Impact of cytogenetic and molecular prognostic markers on the clinical management of chronic lymphocytic leukemia

Alexander W. Hauswirth and Ulrich Jäger
Division of Hematology and Hemostaseology, Department of Medicine I, Medical University of Vienna, Austria
E-mail: ulrich.jaeger@meduniwien.ac.at

The prognosis of patients with B-cell chronic lymphocytic leukemia (B-CLL) has long been determined by the clinical staging systems of Binet and Rai.[1-4] Unfortunately, these systems fail to identify patients in early stages whose disease will rapidly progress. Neither can this approach clearly predict the response of individual patients to specific therapies. In recent years, numerous genetic approaches have provided new markers for prognosis and response prediction.[5] The predictive potential of genetic factors associated with CLL outcome is determined by (i) its accessibility for routine use; (ii) its stability throughout the course of disease; and (iii) its sensitivity and specificity. Prognostic factors are largely dependent on the conventional therapeutic regimens used (i.e. chemotherapy) as well as on the availability of specific targeting drugs. There is increasing evidence that the behavior of the leukemia is not only dependent on genetic factors within the CLL clone, but also on the genetic background of the host in general (*susceptibility*) and in particular of the microenvironment.

We will discuss advances in the genetics of CLL with a special focus on molecular markers. We will first focus on well-established prognostic markers and then review the current status of molecular predictive markers and their potential influence on treatment decisions.

Prognostic markers

Conventional cytogenetics

Karyotypes from CLL cells collected from peripheral blood or bone marrow are difficult to assess. However, stimulation by CD40L or CpG oligonucleotides has, in most cases, yielded positive results.[6] One of the most important findings was that the number of chromosomal translocations has largely been underestimated. Patients with translocations have a poor prognosis with short treatment free and overall survival times.[6] Recent advances were also made in identifying chromosomal regions predisposing for familial CLL. Regions on chromosomes 13q as well as 1, 3, 6, 12, and 17 have been linked to pathogenesis of inherited disease.[7-9] However, while relatives of CLL patients have increased numbers of circulating $CD19^+/CD5^+$ B-cells (a phenomenon called *B-cell monoclonal lymphocytosis*), the risk factors leading to the final development of overt CLL are still unclear.[10] Furthermore, familial CLL does not seem to be associated with a shorter survival in affected individuals.[11] Micro RNAs (MiRs) are also connected to familial disease[12] although their impact is not yet clear.

Fluorescence in situ hybridization

The prognostic value of aberrations has long been established following the hierarchical model described by Döhner *et al.*[13,14] Half of the B-CLL cases have a del 13q, indicating good prognosis. By contrast, 11q deletions are associated with bulky disease, and are associated with short survival times. This is even more pronounced for 17p deletions which predict for very poor outcome.[15,16] Interphase cytogenetics have been extended to a molecular level by microarray analysis in several studies.[17-19] Therefore, a number of genes over- or underexpressed in association with chromosomal aberrations have been identified. Some of these are directly related to the location on deleted or duplicated chromosomes (*gene dosage effect*). However, a number of genes from unaffected chromosomes change their expression, suggesting trans-acting effects. These genes have only partially been evaluated.[20]

Molecular prognostic markers

Molecular markers can be classified according to their presence on the DNA level (mutations, polymorphisms) or on the RNA level (gene expression) (Table 1). Most markers are predictive at diagnosis, but dynamic markers predicting response to therapy, such as minimal residual disease, are also available.

Gene mutations

Immunoglobulin VH-mutational status is now well established as a strong predictor of outcome.[21-28] A 98% sequence homology to germline has been defined as a useful clinical cut-off between good (mutated) or poor (unmutated) prognosis. More detailed analysis has revealed that the 98% threshold is indeed relevant, but that some variation exists.[29] Further analysis has revealed the prognostic relevance of specific VH-families: VH 1-69 cases are always unmutated CLL and mutated cases with a VH 3-21 have a poor outcome.[30,31] In addition, immunoglobulin receptors coding for particular antigen-binding sites can be critical in determining clinical features and outcome for at least some CLL patients.[32]

A number of non-immunoglobulin genes have been searched for mutations in CLL. Germ-line or somatic mutations were found in 5 out of 42 sequenced microRNAs (miR) in 11 out of 75 patients with CLL.[12]

