MDM2 promotor polymorphism and disease characteristics in chronic lymphocytic leukemia: results of an individual patient data-based meta-analysis

Axel Benner,[1] Larry Mansouri,[2] Davide Rossi,[3] Aneela Majid,[4] Kerstin Willander,[5] Anton Parker,[6] Gareth Bond,[7] Sarka Pavlova,[8] Holger Nückel,[9] Olaf Merkel,[10] Paolo Ghia,[11] Emili Montserrat,[12] Mohd Arifin Kaderi,[2,13] Richard Rosenquist,[2] Gianluca Gaidano,[3] Martin J.S. Dyer,[4] Peter Söderkvist,[5] Mats Linderholm,[5] David Oscier,[6] Zuzana Tvaruzkova,[8] Sarka Pospisilova,[8] Ulrich Dührsen,[9] Richard Greil,[10] Hartmut Döhner,[14] Stephan Stilgenbauer,[14] and Thorsten Zenz[15,16] for the European Research Initiative on CLL (ERIC)

[1]Division of Biostatistics, German Cancer Research Center, Heidelberg, Germany; [2]Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Sweden; [3]Division of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy; [4]Department of Cancer Studies and Molecular Medicine, MRC Toxicology Unit, University of Leicester, UK; [5]Department of Clinical and Experimental Medicine, Linköping University, Sweden; [6]Department of Hematology, Royal Bournemouth Hospital, UK; [7]Ludwig Institute for Cancer Research, University of Oxford, UK; [8]University Hospital Brno and Central European Institute of Technology, Masaryk University, Brno, Czech Republic; [9]Department of Hematology, University Hospital, University of Duisburg-Essen, Essen, Germany; [10]Laboratory for Immunological and Molecular Cancer Research, University Clinics of Internal Medicine III with Hematology, Oncology, Hemostaseology, Infectious Disease and Rheumatology, Oncologic Center, Paracelus Medical University, Salzburg, Austria; [11]Laboratory of B Cell Neoplasia, Division of Molecular Oncology, Ospedale San Raffaele, Istituto Scientifico San Raffaele, Fondazione Centro San Raffaele, Università Bita-Salute San Raffaele, Milan, Italy; [12]Institute of Hematology and Oncology, Department of Hematology, Hospital Clínic, IDIBAPS, University of Barcelona, Spain; [13]Department of Biomedical Sciences, Kull Allied Health Sciences, International Islamic University Malaysia, Kuantan, Pahang, Malaysia; [14]Department of Internal Medicine III, University of Ulm, Germany; [15]Department of Translational Oncology, National Center for Tumor Diseases (NCT), German Cancer Research Center (DKFZ), Heidelberg, Germany; and [16]Department of Internal Medicine V, University Hospital Heidelberg, Germany

ABSTRACT

A number of single nucleotide polymorphisms have been associated with disease predisposition in chronic lymphocytic leukemia. A single nucleotide polymorphism in the MDM2 promotor region, MDM2SNP309, was shown to soothe the p53 pathway. In the current study, we aimed to clarify the effect of the MDM2SNP309 on chronic lymphocytic leukemia characteristics and outcome. We performed a meta-analysis of data from 2598 individual patients from 10 different cohorts. Patients' data and genetic analysis for MDM2SNP309 genotype, immunoglobulin heavy chain variable region mutation status and fluorescence *in situ* hybridization results were collected. There were no differences in overall survival based on the polymorphism (log rank test, stratified by study cohort; P=0.76; GG genotype: cohort-adjusted median overall survival of 151 months; TG: 153 months; TT: 149 months). In a multivariable Cox proportional hazards regression analysis, advanced age, male sex and unmutated immunoglobulin heavy chain variable region genes were associated with inferior survival, but not the MDM2 genotype. The MDM2SNP309 is unlikely to influence disease characteristics and prognosis in chronic lymphocytic leukemia. Studies investigating the impact of individual single nucleotide polymorphisms on prognosis are often controversial. This may be due to selection bias and small sample size. A meta-analysis based on individual patient data provides a reasonable strategy for prognostic factor analyses in the case of small individual studies. Individual patient data-based meta-analysis can, therefore, be a powerful tool to assess genetic risk factors in the absence of large studies.

