Scan of 977 nonsynonymous SNPs in CLL4 trial patients for the identification of genetic variants influencing prognosis

Gabrielle S. Sellick,[1] Rachel Wade,[2] Susan Richards,[2] David G. Oscier,[3] Daniel Catovsky,[4] and Richard S. Houlston[1]

[1]Section of Cancer Genetics, Institute of Cancer Research, Sutton; [2]Clinical Trial Service Unit, University of Oxford, Oxford; [3]Department of Haematology, Royal Bournemouth Hospital, Bournemouth; [4]Section of Haemato-Oncology, Institute of Cancer Research, Sutton, United Kingdom

To identify genetic variants associated with outcome from chronic lymphocytic leukemia (CLL), we genotyped 977 nonsynonymous single nucleotide polymorphisms (nsSNPs) in 755 genes with relevance to cancer biology in 425 patients participating in a phase 3 trial comparing the efficacy of fludarabine, chlorambucil, and fludarabine with cyclophosphamide as first-line treatment. Selection of nsSNPs was biased toward those likely to be functionally deleterious. SNP genotypes were linked to individual patient outcome data and response to chemotherapy. The effect of genotype on progression-free survival (PFS) and overall survival (OS) was assessed by Cox regression analysis adjusting for treatment and clinico-pathologic variables. A total of 78 SNPs (51 dominantly acting and a further 27 recessively acting) were associated with PFS (9 also affecting OS) at the 5% level. These included SNPs mapping to the immune-regulation genes *IL16* P434S ($P = .03$), *IL19* S213F ($P = .001$), *LILRA4* P27L ($P = .004$), *KLRC4* S29I ($P = .007$), and *CD5* V471A ($P = .002$); and DNA response genes *POLB* P242R ($P = .04$) and *TOPBP1* S730L ($P = .02$), which were all independently prognostic of immunoglobulin heavy-chain variable region (IgV_H) mutational status. The variants identified warrant further evaluation as promising prognostic markers of patient outcome. To facilitate the identification of prognostic markers through pooled analyses, we have made all data from our analysis publicly available. (Blood. 2008; 111:1625-1633)

Introduction

B-cell chronic lymphocytic leukemia (B-CLL) accounts for approximately 25% of all leukemia and is the most common lymphoid malignancy in Western countries.[1] Although staging systems (Binet, RAi) are useful for predicting prognosis and treatment requirements,[2] there is variability in clinical outcome for patients with apparently the same stage of disease. Hence, accurate assessment of prognosis would be beneficial in the choice of specific therapeutic options, while assessment of the likelihood of response and adverse reactions to chemotherapeutic treatments would allow patient-tailored decisions on drug selection and consequent improvements in survival.

Molecular markers such as immunoglobulin heavy-chain variable region (IgV_H) mutational status have been shown to greatly assist in defining patient prognosis.[3-5] In addition to somatic differences, it is probable that germ-line variation also plays a role in defining individual patient outcome. Studies that have investigated this possibility have to date only evaluated a restricted number of genetic variants in a restricted number of candidate genes—polymorphisms in the coding sequence of *P2×7*,[6] *GNAS*,[7] *SDF-1*,[8] *MTHFR*,[9] *p53*,[10] and polymorphisms in the putative promoter sequences of *TNF*α,[11] *IL-1* and *IL-6*,[12] *BCL-2*,[13] and *BAX*.[14,15] However, many of these purported associations such as those in *P2×7*, *BAX*, and *p53* have been unconfirmed by independent studies.[16-19]

To date, most searches for polymorphic markers of prognosis have been formulated on an "educated guess"; however, without a full understanding of the biology of CLL, definition of additional "suitable" candidate genes is problematic. This makes searches on a "genome-wide" basis an attractive proposition. A further issue with many of the previously reported studies is that they have been based on small numbers of patients ascertained outside the context of a clinical trial and in addition to having limited power, are prone to bias as a consequence of survivorship and other cofounders, especially when based on retrospectively ascertained patients who have received nonuniform treatment.

We have recently conducted an association study based on an analysis of nonsynonymous single nucleotide polymorphisms (nsSNPs) in genes with a priori relevance to cancer biology to identify susceptibility alleles for CLL.[20] As nsSNPs alter the encoded amino acid sequence, they have the potential to directly affect the function of expressed proteins. A total of 425 patients with CLL genotyped in this study were participants in a large phase 3 clinical trial. Linking genetic data to clinical outcome information for these patients has allowed us to test, in an unbiased fashion, the hypothesis that sequence variation defined by these nsSNPs influences the clinical behavior of CLL.

Methods

Patients

The study is based on patients entered in the UK Leukemia Research Fund (LRF) CLL-4 trial. Full details about the design and conduct of the trial are described in previously published material.[21] Briefly, CLL-4 was a randomized phase 3 trial established to compare the efficacy of fludarabine,

Submitted August 30, 2007; accepted November 5, 2007. Prepublished online as *Blood* First Edition paper, November 15, 2007; DOI 10.1182/blood-2007-08-110130.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

chlorambucil, and the combination of fludarabine plus cyclophosphamide as a first-line treatment for Binet stages B, C, and A-progressive CLL. The trial as a whole recruited from a total of 136 centres, primarily from the United Kingdom. Of the 777 patients entered into the trial, the current analysis is based on a random subset of 425 white patients from 110 of the participating centers which had blood samples taken for clinical diagnostic purposes and cell marker studies. Ethical approval for the study was obtained from the Multi-Center Research Ethics Committee in accordance with the Declaration of Helsinki.

Selection of candidate genes, SNP selection and genotyping

Candidate gene selection and SNP genotyping has been described previously.[22] Briefly, nsSNPs mapping to candidate cancer genes were identified by interrogation of the Predicted Impact of Coding SNPs (PICS) database[23] of potential functional SNPs and published work on the resequencing of DNA repair genes. To increase the likelihood of identifying susceptibility alleles, we analyzed nsSNPs in 871 genes with relevance to tumor biology, biasing selection of SNPs to those likely to directly have functional consequences. nsSNPs were genotyped across patient DNAs extracted by standard salt-lysis protocol using customized Illumina Sentrix Bead Arrays according to the manufacturer's protocols (Illumina, San Diego, CA).

To predict the impact of missense variants on protein function, we applied 2 in silico algorithms, polymorphism phenotyping (PolyPhen)[24] and sifting intolerant from tolerant (SIFT).[25] PolyPhen predicts the functional impact of amino acid changes by considering evolutionary conservation, physicochemical differences, and the proximity of the substitution to predicted functional domains and/or structural features. SIFT predicts the functional importance of amino acid substitutions based on the alignment of orthologous and/or paralogous protein sequences. SIFT and PolyPhen scores were classified as intolerant, potentially intolerant, borderline, or tolerant and probably damaging, possibly damaging, potentially damaging, borderline, or benign, respectively, according to the classifications proposed by Xi et al[26] and Ng and Henikoff.[25] Where it was not possible to derive SIFT or PolyPhen metrics, we categorized codon replacements on the basis of Grantham matrix[27] according to the classification of Li et al.[28]

Statistical methods

To examine for systematic biases, we assessed the deviation of the genotype frequencies in the patients from that expected under Hardy-Weinberg Equilibrium (HWE) using a quantile-quantile (Q-Q) plot and estimated an overdispersion factor λ[29] by calculating the ratio of the mean of the smallest 90% of observed test statistics to the mean of the corresponding expected values.