Gene expression

Perhaps the most important value of IgVH mutational status was its ability to discriminate between patient

subgroups for further analysis in microarray studies. Gene expression profiling revealed the association of a number of genes previously thought to be unrelated to CLL. Depending on the use of CD19-selected/T-cell depleted or unpurified B-CLL samples, a variety of markers have been found since the initial experiments of Klein and Rosenwald in 2001.[19,52-54] A comprehensive analysis of more than 500 patients suggests a complex expression pattern with many CLL subgroups and overlaps.[55] Furthermore, Fernàndez *et al*. have shown considerable intra-individual modulation of gene expression patterns during the course of disease.[56]

The first established marker emerging from microarray analysis was the receptor kinase ZAP-70. The prognostic value of this genetic marker has now been well established by FACS-analysis or PCR.[33,34,57-62] ZAP-70 protein expression predicts for treatment-free survival (TFS) and overall survival independently of mutation status. While convincing results were obtained by several groups using different protein detection methods, efforts by the European Initiative in CLL Research (ERIC) to standardize their study approach have proved problematic.[63] Furthermore, investigations into the association with IgVH mutational status have yielded differing results, particularly 17p- samples cluster in the ZAP-70 negative group.[64] Assessment of ZAP-70 mRNA expression by real time PCR requires positive (CD19) or negative selection of B-cells.[33,34] Despite these drawbacks, ZAP-70 is a useful clinical marker and may also serve as a future target for specific signal transduction inhibitors.

Among the markers with an even stronger correlation to IgVH mutational status,[52,53] lipoprotein lipase has been extensively studied.[35-40] Its association with other markers (cytogenetic risk groups, molecular markers) as well as patient outcome (time to treatment, overall survival) is also strong. Lipoprotein lipase is a stable marker which has been studied by real-time PCR in several large series using purified or unpurified CLL cells or even whole blood. No difference between purified and unpurified samples was observed in several studies, indicating its potential for easy and general use. Its specificity regarding IgVH mutational status is 89%, with a sensitivity of 68%, a positive predictive value of 83% and a negative predictive value of 78%.[39] Discordant results are also observed.[36] Lipoprotein lipase can be combined with a downregulated marker (ADAM29) to increase specificity.[35] While lipoprotein lipase protein can also be detected on normal B-cells, its cytoplasmatic expression correlates well with RNA levels.[36] We have used the level of lipoprotein lipase-expression as a discriminator for microarray analysis.[19] Several markers emerging from this experiment have been validated by real time PCR. Among those, septin 10 and dystrophin (DMD) were strongly associated with time to treatment. The prognostic significance of some of these factors have also been confirmed by other groups (septin 10, DMD, AKAP12/gravin)[35-39,52-54] (Table 1). In addition, lipoprotein lipase expression correlated with several functional modules including the MTA3 and fatty acid degradation pathways.[19] Using special statistical methods exploiting the lipoprotein lipase-associated gene expression signature we have shown that lipoprotein lipase-positive CLL cells show similarities to other tissues like fat, muscle and dendritic cells. This body of information suggests that lipoprotein lipase mRNA and protein expression may be of functional importance.

Table 1. Cytogenetic and molecular markers in CLL with prognostic significance.

Cytogenetic markers		*Ref.*
Conventional cytogenetics	Translocations	6
FISH	17p-, 11q-, 13q-, +12	13-19
Molecular markers		
Mutations	IgVH-status	21-32
	Micro RNAs	12
mRNA expression	ZAP-70	33,34
	LPL	35-40
	PEG 10	41
	Sarcoglycan ε	41
	Septin 10	19
	Dystrophin	19
	Activation-induced cytidine deaminase	42-45
	Telomerase	46
	L-selectin	20
	Integrin-β2	20
	CLLU1	47
Minimal residual disease	ASO-PCR	48-51

Expression of other markers was also linked to prognosis

The gene responsible for somatic mutations in immunoglobulins (activation-induced cytidine deaminase, *AICDA*) is overexpressed in high-risk (unmutated) CLL cases.[42-45] We have investigated the prognostic significance of *PEG10*. This maternally-expressed gene is overexpressed in high-risk CLL in parallel with sarcoglycan ε, which resides within the same locus on chromosome 7q21. Knock-down of *PEG10* expression in primary CLL patient cells results in increased apoptotic cell death.[41]

