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

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Acknowledgments: the authors wish to thank Valérie Grandières, Claudine Delsaut and Catherine Vanicatte for their excellent technical assistance.

Funding: this work was supported by the Comité du Pas de Calais de la Ligue contre le Cancer.

Key words: Waldenström's macroglobulinemia, Interleukin 6, polymorphism.

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Citation: Poulain S, Dervite I, Leleu X, Coiteux V, Duthilleul P, Morel P. The IL6(-174G/C) polymorphism is a prognostic factor for survival after treatment initiation in Waldenström macroglobulinemia patients aged 65 years or less. Haematologica 2008; 93:1109-1111. doi: 10.3324/haematol.12530

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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

Table 1. Genotype incidence in different clinical/genetic subgroups.

Subgroup	GG N (%)	GT N (%)	TT N (%)	total
Control[8]	150 (14%)	470 (44%)	445 (41%)	1065
CLL/IC (n=617)	79 (13%)	299 (48%)	239 (39%)	617
CLL (n=479)	54 (12%)	230 (49%)	185 (39%)	467
Binet A	32 (11%)	143 (49%)	118 (40%)	292
Binet B	12 (14%)	49 (56%)	27 (31%)	87
Binet C	2 (7%)	17 (63%)	8 (30%)	27
Female	22 (13%)	80 (48%)	65 (39%)	167
Male	32 (11%)	150 (50%)	118 (39%)	300
17p deletion	2 (9%)	7 (30%)	14 (61%)	23
11q deletion	6 (8%)	38 (53%)	27 (38%)	71
trisomy 12q	6 (8%)	34 (46%)	34 (46%)	74
13q deletion	20 (8%)	125 (49%)	110 (43%)	255
VH mutated	26 (13%)	90 (44%)	87 (43%)	203
VH unmutated	27 (11%)	126 (52%)	90 (37%)	243
2nd malignancy	2 (8%)	13 (54%)	9 (38%)	24
no 2nd malignancy	24 (13%)	92 (48%)	75 (39%)	191
Positive family history	17 (16%)	55 (51%)	35 (33%)	107
Negative family history	9 (9%)	49 (48%)	45 (44%)	103

Table 2. Cox proportional hazards regression on overall survival (OS) and time to first treatment (TFT).

OS (n=467; d[†]=108)

Variable	HR	95% CI
MDM2-SNP309		
T/G:G/G	0.74	0.37-1.45
T/T:G/G	0.74	0.37-1.51
Age (difference of 10 yrs.)	2.03	1.58-2.62
Binet stage		
B:A	1.99	1.19-3.32
C:A	3.64	1.65-8.04
Molecular genetic risk group		
II:I	3.51	1.82-6.77
III:I	6.35	3.13-12.89
IV:I	12.19	5.22-28.45

TFT (n=467; t[‡]=270)

Variable	HR	95% CI
MDM2-SNP309		
T/G:G/G	0.79	0.52-1.21
T/T:G/G	0.87	0.56-1.34
Age (difference of 10 yrs.)	1.07	0.93-1.23
Binet stage		
B:A	2.73	2.00-3.72
C:A	2.71	1.63-4.51
Molecular genetic risk group		
II:I	3.00	2.13-4.23
III:I	3.65	2.43-5.46
IV:I	5.46	3.16-9.44

[†]d: number of deaths; [‡]t: number of patients treated. Four risk groups in decreasing order: IV: 17p-, III: 11q- but no 17p-, II: VH unmutated without 17p- or 11q-, I: VH mutated without 17p- or 11q- .

chained equations (*Hmisc package [accessed July 31, 2007]. http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/Hmisc.*). The Wilcoxon rank sum test was used for pair-wise comparison of age of cancer diagnosis between the two centers. The Kruskal-Wallis test was applied to compare the age distribution between the three MDM2-SNP309 genotypes. Density estimation was made using kernel density estimation with a gaussian kernel. All statistical computations were performed using R, version 2.4.1, together with the R packages: genetics, version 1.2.0, Design, version 2.0-12, and Hmisc, version 3.3-1 (*http://www.R-project.org*).

As shown in Table 1, the genotype frequencies were similar across the control (GG: 14% GT: 44% TT:41%) and patient groups (GG: 13% GT: 48% TT:39%).[8] The frequencies were consistent with Hardy-Weinberg equilibrium. Different patient subgroups showed no significant differences in the distribution of genotypes (Table 1). Because of the high incidence of secondary cancers in CLL we analyzed this in a part of the cohort (n=215). We found no significant differences in the incidence of secondary cancers (LR test, p=0.78). In addition, we studied the relation between a family history (parents, siblings and children) of any cancer and the genotype groups. Table 1 shows the incidence of the SNP genotype in patients with and without a positive family history of cancer (n=210), which also did not show any influence based on the genotype (LR test, p=0.38). For both centers the differences in age at cancer diagnosis according to the SNP309 genotype were not statistically significant (Kruskal-Wallis test, p=0.86 for the Heidelberg subset and p=0.42 for the Ulm subset). When using the 51-year cut-off according to Bond *et al.*, the difference was not statistically significant (Fisher's exact test, p=0.09 for the Heidelberg subset and p=0.85 for the Ulm subset, resp.). We further studied the mRNA levels in a subgroup of the patients (excluding patients with trisomy 12 (gene dosage effect). There was no difference in MDM2 or p53 mRNA expression in the different genotypic groups (Kruskal-Wallis test, p=0.46/p=0.57)(*Online Supplementary Figure S2A*).