Progression-free survival (PFS) and overall survival (OS) of patients were the end points of the analysis. The response recorded for each patient was the best achieved at any time due to first-line treatment. PFS was defined as the survival time from the date of randomization to relapse needing further therapy, progression, or death from any cause. For nonresponders and progressive disease, the date of progression was when no response or progressive disease was recorded. OS was calculated from randomization to death from any cause. Data on age at diagnosis, stage, sex, and treatment were available for all patients, and IgV_H mutation status data were available for a subset of patients, enabling us to examine the significance of each SNP conditional on these covariates. Cox-regression analysis was used to estimate genotype-specific hazard ratios (HRs) and 95% confidence intervals (CIs). For each SNP genotype, HRs were generated using common allele homozygotes as the reference group. *P* values presented correspond to the significance of a test difference among all 3 of the genotype groups (common allele homozygote, heterozygote, and rare allele homozygote). For SNPs where 5 or fewer minor allele homozygotes were observed, minor allele homozygote genotypes were combined with heterozygotes. If this combined frequency was still 5 or less, then the SNP was removed from the analysis. HRs, 95% CIs, and associated *P* values under both dominant and recessive models were also generated. To assess the distribution of test statistics, we generated Q-Q plots. To investigate possible epistatic interactions, the relationship between PFS and each pair of SNPs that showed significant allelic association at the 5% level was evaluated. PFS curves were plotted using Kaplan-Meier estimates. All statistical analyses were undertaken using SAS Version 9.1 (SAS Institute, Cary, NC) and R Version 2.5.0 (The R Foundation for Statistical Computing, www.r-project.org/foundation/main.html).

Table 1. Comparison of patient characteristics and details of drug treatment received by CLL-4 patients genotyped and those excluded from study

	CLL-4 patients genotyped (N = 425), no. (%)	CLL-4 patients excluded (N = 352), no. (%)	*P**
Sex			
Male	312 (73.4)	261 (74.1)	—
Female	113 (26.6)	91 (25.9)	.8
Age			
Less than 60 y	127 (29.9)	127 (36.1)	—
60 to 69 y	168 (39.5)	121 (34.4)	—
Over 70 y	130 (30.6)	104 (29.5)	.2
Stage			
A	104 (24.5)	87 (24.7)	—
B	186 (43.8)	166 (47.2)	—
C	135 (31.8)	99 (28.1)	.5
Died			
Yes	114 (26.8)	88 (25.0)	—
No	311 (73.2)	264 (75.0)	.6
Treatment			
Chlorambucil	206 (48.5)	181 (51.4)	—
Fludarabine	112 (26.4)	82 (23.3)	—
Fludarabine and cyclophosphamide	107 (25.2)	89 (25.3)	.6

— indicates not applicable.
*Chi-square test.

Results

Descriptive statistics

The clinicopathologic characteristics of patients studied are detailed in Table 1. Of the patients genotyped, approximately a third had been diagnosed with CLL before age 60 years, and approximately two-thirds had presented with stage B or C disease. The median follow-up time for patients was 32 months with follow-up to October, 31, 2005. There were 27% deaths in the cohort. Sex, stage at presentation, and drug therapy all significantly influenced PFS. Specifically, being female, having early-stage disease, and the combination of fludarabine and cyclophosphamide as drug therapy were all significantly associated with a more favorable prognosis (Table 2). Only age and stage at presentation significantly affected OS. For 354 (83%) of the 425 patients, we had information on IgV_H mutation status. As in the parent trial, this covariate was prognostically important, being predictive of both PFS and OS ($P < .001$ and .002, respectively).

There was no difference in the demographics, treatment, and follow-up characteristics of the 425 patients genotyped herein compared with the patients entered into CLL-4 but who were excluded from this analysis (Table 1). The observation that conventional staging and other prognosis markers were equally predictive of survival in the complete dataset and the subgroup analyzed provides further evidence that subselection of patients genotyped is unlikely to bias overall study findings.

Relationship between SNP genotype and PFS

Within the parent study, SNP call rates per sample were greater than 99% in patients. Of the 1467 SNPs submitted for analysis,

Table 2. Relationship between treatment, stage, age and sex, and PFS and OS in the patients genotyped

		OS			PFS		
	N	No. deaths	O/E ratio	*P**	Progression or death	O/E ratio	*P**
Treatment							
Chlorambucil	206	48	0.87	.3	155	1.36	< .001
Fludarabine	112	33	1.16	—	75	1.12	—
Fludarabine and cyclophosphamide	107	33	1.08	—	52	0.51	—
Stage							
A	104	20	0.67	< .001	66	0.94	.03
B	186	45	0.88	—	116	0.87	—
C	135	49	1.5	—	100	1.28	—
Age							
Less than 60 y	127	21	0.51	< .001	88	1.01	.8
60 to 69 y	168	42	0.94	—	109	0.96	—
Over 70 y	130	51	1.81	—	85	1.05	—
Sex							
Male	312	88	1.05	.4	218	1.08	.02
Female	113	26	0.87	—	64	0.79	—

O/E ratio indicates observed versus expected ratio.
*Log-rank test for trend.

1218 (83%) were satisfactory genotyped, with mean individual SNP call rates of 99.7% Of the 1218 SNPs loci that satisfactorily genotyped, 241 were either fixed in all patient samples or were observed at subpolymorphic frequencies (ie, having minor allele frequency [MAF] of less than 1%), and hence were excluded from all analyses. Distribution of SNP genotypes was not significantly different to that expected under HWE, with λequal to 1.02 (Figure S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). Of the 977 remaining SNPs mapping to the 755 genes to be analyzed, cell counts of minor allele genotypes were sufficiently infrequent to exclude 57 from the genotype and dominant analyses and 438 from the recessive model analyses. The SNPs for which genotype-survival associations were examined are detailed in Table S1 together with their associated MAFs.

As 10% to 15% of patients with CLL with progressive disease are thought to display deletions involving 17p, we examined this particular locus in detail. Genotypes for the p53 Arg72Pro (rs1042522[30]) were obtained for 421 (99.1%) of the 425 patients, and the distribution of genotypes (239, 154, 28) was not significantly different to those we have previously documented in the United Kingdom population[31] (1471, 1022, 197; $P = .64$).

After adjustment for sex, age, stage of disease at presentation, and drug regime, statistically significant associations between genotype and PFS were identified for 42 SNPs at the 5% level. A Q-Q plot of test statistics showed no large deviation from the expected distribution (Figure S2). Results of all SNPs included in the genotype-survival analysis are detailed in Table S2. On the basis of dominant and recessive models, 78 different nsSNPs were significant, 51 under a dominant model and 27 under a recessive model; none, however, were significant after adjustment for multiple testing using the conservative Bonferroni correction.