Introduction

A number of single nucleotide polymorphisms (SNP) of different genes have been associated with disease predisposition.[1-4] While the potential effects of gene polymorphisms are widely acknowledged, most studies investigating the impact of individual SNPs on prognosis in chronic lymphocytic leukemia (CLL) have been controversial.[5-11] None of the allelic variants described so far contribute to relevant current clinical risk models in CLL.[12,13]

Sample sizes of studies to detect associations between SNP markers and clinical end points should be reasonably large, taking into account the very large number of candidate SNPs. In the absence of large studies, individual patient data (IPD)-based meta-analyses can provide a reliable assessment of possibly prognostic SNP markers. However, few IPD-based meta-analyses on SNP data have been performed so far.

A number of years ago, a single nucleotide polymorphism in the *MDM2* promotor region (IVS1+309) was discovered and shown to soothe the p53 pathway by influencing MDM2 transcript and protein levels.[14,15] Patients with the Li-Fraumeni syndrome develop cancers a decade earlier, when carrying the G/G allele of $MDM2^{SNP309}$. These important findings have led to the investigation of the role of the MDM2 polymorphism in a variety of cancers with conflicting results. A com-

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Correspondence: thorsten.zenz@nct-heidelberg.de

bined meta-analysis of breast, colorectal and lung cancers found no effect of the SNP on predisposition in colorectal and breast cancer, but showed a small but significant predisposition to lung cancer.[16] Most positive findings on the *MDM2*^{SNP309} genotype were related to disease onset rather than outcome.[16] The exceptionally well documented functional consequences of the *MDM2*^{SNP309} and the important prognostic role of p53,[17,18] as well as the particularly striking effects of the SNP in CLL in one study, has led to the study of the *MDM2*^{SNP309} in many centers, providing conflicting results.[5,6,9-11,19]

In the current study, we aimed to clarify the prognostic value of *MDM2*^{SNP309}, as well as the correlation between this SNP and the characteristics of CLL, by using an IPD-based meta-analysis.[20] To this end, we have collected data from 10 European centers contributing pre-determined clinical and genetic information on their patient cohorts. The combined data set is the largest cohort analyzed for the prognostic impact of genetic risk groups in CLL and thus constitutes a powerful data set to approach prognostic models in this disease.

Methods

Cohorts

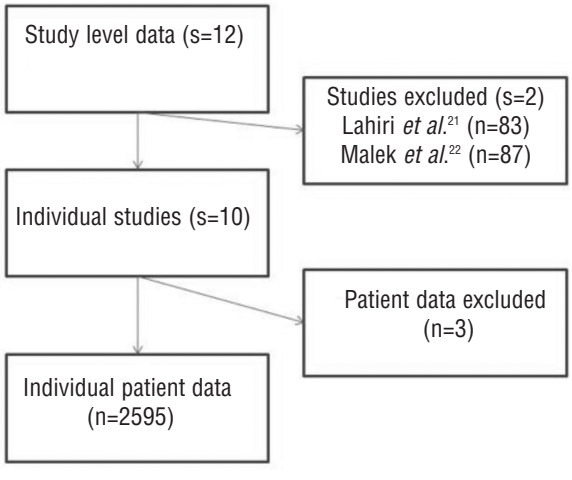
This meta-analysis includes individual patient data from 10 cohorts (n=2598) (Figure 1). The cohorts have, in part, been published previously.[5-9,11] Two data sets were unavailable either because of regulatory issues[21] or due to the decision of the investigator.[22,23] Tables 1 and 2 show the characteristics of the meta-analysis data set. Details are provided in the *Online Supplementary Appendix.*

Statistical analysis

Meta-analyses are applied to combine the results of related studies to produce an overall effect estimate. The large majority of meta-analyses use summary statistics supplied by the trial investigators or extracted from trial reports. Trial-level results are then combined to produce an overall effect estimate. A more powerful and less biased approach collects individual patient data (IPD) directly from the researchers responsible for each trial. Use of IPD has several advantages over the aggregate data approach, including standardization of statistical analyses, the assessment of potential causes of heterogeneity, and the investigation of interactions and non-linear effects. IPD-based meta-analysis is especially useful when time-to-event data are of interest. For survival analysis, the approach uses individual survival times and takes account of censoring. This provides a more powerful and flexible approach compared to the use of aggregate data since power is lost if time is ignored.