We did not observe any influence of the MDM2-SNP309 genotype on overall survival (OS) or time to first treatment (TFT) (Table 2, *Online Supplementary Figure S2B*). As expected, the Binet stage was predictive of clinical outcome (Table 2). We used a molecular genetic risk stratification by defining four risk groups in decreasing order, IV: 17p- (highest risk) (n=23), III: 11q- but no 17p- (n=71), II: VH unmutated without 17p- or 11q- (n=167), I: VH mutated without 17p- or 11q- (n=191). Overall the SNP309 genotype was not statistically significant (OS: p=0.66, TFT: p=0.52) whereas the molecular genetic risk groups were highly significant (OS and TFT: p<0.001; cf. (Table 2). We also did not observe any influence of the genotypes on OS or TFT within the set of patients with mutated or unmutated VH status (*data not shown*).

To summarize, we have genotyped a very large cohort of patients with CLL and were not able to find an effect of the polymorphism on age of disease onset, course or outcome suggesting that the MDM2-SNP 309 has no influence on disease characteristics in CLL. In addition to investigating a large cohort, we also studied the expres-

sion of MDM2 and p53 mRNA and could not detect different MDM2 mRNA levels based on the MDM2-309 genotype, suggesting that MDM2 levels are controlled independently in CLL.

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Acknowledgments: Elsbeth Brückle is gratefully acknowledged for her excellent technical assistance.

Funding: supported by HBFG and Jose Carreras Foundation grants (DJCLS R06/28v).

Key words: chronic lymphocytic leukemia, single nucleotide polymorphism; MDM2-SNP309.

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Citation: Zenz T, Häbe S, Benner A, Kienle D, Döhner H, Stilgenbauer S. The MDM2 -309 T/G promoter single nucleotide polymorphism does not alter disease characteristics in chronic lymphocytic leukemia. Haematologica 2008; 93:1111-1113. doi: 10.3324/haematol.12738

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A possible role for low-dose cyclosporine in refractory immune thrombocytopenic purpura

Management of patients with severe, refractory, chronic immune thrombocytopenic purpura (ITP) is still difficult. However, although there is no consensus on the best treatment strategy, these patients show a persistent, marked thrombocytopenia, and need therapy, owing to the presence of or to an increased risk of bleeding. Various treatments have been attempted: immunosuppressive chemotherapy, high-dose dexamethasone, danazol, combination chemotherapy, which have shown transient response in a variable percentage of cases, but none with any evidence of safe and durable efficacy.[1] Recently, a systematic review of more than 300 patients treated with a monoclonal anti-CD20 antibody, rituximab, many of whom with a severe form of ITP, has shown an overall platelet response of 62.5%, a median response duration of 10.5 months, but also significant toxicities. Therefore, the optimal timing and dose of the drug remain undefined.[2] More recently, good results have been reported with a multiagent induction and maintenance therapy, even if the duration of response was not defined.[3] Furthermore, an increase in platelet counts has been obtained using a thrombopoietin-receptor agonist, eltrombopag, though the durability of the response and the long-term safety of this compound are unknown.[4] Finally, a promising further approach seems to be the active and safe use of low dose rituximab.[5] As reported in published data, there are considerable side-effects associated with the current treatment and the responses of various therapies have not yet been consolidated. Other therapeutic strategies should, therefore, also be considered. There are few data in the literature describing the effects of cyclosporine (CyA) therapy in this setting. A study has been reported in adults in whom high toxicity offsets benefits,[6] and this finding has also been confirmed in children.[7] However, in both studies high doses of CyA (5-10 mg/kg/d) have been used.

We have already reported on long-term salvage therapy with CyA in 12 severe, refractory, chronic ITP[8] with 83.3% of response (10/12), lasting for a median follow-up of 36.8 months. The patients were 9 women and 3 men (median age 66.6 years, range 42-85 years). All patients had previously received 2-3 drug therapies and 8 patients had also undergone splenectomy. Only patients with platelet counts less than 30×10^9/L entered the study. All patients had major or minor bleeding episodes, often transient, but recurrent. Results of a long-term follow-up of the responsive patients are shown in Table 1. The updated median follow-up of approximately 5.5 years (69 months, range 4-13 years), shows that 9 patients had maintained response for the duration of the observation period (Figure 1). A further patient, not considered for the follow-up (n.8) died in complete remission of myocardial infarction during a 3-month course of CyA treatment. Five patients (ns. 1,2,7,9,and 10) had a complete response (platelet counts in normal range), one patient (n. 5) had a partial response (platelet counts between 80 and $150\text{x}10^9$/L), and 2 patients (ns. 3 and 12) had a complete response which had been maintained with continued drug administration. One patient (n. 4) had a drug-dependent complete response for approximately 4.5 years and a partial response for a further 1.5 year, after CyA had been tentatively discontinued and