With such caveats in mind, several potentially interesting findings were observed. Of the SNPs identified to be prognostic (on the basis of nominal $P \leq .05$), 2 have been established to be functional, and a further 28 SNPs are predicted to be functional (Table 3). Collectively, the SNPs predictive of PFS mapped to a restricted number of gene ontologies, and for purposes of clarity, we have restricted our commentary to the genes within pathways for which polymorphic variation in at least one SNP was associated with PFS, specifically genes involved in DNA damage-response/repair and immune regulation.

DNA damage-response genes

DNA polymerase beta (*POLB*; MIM 174760[32]) performs base excision repair required for DNA maintenance, replication, recombination, and drug resistance. Under the Cox proportional hazards model, carrier status for *POLB* P242R variant genotype was significantly associated with a poorer PFS (HR = 1.87; 95% CI: 1.04-3.36; $P = .04$). PFS (3-year) for carriers of the wild-type genotype was 22.2% (95% CI: 7.4%-66.5%) compared with 32.7% (95% CI: 27.9%-38.4%) for those with the wild-type genotype (Figure 1).

Genes participating in homologous recombination include breast cancer 2 (*BRCA2*; MIM 600185) and x-ray repair complementing defective repair in Chinese hamster cells (*XRCC2*; MIM 600375), which encodes a member of the RecA/Rad51-related protein family. Carrier status for *BRCA2* T1915M and *XRCC2* R188H variant genotypes were associated with poorer PFS (HR = 1.59; 95% CI: 1.03-2.44; $P = .04$ and HR = 1.44; 95% CI: 1.06-1.95; $P = .02$, respectively). Patients with the *BRCA2* T1915M wild-type genotype had a 3-year PFS of 33.6% (95% CI: 28.6%-39.4%), whereas the 3-year PFS was 17.8% (95% CI: 6.9%-45.9%) in carriers of wild-type genotype (Figure 2).

Nucleotide excision repair is the major cellular system that repairs bulky DNA adducts caused by mutagens and chemotherapy. This form of DNA repair is in part performed by the proteins encoded by Chinese hamster groups 5 and 6 (*ERCC5*; MIM 133530 and *ERCC6*; MIM 609413). Carrier status for *ERCC5* H1104D and *ERCC6* G399D variant genotypes were both associated with a less favorable PFS (HR = 1.32, 95% CI: 1.03-1.69; $P = .03$ and HR = 1.35, 95% CI: 1.05-1.73; $P = .02$). For *ERCC5* H1104D, carriers with the wild-type genotype had a 3-year PFS of 13.8% (95% CI: 5.1%-37.7%) compared with 33.9% (95% CI: 29.0%-39.7%) for noncarriers (Figure 3).

Other DNA damage-response/repair gene SNPs displaying an association with PFS included those mapping to postmeiotic segregation increased 2 (*PMS2*; MIM 600259), which participates in mismatch repair; methylguanine-DNA methyltransferase (*MGMT*; MIM 156569); and topoisomerase (DNA) II binding

Table 3. SNPs showing significant genotypic association with PFS

Gene	Symbol	SNP	Reference allele (dbSNP)	N*	HR	95% CI	*P*
Dominantly acting alleles							
Sec23 homolog B	*SEC23B*	H489Q	—	C	93	0.60	0.45-0.81	.001†
Leukocyte immunoglobulin-like receptor, subfamily A member 4	*LILRA4*	P27L‡	rs2241384	C	285	1.44	1.12-1.85	.004†
Chondroitin sulfate galnact-2	*GALNACT-2*	P479S‡	rs2435381	C	237	0.70	0.55-0.89	.004†
Cubilin (intrinsic factor-cobalamin receptor)	*CUBN*	S253F	rs1801222	C	151	1.42	1.11-1.83	.006†
Killer cell lectin-like receptor subfamily C, member 4	*KLRC4*	S29I‡	rs1841958	G	195	0.72	0.57-0.91	.007†
Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	*LHPP*	R94Q	rs6597801	G	344	1.49	1.11-1.99	.008
Melanoma inhibitory activity 2	*MIA2*	D547H	rs10134365	G	269	1.39	1.09-1.78	.008
ARP10 protein	*ARP10*	K121N	rs139299	G	114	1.47	1.11-1.95	.008†
Dystonin	*DST*	Q1634R	rs4712138	A	153	1.39	1.08-1.79	.011†
Myosin binding protein C, cardiac	*MYBPC3*	V158M‡	rs3729986	G	350	1.47	1.09-1.99	.012†
Sema domain, immunoglobulin domain, short basic domain, secreted	*SEMA3C*	V337M‡	rs1527482	G	419	3.15	1.28-7.75	.013
Zinc finger protein 169	*ZNF169*	Q583H‡	rs12350212	G	344	1.46	1.08-1.97	.013†
Serologically defined colon cancer antigen 3-like	*SDCCAG3L*	I437T	rs2000746	T	218	0.74	0.58-0.94	.013
Phospholipase A2, group VII	*PLA2G7*	A379V	rs1051931	C	281	0.72	0.56-0.94	.014†
Apolipoprotein E	*APOE*	R176C‡§	rs7412	C	352	1.46	1.08-1.98	.015†
BRCA1-associated RING domain 1	*BARD1*	S378R‡	rs2229571	C	154	0.75	0.59-0.95	.019†
General transcription factor IIE, polypeptide 1, alpha 56 kDa	*GTF2E1*	P366S	rs3732401	C	387	0.56	0.34-0.91	.02†
X-ray repair complementing defective repair in Chinese hamster cells 2	*XRCC2*	R188H	rs3218536	G	354	1.44	1.06-1.95	.02
Excision repair cross-complementing rodent repair deficiency, complementation group	*ERCC6*	G399D	rs2228528	G	299	1.35	1.05-1.73	.021
Low-density lipoprotein receptor–related protein 4	*LRP4*	G1554S‡	rs2306029	G	133	1.37	1.05-1.78	.021†
Erbb2 interacting protein	*ERBB2IP*	S274L‡	rs3213837	C	291	1.34	1.04-1.72	.022
Low-density lipoprotein–related protein 2	*LRP2*	L4210I‡	rs4667591	C	262	1.32	1.04-1.68	.024
Cancer susceptibility candidate 5	*CASC5*	M598T	rs11858113	T	139	0.75	0.59-0.96	.025†
Tyrosinase	*TYR*	S192Y‡	rs1042602	C	159	0.76	0.60-0.97	.027
Thrombospondin 1	*THBS1*	N700S‡§	rs17632786	A	325	0.72	0.54-0.96	.027†
Excision repair cross-complementing rodent repair deficiency, complementation group	*ERCC5*	H1104D	rs17655	C	256	1.32	1.03-1.69	.028
Transmembrane protease, serine 4	*TMPRSS4*	G206V	rs1941635	G	395	1.57	1.05-2.35	.028†
E2F transcription factor 2	*E2F2*	G205R	rs3218170	G	412	1.98	1.08-3.62	.028
Laminin, alpha 2	*LAMA2*	V1138M	rs2306942	G	378	1.51	1.04-2.18	.028
Interleukin-16 (lymphocyte chemoattractant factor)	*IL16*	P434S	rs4072111	C	334	0.72	0.53-0.97	.031†
Carboxypeptidase A4	*CPA4*	G303C‡	rs2171492	G	153	0.76	0.6-0.98	.032
RAD18 homolog	*RAD18*	Q302R	rs373572	A	223	1.29	1.02-1.64	.034
Cytochrome P450, family 39, subfamily A, polypeptide 1	*CYP39A1*	P23R	rs12192544	C	258	1.3	1.02-1.65	.034†
Keratin 1	*KRT1*	A454S‡	rs17678945	G	395	1.59	1.03-2.44	.035
Polymerase (DNA-directed), beta	*POLB*	P242R‡	rs3136797	C	409	1.87	1.04-3.36	.036†
Breast cancer 2, early onset	*BRCA2*	T1915M	rs4987117	C	393	1.59	1.03-2.44	.036
PMS2 postmeiotic segregation increased 2	*PMS2*	M622I	rs1805324	G	411	0.39	0.16-0.95	.037
Ubiquitin specific protease 47	*USP47*	V75G‡	rs11022079	T	205	1.28	1.01-1.63	.039
Zinc finger protein 311	*ZNF311*	K511Q	rs6456880	A	168	1.29	1.01-1.65	.039
Zinc finger CCCH-type containing 3	*ZC3H3*	G452S	rs4874147	G	245	1.30	1.01-1.66	.040
Protein tyrosine phosphatase, nonreceptor type 13	*PTPN13*	Y2062D	rs989902	T	146	0.78	0.61-0.99	.041†
Homeodomain interacting protein kinase 4	*HIPK4*	R302Q‡	rs11670988	G	347	1.37	1.01-1.87	.042†
RAD1 homolog (*Saccharomyces pombe*)	*RAD1*	E281G	rs1805327	A	357	0.71	0.50-0.99	.042
Cytochrome P450, family 2, subfamily C, polypeptide 9	*CYP2C9*	I359L‡	rs1057910	A	370	1.42	1.01-2.00	.042†