In our study, we apply an approach based on a single proportional hazards regression model stratified by trial. In order to incorporate random effects, a mixed-effect regression model is used. The primary end point of the IPD-based meta-analysis study presented here was overall survival (OS), and the second was time to first treatment (TFT). OS was defined as the time from diagnosis to death (event) or last follow up. TFT was defined as the time interval between the date of diagnosis and date of first CLL-specific treatment or death. TFT was censored at last treatment-free follow-up date. The log rank test, stratified by center, was used to compare survival curves with respect to *MDM2*^{SNP309} genotypes. For multivariable survival analysis, the proportional hazards regression model of Cox was applied including patients' sex, age, *IGVH* gene mutation status and FISH results. For the *MDM2*^{SNP309}, an additive genetic model was used with values 0, 1, and 2, indicating the number of copies of the variant G allele for each patient. A stratified approach was used, allowing each center to have a different base-line hazard. We applied mixed effects Cox proportional hazards models with a random center effect to consider between-center heterogeneity.[24] Univariable survival distributions according to genotype are provided as direct adjusted survival curves, adjusted for cohort.[25] Forest plots were used to present results for individual centers and the combined analysis. Cochran's Q was applied to test heterogeneity, whereas the I^2 index was used to quantify the degree of heterogeneity between cohorts. Bias and systematic heterogeneity was checked by contour-enhanced funnel plots, where the standard error is plotted against the log hazard ratio estimate.[26] The contour enhanced funnel plots also indicate regions of statistical significance at levels of 0.01, 0.05 and 0.1, testing the null hypotheses that log hazard ratios are equal to zero.

Figure 1. Summary of individual studies as reported.



Results

A total of 2598 patients were included in this study, gathered from 10 different single-center series from Europe. The comparison of the base-line characteristics of different cohorts is shown in Table 1, while time-to-event data (survival time, time to first treatment) are shown in Table 2. Median overall survival from diagnosis ranged from a median of 104 months to 301 months. Not surprisingly, there was a difference in outcome of the cohorts with respect to overall survival and time to first treatment reflecting the patient cohort differences and the respective referral patterns. As expected, the most important known genetic risk factors (17p-, 11q-, unmutated *IGHV* genes) showed hierarchical impact on OS (*data not shown*).

MDM2^{SNP309} genotype and disease characteristics

There was no significant difference in genotype distribution among cohorts (P=0.11). Furthermore, the *MDM2*^{SNP309} was not associated with particular disease characteristics, such as age of onset, 17p-, 11q-, +12q, or IGHV gene mutation status (Table 3). To assess the potential influence of the genotype on the patients' prognosis,

we analyzed TFT and OS using an additive genetic model. Results of the analyses of OS in individual cohorts and the summarizing IPD meta-analysis are illustrated by the forest plot shown in Figure 2. The funnel plot of individual studies was roughly symmetrical, indicating no evidence of publication bias in this study (Figure 3). Results for TFT were comparable. To account for heterogeneity, meta-analysis was carried out stratified for study cohorts.

Table 1. Comparison of clinical and biological characteristics between the ten study cohorts.

