in 5% of MZL cases, comprising a splenic and an extranodal MZL case. Additional studies are needed to clarify how these mutations affect Notch signaling and oncogenesis in these lymphomas.

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The IL6(-174G/C) polymorphism is a prognostic factor for survival after treatment initiation in Waldenström macroglobulinemia patients aged 65 years or less

Waldenström macroglobulinemia (WM) is characterized by the production of serum monoclonal immunoglobulin (Ig) M and by lymphoplasmacytic bone marrow infiltration.[1] Upregulation of interleukin 6 (IL6) has been demonstrated in WM,[2,3] in accordance with the increased IL6 serum concentrations previously observed.[4,5] Moreover, clonal blood B cells from patients with WM differentiated spontaneously *in vitro* to plasma cells via an autocrine pathway involving IL6.[6] Subjects with the C allele in the IL6–17[4] promoter region had lower serum IL6 concentration.[7,8] Therefore, we aimed to investigate the prognostic value of the IL6(-174G/C) polymorphism in WM.

One hundred patients with WM (M/F ratio: 1.7, median age: 67 years, range: 39-91) entered the study with the following inclusion criteria: (i) proven WM and (ii) initiation of therapy before January 2006 only in symptomatic patients according to the second international workshop on WM recommendations; (iii) informed consent obtained according to the protocol submitted to the Institution Review Board. Seventy-five patients required first-line therapy (at diagnosis: 48 patients and 4-114 months later: 27). Fifty-seven patients received chlorambucil, 3 combination chemotherapy, 9 fludarabine alone, 3 fludarabine in combination with other chemotherapy, and 3 rituximab. Median follow-up was 42 months (range: 17-180 months) in alive patients with a stopping date set at January 1st, 2007. IL6 genotype was assessed using an allelic discrimination assay performed on an ABI PRISM 7000 (Applied Biosystems, USA) as previously described.[9] The clinical and laboratory characteristics of the different genotypic groups and survival curves were compared using the Yates modified χ^2 test or the Fisher exact test and the log-rank test with bootstrap resampling (1,000 replicates) respectively. Characteristics associated with a significant prognostic value were introduced in a Cox proportional hazard model, after assessment of the validity of the assumption of this model. These analyses and differences in the distribution of age in genotypic subgroups prompted us to assess the prognostic value of the IL6 polymorphism in subgroups defined by age. All statistical analyses were carried out using the Splus 6.2 (MathSoft, Cambridge, MA,