HRs adjusted for age, sex, stage of disease, and treatment.
db SNP indicates database of single nucleotide polymorphisms.
*Number of patients with at-risk genotype.
†Significant after adjustment for IgV_H mutation status.
‡Predicted to be intolerant/potentially intolerant, damaging/probably damaging, or radical/moderately radical based on SIFT/POLYPHEN/Grantham scores.
§Established to be functional.

Table 3. SNPs showing significant genotypic association with PFS (Continued)

Gene	Symbol	SNP		Reference allele (dbSNP)	N*	HR	95% CI	*P*
Forkhead box N1	*FOXN1*	R69C‡	rs2071587	C	350	1.37	1.01-1.85	.042
Ectonucleotide pyrophosphatase/phosphodiesterase 5	*ENPP5*	V171I	rs6926570	G	117	0.76	0.58-0.99	.043
FRAS1-related extracellular matrix protein 2	*FREM2*	F1070S	rs2496425	T	231	1.28	1.01-1.62	.044
Serine/threonine kinase 6	*STK6*	I31F	rs2273535	A	261	0.78	0.61-0.99	.045†
O-6-methylguanine-DNA methyltransferase	*MGMT*	L84F	rs12917	C	327	0.75	0.56-0.99	.045
Carbonyl reductase 3	*CBR3*	V244M	rs1056892	G	186	1.28	1.00-1.63	.046
Zinc finger protein 527	*ZNF527*	H181R‡	rs4452075	A	295	0.76	0.59-1.00	.047
Recessively acting alleles								
Interleukin-19	*IL19*	S213F	rs2243191	C	16	2.53	1.46-4.38	.001†
Cadherin 11, type 2, OB-cadherin	*CDH11*	T255M	rs35195	C	47	1.73	1.22-2.45	.002†
Cathepsin B	*CTSB*	L26V	rs12338	C	71	0.57	0.40-0.82	.002†
CD5 antigen (p56–62)	*CD5*	V471A‡	rs13328900	T	104	1.50	1.16-1.96	.002†
Extra spindle poles–like 1 (*S cerevisiae*)	*ESPL1*	R614S‡	rs1318648	A	58	1.60	1.15-2.22	.006
Ectonucleotide pyrophosphatase/phosphodiesterase 5	*ENPP5*	V171I	rs6926570	G	86	0.64	0.46-0.88	.006†
Glypican 1	*GPC1*	G500S	rs2228331	G	32	0.48	0.28-0.81	.006†
Matrix metalloproteinase 10 (stromelysin 2)	*MMP10*	G65R‡	rs17293607	G	8	2.92	1.36-6.27	.006†
Dual specificity phosphatase 13	*DUSP13*	C206Y‡	rs3088142	G	77	0.63	0.45-0.88	.007
Sucrase-isomaltase (alpha-glucosidase)	*SI*	A231T	rs9283633	G	71	1.51	1.12-2.05	.008
Neural precursor cell–expressed, developmentally down-regulated 4	*NEDD4*	Q260R	rs2303580	A	15	0.35	0.16-0.79	.012†
Reticulon 3	*RTN3*	A6E	rs11551944	C	9	2.44	1.19-5.00	.015†
Carnitine palmitoyltransferase II	*CPT2*	M647V‡	rs1799822	A	27	1.74	1.11-2.73	.016†
Monoacylglycerol O-acyltransferase 1	*MOGAT1*	S162P	rs1868024	T	21	1.91	1.13-3.23	.016
Extra spindle poles–like 1 (*S cerevisiae*)	*ESPL1*	D25A	rs6580942	A	44	1.58	1.09-2.29	.017
Topoisomerase (DNA) II binding protein 1	*TOPBP1*	S730L	rs17301766	C	18	1.89	1.12-3.19	.018†
Zinc finger protein 643	*ZNF643*	C13Y	rs2272994	G	16	0.45	0.23-0.87	.019
SET domain, bifurcated 2	*SETDB2*	G117E	rs7998427	G	64	1.45	1.05-2.00	.023†
Zinc finger protein 573	*ZNF573*	A166G‡	rs3752365	C	24	1.71	1.08-2.70	.024
A kinase (PRKA) anchor protein (yotiao) 9	*AKAP9*	M463I	rs6964587	G	65	1.43	1.05-1.97	.025
BCL2-like 13 (apoptosis facilitator)	*BCL2L13*	P360S	rs9306198	C	6	2.68	1.09-6.60	.032†
Dynein, axonemal, heavy polypeptide 9	*DNAH9*	N2195S	rs3744581	A	23	1.68	1.03-2.72	.037
A kinase (PRKA) anchor protein 10	*AKAP10*	R249H	rs2108978	G	63	1.41	1.02-1.94	.038†
DEAD (Asp-Glu-Ala-Asp) box polypeptide 27	*DDX27*	G206V	rs11908296	G	22	1.70	1.03-2.80	.038
Inhibin, beta C	*INHBC*	R322Q‡	rs2229357	G	22	1.75	1.02-2.98	.041
Sterile alpha motif and leucine zipper containing kinase AZK	*ZAK*	S531L	rs3769148	C	95	0.75	0.56-1.00	.047
Eosinophil peroxidase	*EPX*	Q122H	rs11652709	G	35	0.64	0.41-0.99	.047
Four and a half LIM domains 5	*FHL5*	S243R‡	rs9373985	C	43	0.65	0.42-1.00	.049