Factor	Overall (n=2598)	Brno (n=164)	Essen (n=166)	Heidelberg (n=225)	Leicester (n=402)	Linkoping (n=210)	Novara (n=331)	Salzburg (n=140)	Uppsala (n=418)	Ulm (n=242)	Bournemouth (n=300)	P
Age												< 0.001
Median	63	58.6	61.0	60.9	65.0	62.6	68.2	61.8	64.6	56.8	65.6	
Range	20.9 - 96.6	32.1 - 79.4	30.0 - 93.0	29.9 - 85.6	33.5 - 96.6	38.3 - 87.0	20.9 - 92.4	36.7 - 85.9	32.6 - 88.3	25.4 - 79.6	37.1 - 93.3	
n.a.	43		15		1		1				26	
Sex												0.001
Female	939 (36.2%)	61 (37.2%)	53 (31.9%)	90 (40.0%)	136 (33.8%)	64 (30.5%)	156 (47.1%)	51 (36.4%)	142 (34.0%)	77 (31.8%)	109 (36.7%)	
Male	1656 (63.8%)	103 (62.8%)	113 (68.1%)	135 (60.0%)	266 (66.2%)	146 (69.5%)	175 (52.9%)	89 (63.6%)	276 (66.0%)	165 (68.2%)	188 (63.3%)	
n.a.	3										3	
Binet stage												< 0.001
A	1392 (68.3%)	123 (77.8%)	117 (70.5%)	151 (74.0%)	108 (56.2%)	101 (52.3%)	256 (77.3%)		254 (73.2%)	141 (69.8%)	141 (57.6%)	
B	400 (19.6%)	13 (8.2%)	36 (21.7%)	41 (20.1%)	56 (29.2%)	41 (21.2%)	41 (12.4%)		66 (19.0%)	46 (22.8%)	60 (24.5%)	
C	246 (12.1%)	22 (13.9%)	13 (7.8%)	12 (5.9%)	28 (14.6%)	51 (26.4%)	34 (10.3%)		27 (7.8%)	15 (7.4%)	44 (18.0%)	
n.a.	560	6		21	210	17		140	71	40	55	
IGHV gene mutation status												< 0.001
Mutated	1345 (55.1%)	57 (35.8%)	51 (50.0%)	99 (45.0%)	276 (69.5%)	56 (31.8%)	201 (61.7%)	83 (65.9%)	241 (58.8%)	104 (46.0%)	177 (59.0%)	
Unmutated	1097 (44.9%)	102 (64.2%)	51 (50.0%)	121 (55.0%)	121 (30.5%)	120 (68.2%)	125 (38.3%)	43 (34.1%)	169 (41.2%)	122 (54.0%)	123 (41.0%)	
n.a.	156	5	64	5	5	34	5	14	8	16		
del 17p13												< 0.001
no	1967 (93%)	149 (90.9%)	130 (92.9%)	217 (96.4%)	266 (95.0%)		286 (89.4%)	118 (84.3%)	308 (93.3%)	227 (93.8%)	266 (96.7%)	
yes	149 (7%)	15 (9.1%)	10 (7.1%)	8 (3.6%)	14 (5.0%)		34 (10.6%)	22 (15.7%)	22 (6.7%)	15 (6.2%)	9 (3.3%)	
n.a.	482		26		122	210	11		88		25	
del 11q22.q23												< 0.001
no	1817 (87.6%)	130 (79.3%)	128 (91.4%)	193 (85.8%)	241 (86.1%)		297 (92.8%)	92 (97.9%)	286 (86.7%)	203 (83.9%)	247 (88.5%)	
yes	257 (12.4%)	34 (20.7%)	12 (8.6%)	32 (14.2%)	39 (13.9%)		23 (7.2%)	2 (2.1%)	44 (13.3%)	39 (16.1%)	32 (11.5%)	
n.a.	524		26		122	210	11	46	88		21	
trisomy 12q13												0.002
no	1715 (83.2%)	137 (84.0%)	124 (88.6%)	180 (80.0%)	227 (81.1%)		257 (80.3%)	79 (84.0%)	291 (88.2%)	213 (88.0%)	207 (77.2%)	
yes	347 (16.8%)	26 (16.0%)	16 (11.4%)	45 (20.0%)	53 (18.9%)		63 (19.7%)	15 (16.0%)	39 (11.8%)	29 (12.0%)	61 (22.8%)	
n.a.	536	1	26	0	122	210	11	46	88	0	32	
MDM2 SNP309 genotype												0.11
GG	318 (12.2%)	27 (16.5%)	26 (15.7%)	23 (10.2%)	43 (10.7%)	17 (8.1%)	49 (14.8%)	20 (14.3%)	43 (10.3%)	31 (12.8%)	39 (13.0%)	
TG	1195 (46%)	82 (50.0%)	77 (46.4%)	111 (49.3%)	175 (43.6%)	98 (46.7%)	159 (48.0%)	58 (41.4%)	190 (45.5%)	119 (49.2%)	126 (42.0%)	
TT	1084 (41.7%)	55 (33.5%)	63 (38.0%)	91 (40.4%)	183 (45.6%)	95 (45.2%)	123 (37.2%)	62 (44.3%)	185 (44.3%)	92 (38.0%)	135 (45.0%)	
n.a.	1				1							

Table 2. Comparison of time-to-event characteristics between the ten study cohorts.

