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,

Table 1. Clinical characteristics of 75 patients at the time of initiation of therapy according to IL6(-174G/C) polymorphism.

	IL6(-174G/G)	IL6(-174G/C)	IL6(-174C/C)
Number of patients	22 (29%)	42 (56%)	11 (15%)
Median age and ranges (years)	64 (39-84)*	68 (44-91)*	73 (64-79)*
Gender			
Male	18 (82%)	28 (67%)	8 (73%)
Female	4 (18%)	14 (33%)	3 (27%)
Treatment initiation criteria			
Cytopenia	11 (50%)	23 (55%)	7 (64%)
Organomegaly	4 (18%)	8 (19%)	2 (18%)
Splenomegaly	1 (5%)	2 (5%)	0
Hyperviscosity	6 (27%)	7 (17%)	0
IgM related disorder	6 (27%)	2 (5%)	3 (27%)
Constitutional symptoms	4 (18%)	5 (12%)	3 (27%)
Hemoglobin level (g/dL) +			
>11.5	8 (36%)	13 (35%)	4 (37%)
≤ 11.5	14 (64%)	24 (65%)	7 (63%)
Platelet count (10^9/L) +			
>100	18 (86%)	33 (89%)	10 (92%)
≤ 100	3 (14%)	4 (11%)	1 (8%)
β2-microglobulin (mg/L) +			
≤ 3	13 (68%)	20 (74%)	5 (50%)
> 3	6 (32%)	7 (26%)	5 (50%)
Serum monoclonal protein concentration (g/L) +			
>70	2 (9%)	1 (3%)	0
≤ 70	20 (91%)	36 (97%)	11 (100%)
Serum albumin concentration (g/L) +			
>35	15 (71%)	29 (85%)	9 (82%)
≤ 35	6 (29%)	5 (15%)	2 (18%)
International Scoring System (ISSWM) +			
Low-risk	8 (42%)	9 (31%)	1 (11%)
Intermediate	7 (37%)	15 (52%)	5 (56%)
High risk	4 (21%)	5 (17%)	3 (33%)
Number of treatments			
1	15 (68%)	33 (79%)	9 (82%)
2 or more	7 (32%)	9 (21%)	2 (18%)

*pts: patients. * p=0.007 for the comparison between IL6(-174G/G) and other genotypes: IL6-(174C/G) or (C/C). No other statistically significant difference was observed between genotypic subgroups. +: the clinical characteristics at the time of initiating therapy were missing in some patients referred to our institutions several years later.*

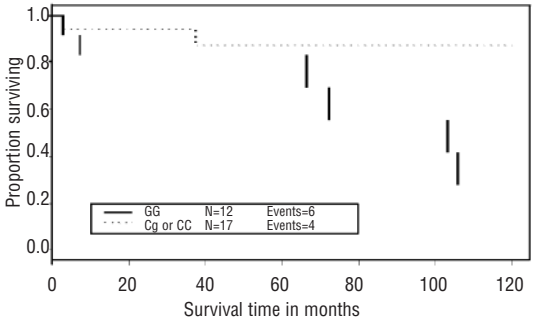

Figure 1. Actuarial survival curves of patients with Waldenström's macroglobulinemia aged 65 years or less, according to their IL6 (-174G/C) polymorphism status. The solid line indicates the IL6(-174 G/G) patients, the dashed line IL6(-174 G/C) or IL6(-174 C/C) patients (p=0.02).

USA) software.

The distribution of the IL6-174 polymorphism genotypes for the 100 patients were as follows: G/G, 33%; G/C, 52%; and C/C, 15%. The frequency of the IL6-174G allele was 0.59. This distribution did not differ from that expected from Hardy Weinberg equilibrium and agreed with previous reports.[7,9-11] The IL6(-174G/C) genotype of the 75 patients who received treatment and their main clinical characteristics at the time of first treatment initiation are reported in Table 1. Patients homozygous for the G allele (G/G) were younger than remaining patients (p=0.007). Mean monoclonal component concentration was 32.7 g/L in IL6(-174GG) patients and 23.3 g/L in the remaining patients (p=0.10). Thirty-two of the 75 patients (43%) who received treatment had died at the stopping date and the median survival after treatment initiation was 106 months (95CI: 74-197 months). Advanced age >65 years (p=0.005) and intermediate or high-risk according to the International Scoring System for WM (ISSWM, p=0.05) were associated with an adverse prognostic value for survival after treatment initiation. Multivariate analysis demonstrated the presence of an interaction between IL6(-174G/C) polymorphism and age. Indeed, the IL6(-174G/G) genotype was associated with an adverse prognostic value only in patients aged 65 years or less (6-year survival probability after treatment initiation: 55% for IL6(-174G/G) *vs.* 87% for IL6(-174C/G) or (C/C) patients, p=0.02, Figure 1). Three of the 25 remaining untreated patients had died from causes not related to the disease and the IL6(-174G/C) genotype had no prognostic value for the overall survival in the 100 patients.