HRs adjusted for age, sex, stage of disease, and treatment.
db SNP indicates database of single nucleotide polymorphisms.
*Number of patients with at-risk genotype.
†Significant after adjustment for IgV_H mutation status.
‡Predicted to be intolerant/potentially intolerant, damaging/probably damaging, or radical/moderately radical based on SIFT/POLYPHEN/Grantham scores.
§Established to be functional.

protein 1 (*TOP53*; MIM #07760), with carrier status for *PMS2* M622I and *MGMT* L84F being associated with a better prognosis than those with wild-type alleles (HR = 0.39, 95% CI: 0.16-0.95; $P = .04$ and HR = 0.75, 95% CI: 0.66-0.99; $P = .04$). Conversely, homozygosity for *TOPBP1* S730L variant genotype was associated with a poorer outlook (HR = 1.89, 95% CI: 1.12-3.19; $P = .02$).

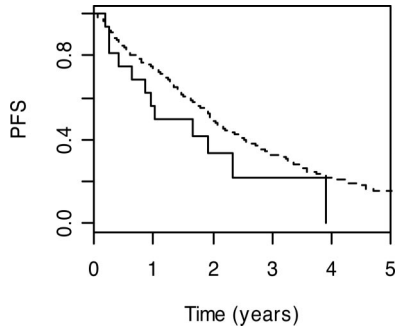

Figure 1. Kaplan-Meier curves for *POLB* P242R. PFS curves associated with wild-type genotype are shown as a broken line. The solid line depicts survival curve for those with at risk variant genotype.

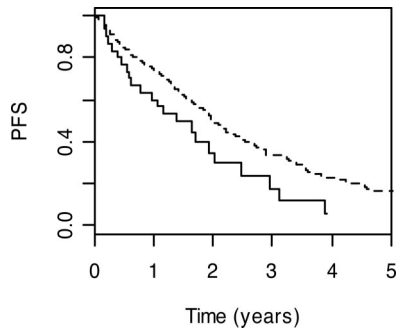

Figure 2. PFS curves for *BRCA2* T1915M. Lines are as defined in Figure 1.

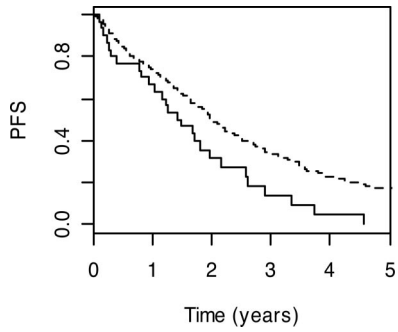

Figure 3. PFS curves for *ERCC5* H1104D. Lines are as defined in Figure 1.

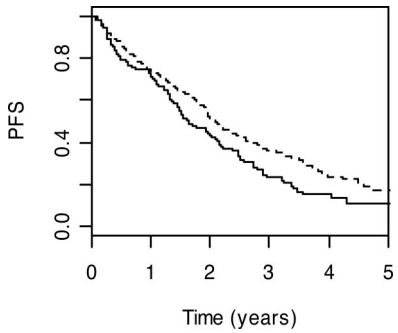

Figure 5. PFS curves for *LILRA4* P27L. Lines are as defined in Figure 1.

Immune regulation genes

Preeminent among immune regulation pathway SNPs were those mapping to IL-19 (*IL19*; MIM 605687) and IL-16 genes (*IL16*; MIM 603035). Although based on relatively small numbers, homozygosity for *IL19* S213F variant genotype was associated with a worse PFS (HR = 2.53, 95% CI: 1.46-4.38; $P = .001$). Conversely, carrier status for *IL16* P434S was associated with a more favorable prognosis (HR = 0.72, 95% CI: 0.53-0.97; $P = .03$); 3-year PFS was 47.3% (95% CI: 37.1%-60.3%) compared with 28.4% (95% CI: 23.4%-34.6%; Figure 4). Under the recessive model, homozygosity for lymphocyte antigen CD5 (MIM 153340) V471A variant genotype was associated with a poorer prognosis (HR = 1.50; 95% CI: 1.16-1.96; $P = .002$). Also associated with poorer PFS were carriers of leukocyte immunoglobulin-like receptor, subfamily A member 4 (*LILRA4*; MIM 607517) P27L variant genotype (HR = 1.44, 95% CI: 1.12-1.85; $P = .004$); 3-year PFS was 23.3% (95% CI: 16.2%-33.6%) compared with 36.4% (95% CI: 30.6%-43.3%; Figure 5) and sema domain, immunoglobulin domain (Ig) short basic domain, secreted, 3C (*SEMA3C*; MIM 602645) V337M genotype (HR = 3.15, 95% CI: 1.28-7.75; $P = .01$). In contrast, carrier status for killer cell lectin-like receptor subfamily C, member 4 (*KLRC4*; MIM 602893) S29I variant genotype was associated with a better PFS (HR = 0.72, 95% CI: 0.57-0.91; $P = .007$); 3-year PFS was 38.2% (95% CI: 31.4%-46.5%) compared with 26.1% (95% CI: 20.0%-34.0%; Figure 6).

Relationship between SNP genotype and OS

A total of 9 of the 78 SNPs associated with PFS also showed an association with OS: *SEC23B* H489Q, *GALNACT-2* P479S, *ENPP5* V171I, *SEMA3C* V337M, *GTF2E1* P366S, *INHBC* R322, *LAMA2* V1138M, *ZNF527* H181R, and *BRCA2* T1915M.

Impact of SNP genotype adjusting for IgV_H mutational status

For 354 (83%) of the 425 patients, we had information on IgV_H mutation status, allowing us to evaluate the impact of polymorphic variation adjusting for this significant covariate. In a multivariate analysis incorporating information on IgV_H mutation status, 38 of the 78 SNPs were independently prognostic (Table 3). Among these 38 SNPs were 5 mapping to immune regulation genes *IL16* P434S, *IL19* S213F, *LILRA4* P27L, *KLRC*4 S29I, and *CD5* V471A and 2 within DNA response genes *POLB* P242R and *TOPBP1* S730L.