	Overall (n=2598)	Brno (n=164)	Essen (n=166)	Heidelberg (n=225)	Leicester (n=402)	Linkoping (n=210)	Novara (n=331)	Salzburg (n=140)	Uppsala (n=418)	Ulm (n=242)	Bournemouth (n=300)	P
Follow-up												
Median	97.3	89.8	61	68.5	79.8	106.6	73.9	67	117.3	56.9	145.3	
n.a.	7				5						2	
Overall survival												< 0.001
Median	143.5	135.5	297	104.2	184.2	112.6	156.6	207	114.9	300.9	132.5	
CI95	135 - 153.8	114.6 - Inf.	199 - Inf.	97.9 - 132.8	163.4 - 228.3	92.9 - 143.5	137 - Inf.	165 - Inf.	106.2 - 125.7	170.6 - Inf.	117.1 - 157	
n.a.	7				5						2	
Time to first treatment												< 0.001
Median	44.9	42.7	82	50.1	18.2	20.7	77.3	74	47	40	44.6	
CI95	41.6 - 48.4	34 - 54	52 - 119	43.5 - 63	11.3 - 25.5	14 - 27.6	55.5 - 99.2	62 - 106.1	26.7 - 66.7	31.6 - 55.1	34 - 60.4	
n.a.	367				245	7			105		10	

Stratified log rank tests showed no significant differences based on the polymorphism with respect to OS and TFT (P=0.76 and P=0.58, respectively). Specifically, the GG genotype group had a cohort-adjusted median OS of 151 months compared to 153 months for the TG genotype group and 149 months for the TT genotype group (Figure 4). Cohort-adjusted median TFT was 43 months for the GG genotype group, and 42 months for the TG and the TT genotype group.

Because of the known interaction of MDM2 with the p53 pathway, we also assessed the impact of the SNP in the group with and the group without 17p deletion. In the subset of patients without 17p deletion, the survival distribution in the sets defined by the three genotypes were comparable (stratified log rank test: P=0.58). Accordingly, no statistically significant impact of the genotype could be demonstrated in the subset with 17p deletion (stratified log rank test: P=0.56). Similar results were obtained for TFT.

Multivariable meta-analysis

We performed a cohort-stratified Cox proportional hazards regression analysis to assess the impact of the *MDM2* genotype on overall and treatment-free survival considering known prognostic factors. The first model included *MDM2* genotype, sex, age and *IGHV* gene mutation status as well as the stratification according to cohorts. As shown in Table 4, advanced age, male sex and unmutated *IGHV* genes were significantly associated with inferior survival but not the *MDM2* genotype (P=0.31). Results for TFT were comparable.

In a separate model, we included cytogenetic aberra-

Table 3. Association of the *MDM2*^{SNP309} genotype and patients' characteristics.

Factor	Overall	GG	TG	TT	P
Age					0.68
Median	63	63.9	62.6	63.4	
Range	20.9 - 96.6	31.0 - 88.3	25.4 - 96.6	20.9 - 93.0	
n.a.	43	4	26	15	
Sex					0.68
F	939 (36.2%)	114 (35.8%)	442 (37.0%)	382 (35.3%)	
M	1656 (63.8%)	204 (64.2%)	751 (63.0%)	701 (64.7%)	
n.a.	3	0	2	1	
Binet stage					0.50
A	1392 (68.3%)	172 (66.7%)	649 (68.2%)	571 (69.0%)	
B	400 (19.6%)	60 (23.3%)	182 (19.1%)	158 (19.1%)	
C	246 (12.1%)	26 (10.1%)	121 (12.7%)	99 (12.0%)	
n.a.	560	60	243	256	
IGHV gene status					0.53
Mutated	1345 (55.1%)	159 (52.1%)	617 (55.2%)	569 (55.8%)	
Unmutated	1097 (44.9%)	146 (47.9%)	500 (44.8%)	451 (44.2%)	
n.a.	156	13	78	64	
del 17p13					0.91
no	1967 (93%)	252 (92.3%)	917 (93.0%)	798 (93.1%)	
yes	149 (7%)	21 (7.7%)	69 (7.0%)	59 (6.9%)	
n.a.	482	45	209	227	
del 11q22.q23					0.64
no	1817 (87.6%)	237 (89.1%)	841 (87.0%)	739 (87.9%)	
yes	257 (12.4%)	29 (10.9%)	126 (13.0%)	102 (12.1%)	
n.a.	524	52	228	243	
trisomy 12q13					0.17
no	1715 (83.2%)	221 (84.0%)	815 (84.5%)	679 (81.3%)	
yes	347 (16.8%)	42 (16.0%)	149 (15.5%)	156 (18.7%)	
n.a.	536	55	231	249	