For the first time, we describe an influence of the IL6(-174GG) polymorphism, previously associated with high expression level of IL6, on the outcome of WM after treatment initiation in patients aged less than 65 years. This observation was achieved in a large biological series of WM patients with prolonged follow-up and validated with bootstrap resampling, given the rarity of the disease. Previous results may support this finding: IL6 is expressed by tumoral cells,[2,3] IL6 favor the differentiation of blood B cells from WM patients via an autocrine pathway.[5,6] Moreover, IL6 induces rapid Akt phosphorylation[5,12] that promotes survival and growth of tumor cells.[5,12] Unfortunately, serum was not available and, thus, IL6 measurements could not be performed. Conflicting results were reported on the relationships between serum IL6 concentration and IL6(-174G/C) polymorphism, using various experimental protocols.[7-9] The numerous circumstances influencing IL6 levels, especially advanced age, the high clearance of serum IL6[10,11] and probable tumoral specific deregulations of IL6 in bone marrow support the analysis of the IL6(-174G/C) polymorphism, in place of serum analysis, to assess the overall expression profile of IL6. We cannot exclude a linkage dysequilibrium between the informative IL6 locus and one or more other informative genes that contribute to the pathogenesis of the disease. However, the presence of the C allele in the IL6–174 promoter region has also been associated with improved outcome in patients with breast cancer.[10] The IL6(-174GG) genotype was associated with young age. This pattern of distribution of age may counteract the adverse prognostic value associated with the IL6(-174GG) genotype in the 75 symptomatic patients. We recorded deaths not related to the disease in asymptomatic patients, whatever their IL6(-174G/C) polymorphism status. These findings probably explain the lack of prognostic value of the polymorphism in the whole series.

The present results should be viewed as exploratory and require confirmation in larger prospective studies. They indicate that IL6(-174G/G) genotype may be a new adverse prognostic factor in WM patients aged less than 65

years who require therapy. This finding supports the clinical significance of the genetic background of the IL6 pathway in WM.

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The MDM2 –309 T/G promoter single nucleotide polymorphism does not alter disease characteristics in chronic lymphocytic leukemia

A SNP in the MDM2 promotor region was identified and shown to directly influence MDM2 transcript levels with the subsequent attenuation of the p53 pathway.[1] Patients with Li-Fraumeni syndrome and with the SNP309 (G/G) developed cancers earlier than those individuals with a T/T genotype.[1] This has led to the investigation of the role of the MDM2 polymorphism in a variety of cancers with mixed results.[2] A recent meta-analysis found no effect of the polymorphism in colorectal and breast cancer, but showed a small and significant predisposition to lung cancer in carriers of the GG genotype.[3] A previous study focussed on childhood ALL and found that children with the MDM2-SNP309G genotype developed ALL at a younger age.[4] A very recent small study on 83 patients with CLL failed to show an impact of the MDM2 polymorphism but the limited cohort did not allow for a detailed analysis.[5]

Because of the well documented functional consequences of the MDM2 309 SNP and the important prognostic role of p53 in patients with CLL,[6] we studied the MDM2 SNP309 in a large cohort of CLL patients. Because of the high incidence of trisomy 12 (MDM2 locus) a possible effect of the polymorphism might be expected to be increased.

We studied the MDM2 SNP309 genotype in 617 patients with CLL. Detailed analysis was carried out on a cohort of 467 consecutive patients with clinical information from two centers in Germany (1990-1998 University of Heidelberg; n=225, thereafter from the University of Ulm; n=242) with available DNA.[6,7]

We used a DHPLC based method to detect the sequence variation at MDM2-SNP309. The PCR and heteroduplex protocols are available on request. The method was established on 20 samples which were also analyzed by sequencing. The optimal separation of the genotypes including the homozygous variants were seen at 64.2° and 66.5°. The different chemical properties of the T and G bases led to a clearly distinguishable and reproducible profile at 66.5° (*Online Supplementary Figure S1*). All samples in a prospective training set (n=80) were called correctly in the presence of no further sequence variation. RQ-PCR analysis was carried out as previously described.[7]

Hardy-Weinberg equilibrium was assessed by use of Fisher's exact test. The possible effect of MDM2-SNP309 genotype on the incidence of secondary cancers and a positive family history was analyzed by a conditional logistic regression analysis, stratified by center (likelihood ratio test). Prognostic factors for overall survival (OS) and time to first treatment (TFT) were analyzed using Cox's proportional hazards regression (by center). Besides the SNP, the models included age at diagnosis, Binet stage and a molecular genetic risk stratification factor. Missing values of explanatory variables were replaced using a multiple imputation technique using