Relationship between SNP genotypes and chemotherapy

In view of the differences in pharmacology of chlorambucil, fludarabine, and cyclophosphamide, we examined for potential interactive effects between SNPs and PFS for the different drugs separately. Table S3 details the relationship between SNPs significant at the 5% level and PFS. A total of 5 SNPs associated with PFS were common to patients treated with chlorambucil or fludarabine (*DST* L22S, *LILRA4* P27L, *SEC23B* H489Q, *XRCC2* R188H, and *ZAK* S531L), 3 SNPs were common to patients treated with either chlorambucil or fludarabine with cyclophosphamide (*APBB3* C236R, *ENPPS* I171V, and *C21orf57* S2L), and 4 SNPs were common to patients treated with either fludarabine or fludarabine with cyclophosphamide (*DDX27* G206V, *DPYD* S534N, WNT16 G72R, and *DHX16* D566G).

Discussion

Using SNP genotype data generated on 425 patients within the CLL-4 trial, we have sought to identify a number of promising genetic variants with prognostic potential. A major strength of our study is its large size and the fact that we have analyzed patients entered into a phase 3 randomized trial, thereby minimizing bias.

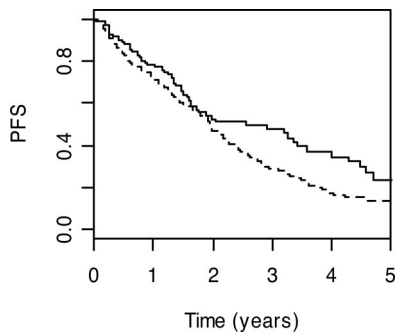

Figure 4. PFS curves for *IL16* P434S. Lines are as defined in Figure 1.

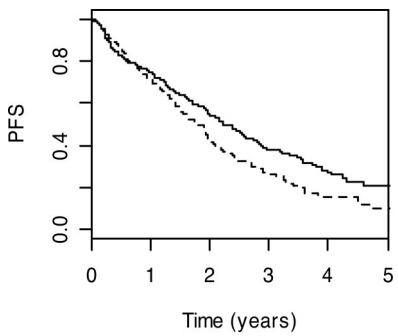

Figure 6. PFS curves for *KLRC4* S29I. Lines are as defined in Figure 1.

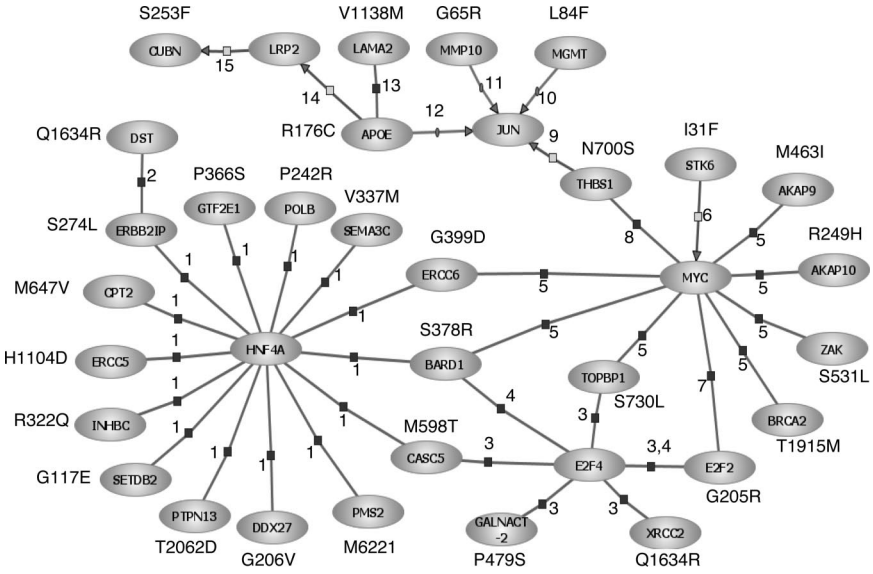

Figure 7. Interrelationships between genes involved in the DNA damage-response, immune regulation, and cell-signaling pathways for SNPs associated with PFS in CLL. Interactions were established using Pathway Assist software and are color-coded: blue indicates expression; gray, regulation; and red, protein binding. Supporting publications are indicated with the corresponding National Center for Biotechnology Information (NCBI) Entrez PubMed ID in parentheses. 1, binding (14988562); 2, binding (11375975); 3, binding (15525513); 4, binding (11799067); 5, binding (12808131); 6, regulation (14744757); 7, binding (11279041); 8, binding (15735755); 9, regulation (10490955); 10, promoter binding (10942771); 11, promoter binding (1480019); 12, promoter binding (8018731); 13, binding (7498414); 14, regulation (10487844); and 15, regulation (11595644).

While not all of the patients entered into CLL-4 trial were analyzed, there were no salient differences in the demography of patients genotyped and those excluded from the analysis; hence, it is unlikely that any spurious biases will have influenced study findings. Furthermore, our analysis is unlikely to be confounded by population stratification. We have been able to adjust for the major molecular covariate, IgV_H mutation status, and identify a number of SNPs independently predictive of PFS, including variants of *IL16, IL19, CD5*, and *POLB*. We did not, however, find evidence that *MTHFR* A222V or *p53* Arg72Pro genotype influences prognosis as previously purported.

Despite the strong biologic plausibility for several individual associations as discussed herein, and the fact that our study is large compared with contemporaneous ones, these types of analyses are prone to false discovery as a consequence of multiple comparisons. Hence, although the signal generated from our gene level analyses suggests that we observed an approximately 8% greater signal over that expected on the basis of the dominant model, our nsSNPs findings are consistent with chance after adjusting for the number of tests used.

Although our observations must be considered preliminary and hypothesis-generating, it is intriguing that a large proportion of the SNPs we have identified as having prognostic relevance resides in genes mediating the immune response, extracellular matrix interactions, or DNA damage-response/repair. Especially relevant to CLL was the observation that variation in *CD5* defined by the V471I polymorphism was a determinant of PFS. Although in part speculative, through interrogation of the Pathway Architecture program (Stratagene, La Jolla, CA), 30 (38%) of the SNPs associated with prognosis were found within interrelated genes encoding pivotal components of the DNA damage-response, immune regulation, and cell-signaling pathways (Figure 7). Biologically, it is eminently plausible that variation in these genes will affect prognosis in the case of *CD5* and the interleukin genes, as levels of interleukins have been documented to influence clinical behavior.[34,35] Furthermore, defects in the TP53 tumor suppressor gene pathway are known to be important in CLL, as *p53* inactivation is associated with aggressive disease.[36,37] It is intriguing that we identified an association with *APOE*, as the expressed protein has cytostatic and cytotoxic activity; moreover, apolipoprotein levels and activity of aplolipoprotein receptors have been linked to prognosis in B-CLL.[38-40] The prior probability of identifying a significant association with prognosis for a series of SNPs mapping to a single DNA repair pathway is intuitively small, thereby providing a measure of robustness to our observations. However, ultimately, replication in independent studies is required to either validate or refute study findings.

Our observation that polymorphic variation in a number of the DNA repair genes (*ERCC5*, *BRCA2*, and *POLB*) influences survival of patients with cancer is not without precedent. Outside the context of CLL variation, *ERCC5* D1104H has been reported to influence esophageal[41] and lung[42] cancer, and *POLB* P242R has been reported to influence lung cancer prognosis[42] in the same manner, suggesting generic effects on outcome from malignancy.