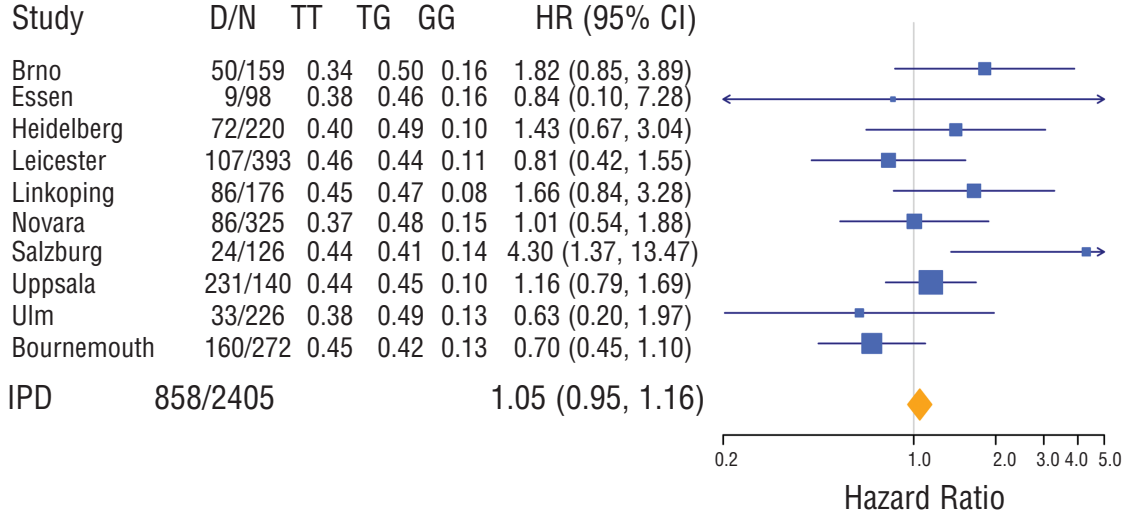


Figure 2. Forest plot of the IPD-based meta-analysis for overall survival. The plot shows the individual results of univariable Cox regression models for each study cohort together with the meta-analysis result with respect to the *MDM2*^{SNP309} genotype using an additive genetic model. The estimated hazard ratios, HR, together with their 95% confidence intervals are shown numerically and graphically. In addition the numbers of events, D, and observations, N, as well as the proportions of the three genotypes are provided. The results of individual studies are shown as squares centered on each cohort's point estimate of the hazard ratio (HR). Size of a square represents the precision of the estimate (the inverse of the squared standard error). 95% confidence intervals are represented by horizontal lines. The overall HR estimate from the IPD meta-analysis and its confidence interval are shown at the bottom, represented by a diamond. Testing heterogeneity resulted in Q(df=9) = 14.9, P = 0.10, with I^2 =39%.

tions (17p-, 11q-, +12q). In this model, sex, age, *IGHV* gene mutation status, and cytogenetic aberrations were associated with increased risk of death. Again, the *MDM2*^{SNP309} genotype was not significantly associated with overall survival (P=0.28; Table 4). Considering treatment-free survival, age (P=0.10) and the *MDM2*^{SNP309} genotype (P=0.85) were the only factors that were not statistically significant.