While we have evaluated only nsSNPs with a higher probability of being directly causal, there is currently direct evidence for functionality only for a minority, such as *APOE* R176C and *THBS1* N700S.[43,44] Evidence for others is provided by in silico predictions regarding the consequences of amino acid changes. While such predictions have been shown to successfully categorize a high proportion of amino acid substitutions in benchmarking studies,[26,45,46] it is acknowledged that assigning functionality to SNPs on the basis of metrics is in essence speculative. Moreover, it is probable that some associations are a consequence of linkage disequilibrium with causal variants.

As the patients analyzed in our study were part of a large clinical trial, this has provided a unique opportunity to examine differential effects of SNP on PFS according to specific chemotherapy. On the basis that chlorambucil and cyclophosphamide are both alkylating agents, we have looked for SNPs common to both patient groups. Although variants such as *XRCC2* R188H are attractive candidates for conferring differential response to treatment, there are currently no data for the role for variation in this or the other genes identified as a determinant of drug response. Interestingly, the S534N variant of dihydropyrimidine dehydrogenase (*DPYD*), shown to influence response to fludarabine, was related to PFS ($P = .03$ and .002 for PFS in fludarabine-treated patients and fludarabine plus cyclophosphamide-treated patients, respectively). *DPYD* is the initial and rate-limiting factor in the pathway of uracil and thymidine catabolism, with genetic deficiency being well recognized to result in an error in pyrimidine metabolism.[47] In addition, cyclophosphamide has been shown to

augment the tumor effect of pyrimidine agents such as 5-fluorouracil through inhibition of *DPYD*.[48] In this respect, although fludarabine acts as a pyrimidine analog, it is interesting that a comparable effect was observed.

Here, we have restricted our search for SNPs associated with disease outcome by assessing those which have the potential to affect the behavior of the expressed protein through change in the amino sequence. Epigenetic silencing of *DAPK1* by promoter methylation has recently been shown to occur in most patients with CLL[49]; hence, expression of this gene is a key player in CLL. Intriguingly, a rare variant in the promoter of *DAPK1* was demonstrated to affect *DAPK1* expression.[49] It is therefore highly probable that in addition to nsSNPs, noncoding variants influencing expression of key genes such as *DAPK1* will also be determinants of patient outcome and worthy of evaluation.

Our study provides evidence that inherited variation influences the clinical outcome from CLL and potentially provides additional insight into the biological determinants of prognosis. Furthermore, it also serves to highlight the statistical problem of searching for genetic associations when the impact of any variant is likely to be at best modest. Hence, stipulating significance levels of less than 10^{-5} for an analysis of clinical trial data is unrealistic, because to have 80% power to demonstrate a 5% difference in survival, which is clinically relevant, requires at least 4800 patient samples to be analyzed, even if the frequency of the at risk genotype is 50%. Moreover, imposing very stringent statistical thresholds can create the serious issue of type II errors. To facilitate the identification of prognostic markers through pooled analyses, we have therefore made data from our study publicly accessible (Tables S1-S3, Figures S1,S2).

Finally, although germ-line variants are unlikely to replace staging schemes and conventional markers such as IgV_H mutation status in the short term, they have potential to assist in distinguishing different outcome patterns among patients with the same stage of disease where 10% differences are clinically relevant, thereby opening up the possibility of a rational, targeted approach to treatment based on a combination of genotype and tumor characteristics of a patient.

Acknowledgments

We are grateful to all patients and clinicians participating in the LRF CLL4 trial. We thank Emily Webb for additional statistical analyses and Peter Broderick for his expertise in use of the Pathway Assist Software.

This work was undertaken primarily with grant support from Leukemia Research. Additional funding was provided by the Arabib Foundation and Cancer Research UK.

Authorship

Contribution: G.S.S. designed research, performed genetic analyses, and analyzed and interpreted data; R.W. and S.R. performed statistical analyses; D.G.O. performed hematologic assays; D.C. designed and conducted the clinical trial; and R.H. designed research, performed research, analyzed and interpreted data, and drafted the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Richard S. Houlston, Section of Cancer Genetics, Institute of Cancer Research, 15 Cotswald Road, Sutton, Surrey SM2 5NG, UK; e-mail: richard.houlston@icr.ac.uk.

References

1. Stevenson FK, Caligaris-Cappio F. Chronic lymphocytic leukemia: revelations from the B-cell receptor. Blood. 2004;103:4389-4395.
2. Binet JL. Prognostic factors in chronic lymphocytic leukaemia. Haematologica. 1999;84:96-97.
3. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343:1910-1916.
4. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94:1848-1854.
5. Del Poeta G, Maurillo L, Venditti A, et al. Clinical significance of CD38 expression in chronic lymphocytic leukemia. Blood. 2001;98:2633-2639.
6. Thunberg U, Tobin G, Johnson A, et al. Polymorphism in the P2X7 receptor gene and survival in chronic lymphocytic leukaemia. Lancet. 2002;360:1935-1939.
7. Frey UH, Nuckel H, Sellmann L, et al. The GNAS1 T393C polymorphism is associated with disease progression and survival in chronic lymphocytic leukemia. Clin Cancer Res. 2006;12:5686-5692.
8. Pemberton NC, Paneesha S, Hiller L, et al. The SDF-1 G > A polymorphism at position 801 plays no role in multiple myeloma but may contribute to an inferior cause-specific survival in chronic lymphocytic leukemia. Leuk Lymphoma. 2006;47:1239-1244.
9. Nuckel H, Frey UH, Durig J, Duhrsen U, Siffert W. Methylenetetrahydrofolate reductase (MTHFR) gene 677C>T and 1298A>C polymorphisms are associated with differential apoptosis of leukemic B cells in vitro and disease progression in chronic lymphocytic leukemia. Leukemia. 2004;18:1816-1823.
10. Kochethu G, Delgado J, Pepper C, et al. Two germ line polymorphisms of the tumour suppressor gene p53 may influence the biology of chronic lymphocytic leukaemia. Leuk Res. 2006;30:1113-1118.
11. Au WY, Fung A, Wong KF, Chan CH, Liang R. Tumor necrosis factor alpha promoter polymorphism and the risk of chronic lymphocytic leukemia and myeloma in the Chinese population. Leuk Lymphoma. 2006;47:2189-2193.
12. Hulkkonen J, Vilpo J, Vilpo L, Koski T, Hurme M. Interleukin-1 beta, interleukin-1 receptor antagonist and interleukin-6 plasma levels and cytokine gene polymorphisms in chronic lymphocytic leukemia: correlation with prognostic parameters. Haematologica. 2000;85:600-606.
13. Nuckel H, Frey UH, Bau M, et al. Association of a novel regulatory polymorphism (−938C>A) in the BCL2 gene promoter with disease progression and survival in chronic lymphocytic leukemia. Blood. 2007;109:290-297.
14. Saxena A, Moshynska O, Sankaran K, Viswanathan S, Sheridan DP. Association of a novel single nucleotide polymorphism, G(−248)A, in the 5′-UTR of BAX gene in chronic lymphocytic leukemia with disease progression and treatment resistance. Cancer Lett. 2002;187:199-205.
15. Starczynski J, Pepper C, Pratt G, et al. Common polymorphism G(−248)A in the promoter region of the bax gene results in significantly shorter survival in patients with chronic lymphocytic Leukemia once treatment is initiated. J Clin Oncol. 2005;23:1514-1521.
16. Starczynski J, Pepper C, Pratt G, et al. The P2X7 receptor gene polymorphism 1513 A->C has no effect on clinical prognostic markers, in vitro sensitivity to fludarabine, Bcl-2 family protein expression or survival in B-cell chronic lymphocytic leukaemia. Br J Haematol. 2003;123:66-71.
17. Nuckel H, Frey UH, Durig J, Duhrsen U, Siffert W. 1513A/C polymorphism in the P2X7 receptor gene in chronic lymphocytic leukemia: absence of correlation with clinical outcome. Eur J Haematol. 2004;72:259-263.
18. Sturm I, Bosanquet AG, Hummel M, Dorken B, Daniel PT. In B-CLL, the codon 72 polymorphic variants of p53 are not related to drug resistance and disease prognosis. BMC Cancer. 2005;5:105.
19. Skogsberg S, Tobin G, Krober A, et al. The G(−248)A polymorphism in the promoter region of the Bax gene does not correlate with prognostic markers or overall survival in chronic lymphocytic leukemia. Leukemia. 2006;20:77-81.
20. Rudd MF, Sellick GS, Webb EL, Catovsky D, Houlston RS. Variants in the ATM-BRCA2-CHEK2 axis predispose to chronic lymphocytic leukemia. Blood. 2006;108:638-644.
21. Catovsky D, Richards S, Matutes E, et al. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. Lancet. 2007;370:230-239.
22. Rudd MF, Williams RD, Webb EL, Schmidt S, Sellick GS, Houlston RS. The predicted impact of