Discussion

The current study used individual patient data to assess the role of a particular host genetic factor in CLL. In contrast to other large studies, we focused on the prognostic impact of the MDM2 polymorphism. The size of the cohort allowed us to perform subgroup analysis with sufficient statistical power to account for the biological heterogeneity of CLL.

In response to cellular stress, the p53 protein is stabilized and regulates the activity of key effectors of cellular processes, such as DNA repair, cell-cycle arrest, senescence, and apoptosis. These p53-mediated responses are crucial both in reducing cancer frequency and in mediating the response of commonly used cancer therapies. Not surprisingly, p53 loss or mutation leads to very poor outcome in hematologic malignancies, and the impact is particularly striking in CLL. There is a large body of evidence suggesting that the p53 pathway harbors functional inherited single-nucleotide polymorphisms (SNPs) that affect p53 signaling in cells, resulting in differences in cancer risk and clinical outcome in humans (reviewed by Grochola *et al.*[27]). Recent elegant work with mice carrying the *MDM2*^{SNP309G} allele showed that *MDM2*$^{SNP309G/G}$ cells exhibit elevated mdm2 levels, reduced p53 levels, and decreased apoptosis with the *MDM2*^{SNP309G} allele potentiating the tumor phenotype, and altered the tumor spectrum in mice inheriting a p53 hot-spot mutation.[28]

Although the number of studies on the prognostic impact of individual SNPs in cancer, including CLL, is rapidly increasing, the validation of the results obtained in these studies is often disappointing. Meta-analysis has become an essential part of the process of SNP evaluation. In CLL, IPD-based meta-analysis have so far been limited

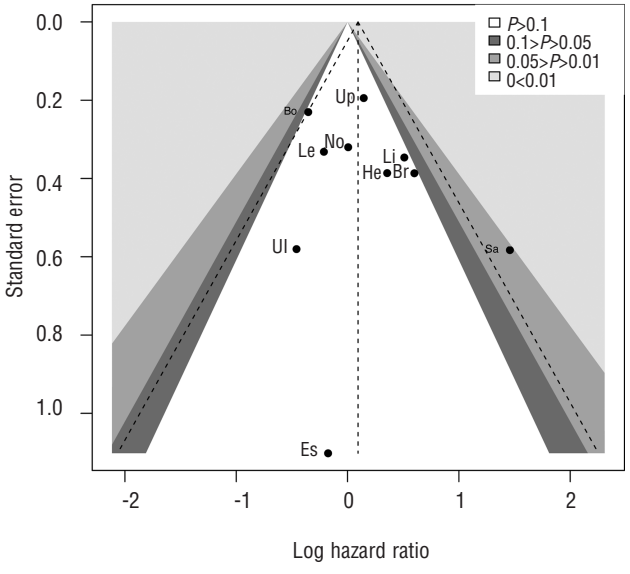


Figure 3. Contour-enhanced funnel plot of the OS analysis. The black dots indicate the cohort-specific estimates of the log hazard ratio and their standard errors; the vertical dashed line shows the summary estimate. Cohorts are indicated by the first two letters of their names. The shaded regions reveal the *P* value in each study relating to the test of the null hypothesis that the log (hazard ratio) is zero. The funnel plot of the OS analyses with respect to *MDM2*^{SNP309} genotypes is roughly symmetrical, indicating no evidence of publication bias in this study.

Table 4. Cox regression models for overall survival, stratified by study center. Stratified Cox proportional hazards model including covariates age, sex, IGHV mutation status and MDM2^{SNP309} (10 centers) *and* extended model, additionally including cytogenetic aberrations (17p-,11q- and +12q; 9 centers).