coding single nucleotide polymorphisms database. Cancer Epidemiol Biomarkers Prev. 2005; 14:2598-2604.

23. Predicted Impact of Coding SNPs (PICS) database. Available at: http://www.icr.ac.uk/research/research_sections/cancer_genetics/cancer_genetics_teams/molecular_and_population_genetics/software_and_databases/index.shtml. Accessed August 2008.
24. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res. 2002;30:3894-3900.
25. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. Genome Res. 2001;11:863-874.
26. Xi T, Jones IM, Mohrenweiser HW. Many amino acid substitution variants identified in DNA repair genes during human population screenings are predicted to impact protein function. Genomics. 2004;83:970-979.
27. Grantham R. Amino acid difference formula to help explain protein evolution. Science. 1974; 185:862-864.
28. Li WH, Wu CI, Luo CC. Nonrandomness of point mutation as reflected in nucleotide substitutions in pseudogenes and its evolutionary implications. J Mol Evol. 1984;21:58-71.
29. Clayton DG, Walker NM, Smyth DJ, et al. Population structure, differential bias and genomic control in a large-scale, case-control association study. Nat Genet. 2005;37:1243-1246.
30. National Center for Biotechnology Information. Entrez SNP. http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp. Accessed July 2007.
31. Webb EL, Rudd MF, Sellick GS, et al. Search for low penetrance alleles for colorectal cancer through a scan of 1467 non-synonymous SNPs in 2575 cases and 2707 controls with validation by kin-cohort analysis of 14 704 first-degree relatives. Hum Mol Genet. 2006;15:3263-3271.
32. National Center for Biotechnology Information. Online Mendelian Inheritance in Man (OMIM). http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim. Accessed July 2007.
33. National Center for Biotechnology Information. Entrez PubMed. http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed. Accessed July 2007.
34. Wierda WG, Johnson MM, Do KA, et al. Plasma interleukin 8 level predicts for survival in chronic lymphocytic leukaemia. Br J Haematol. 2003;120: 452-456.
35. Fayad L, Keating MJ, Reuben JM, et al. Interleukin-6 and interleukin-10 levels in chronic lymphocytic leukemia: correlation with phenotypic characteristics and outcome. Blood. 2001;97:256-263.
36. Stankovic T, Hubank M, Cronin D, et al. Microarray analysis reveals that TP53- and ATM-mutant B-CLLs share a defect in activating proapoptotic responses after DNA damage but are distinguished by major differences in activating prosurvival responses. Blood. 2004;103:291-300.
37. Guarini A, Gaidano G, Mauro FR, et al. Chronic lymphocytic leukemia patients with highly stable and indolent disease show distinctive phenotypic and genotypic features. Blood. 2003;102:1035-1041.
38. Juliusson G, Vitols S. Impaired low-density lipoprotein receptor activity in chronic B-lymphocytic leukaemia cells. Eur J Haematol. 1988;40: 18-24.
39. Chapman-Shimshoni D, Yuklea M, Radnay J, Shapiro H, Lishner M. Simvastatin induces apoptosis of B-CLL cells by activation of mitochondrial caspase 9. Exp Hematol. 2003;31:779-783.
40. Bomstein Y, Yuklea M, Radnay J, et al. The anti-apoptotic effects of blood constituents in patients with chronic lymphocytic leukemia. Eur J Haematol. 2003;70:290-295.
41. Wu X, Gu J, Wu TT, et al. Genetic variations in radiation and chemotherapy drug action pathways predict clinical outcomes in esophageal cancer. J Clin Oncol. 2006;24:3789-3798.
42. Matakidou A, el Galta R, Webb E, et al. Genetic variation in the DNA repair genes is predictive of outcome in lung cancer Hum Mol Genet. 2007; 6:2333-2340.
43. Stenina OI, Ustinov V, Krukovets I, Marinic T, Topol EJ, Plow EF. Polymorphisms A387P in thrombospondin-4 and N700S in thrombospondin-1 perturb calcium binding sites. FASEB J. 2005;19:1893-1895.
44. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis. 1988;8:1-21.
45. Savas S, Kim DY, Ahmad MF, Shariff M, Ozcelik H. Identifying functional genetic variants in DNA repair pathway using protein conservation analysis. Cancer Epidemiol Biomarkers Prev. 2004;13: 801-807.
46. Sunyaev S, Ramensky V, Bork P. Towards a structural basis of human non-synonymous single nucleotide polymorphisms. Trends Genet. 2000; 16:198-200.
47. Johnson MR, Wang K, Diasio RB. Profound dihydropyrimidine dehydrogenase deficiency resulting from a novel compound heterozygote genotype. Clin Cancer Res. 2002;8:768-774.
48. Nio Y, Iguchi C, Kodama H, et al. Cyclophosphamide augments the anti-tumor efficacy of uracil and tegafur by inhibiting dihydropyrimidine dehydrogenase. Oncol Rep. 2007;17:153-159.
49. Raval A, Tanner SM, Byrd JC, et al. Downregulation of death-associated protein kinase 1 (DAPK1) in chronic lymphocytic leukemia. Cell. 2007;129:879-890.