	Effect	Hazard ratio	95% CI	*P*
Age	10 years	1.94	1.80 - 2.09	< 0.001
Sex	M:F	1.61	1.39 - 1.86	< 0.001
IGHV gene mutation status	UM:M	3.18	2.74 - 3.68	< 0.001
MDM2^{SNP309}	dG*	1.11	0.91 - 1.35	0.31

	Effect	Hazard ratio	95% CI	*P*
Age	10 years	1.94	1.78 - 2.12	< 0.001
Sex	M:F	1.58	1.32 - 1.88	< 0.001
IGHV gene mutation status	UM:M	3.06	2.55 - 3.68	< 0.001
17p13-	yes:no	3.14	2.45 - 4.03	< 0.001
11qq23-	yes:no	1.48	1.19 - 1.84	< 0.001
+12q13	yes:no	1.44	1.19 - 1.76	< 0.001
MDM2^{SNP309}	dG*	1.14	0.90 - 1.45	0.28

**dG: additional copy of G allele.*

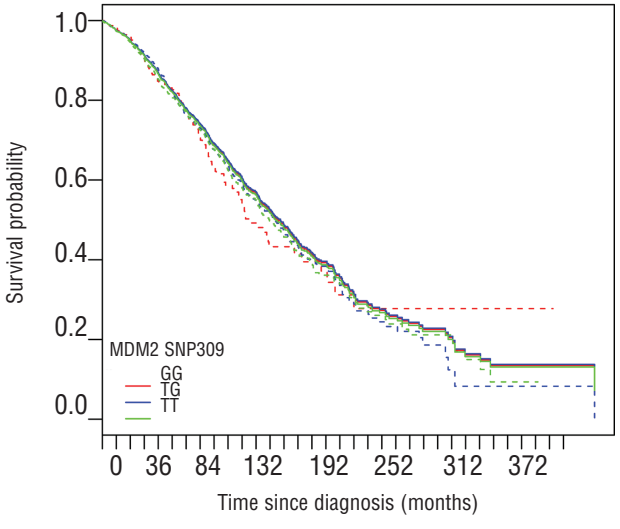


Figure 4. Estimated distributions of overall survival. The solid lines show the direct adjusted estimates of survival curves corresponding to *MDM2* genotype. Adjustment was made with respect to the different cohorts of the study. Dashed lines represent the unadjusted Kaplan-Meier estimates.

to clinical trial analysis focusing on clinical questions such as early *versus* late treatment,[29] immunoglobulin prophylaxis[30] or response to treatment.[31,32] This is, therefore, the first IPD based meta-analysis in which the clinical and prognostic significance of a biomarker such as *MDM2* gene polymorphism is evaluated. To that purpose, we have compiled a unique data set of 2598 patients from 10 centers using IPD-based meta-analysis to analyze the association between *MDM2*^{SNP309} polymorphism, genetic and clinical features and clinical outcome in CLL. We observed a strong heterogeneity in the cohorts studied, likely to be due to referral patterns and health care differences across countries. The incidence of the *MDM2*^{SNP309} genotype in different studies argues for comparability of the different techniques used to detect it.

In contrast to single center studies, we did not find a correlation between *MDM2*^{SNP309}, disease characteristics and outcome, including patients' age at diagnosis, genetic lesions, *IGHV* mutations, TFT or overall survival. On the other hand, the current study provides a unique dataset to estimate the impact of genetic aberrations and *IGHV* mutational status for outcome of patients with CLL. There are additional smaller studies that we have not been able to include in the analysis for various reasons;[21-23,33] based on the limited number of cases involved the omission is unlikely to affect the results.

In our analysis, we have used an additive genetic model considering the number of copies of the variant G allele and stratification by study cohort. We have also analyzed the data using mixed effects Cox models with random cohort effect providing comparable results (*data not shown*). One shortcoming of our study is the lack of data on TP53 mutations, which are now accepted as markers of poor prognosis in CLL but which were not widely available when the series analyzed here were investigated. The analysis within the group of patients with 17p deletion did not show an impact of the MDM2^{SNP309} suggesting that, in CLL, interaction with p53 status is unlikely. While no sample exchange and validation experiments were performed, the incidence of the SNP allele distribution was similar across centers suggesting that technology reproducibility was not a confounding factor. While comprehensive data on the mRNA or Protein expression of mdm2 in CLL are missing, a larger study detected no impact of the MDM2^{SNP309} on mRNA levels.[11] Based on our data, further meta-analyses are encouraged; this will help classify and develop the hierarchy of relevant prognostic factors in this disease. This is particularly important as, with the identification of novel mutations (e.g. SF3B1, NOTCH1, BIRC3, ATM, MYD88), the number of prognostic factors has increased, necessitating large cohorts to avoid over-fitted models.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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