Another important functional target is the telomerase gene which is overexpressed in CLL cells of patients with poor prognosis.[46] Additional prognostic factors include L-selectin and integrin-β2.[20] A very CLL-specific gene is *CLLU1* whose mRNA expression level can predict time to initiation of treatment and survival in CLL patients.[47]

One of the most significant findings in the field of CLL research was the detection of certain microRNA genes which are over- or underexpressed in conjunction with certain chromosomal aberrations.[12,18] Specific miR expression signatures are correlated with IgVH mutational status, ZAP-70 expression and treatment-free survival indicating prognostic importance.

Minimal residual disease

Molecular monitoring of residual CLL cells during therapy has been used as a dynamic marker during therapy. Eradication of MRD below detection levels of tailored PCR (ASO-PCR) or multicolour FACS is a predictor of favourable outcome.[48,49] This has been shown for induction therapy with the antibody alemtuzumab as well as for autologous or allogeneic stem cell transplantation.[50,51]

Prediction of response and influence on treatment decisions

Cytogenetics/FISH

Chromosome 17p deletions or p53 mutations result in poor response to fludarabine and rituximab which can be overcome by therapy with alemtuzumab[65,66] (Table 2). Alemtuzumab is particularly effective when combined with high-dose steroids.[67] A novel apoptogenic mechanism, cytosolic Histone H1.2 release, was also shown to be p53 dependent.[68] The predictive value of trisomy 12 is abrogated when fludarabine-containing regimens are used (*S. Stilgenbauer, personal communication*). Patients with deletions in 17p or 11q have been shown to respond to flavopiridol.[69] While combination chemoimmunotherapy with rituximab, pentostatin and cyclophosphamide was not effective in 17p- patients, it was very effective in patients with 11q deletions.[70] These novel data will obviously have consequences for treatment selection and expected response and will pave the way to tailored therapy.

Minimal residual disease

Minimal residual disease detection may not only be used as a measure of outcome, but could potentially serve to guide therapy in individual patients. In particular, response assessment after induction therapy could be used to tailor maintenance treatment with alemtuzumab or rituximab.[51,78] Since the clinical outcome of patients who become minimal residual disease-negative is substantially improved these molecular data may also impact on transplant decisions. Thus, minimal residual disease data will contribute to challenge the paradigm that CLL should not be treated aggressively.[49,78-81]

In vitro evidence for response to specific targeting drugs

Molecular markers have led to the detection of potentially active novel agents or off-label use of known drugs against CLL (Table 2). While these data are still based on *in vitro* or *ex vivo* observations, some of the agents used are close to clinical testing. Inhibitors of activated heat shock protein 90 (HSP90) have been shown to influence survival of B-cells expressing high levels of ZAP-70.[72] Specific ZAP-70 inhibitors are currently being developed. Since there is strong evidence for the functional importance of lipoprotein lipase in high-risk CLL, it is noteworthy that lipoprotein lipase inhibitors are already on the market for metabolic disorders. There is preliminary evidence that one of these agents (orlistat) leads to apoptosis in B-CLL cells.[73] Since orlistat has low toxicity, its off-label use in B-CLL seems warranted. We have shown that PI3-kinase/AKT inhibitors (Wortmannin, LY294002) also effectively induce apoptosis of primary B-CLL cells.[74] A number of genes important for CLL survival have been knocked down by siRNA technology. Thus, even miR genes or others may eventually serve as targets for therapeutic interventions.[12,18]

Molecules detected by molecular methods may also serve as targets for T-cell immunotherapy. One such example is the CLL specific antigen fibromodulin which allows expansion of specific $CD8^+$ autologous T lymphocytes *in vitro*.[75]

Pharmacogenomics

In addition to general static and dynamic prognostic markers related to the disease, several genetic markers associated with response to specific therapies have been investigated (Table 2). Polymorphisms of the FC recep-

Table 2. Impact of selected genetic markers on treatment decisions.

	Prognostic	*Influence of treatment decision, response to specific drugs*	*Reference*
Cytogenetics/FISH			
17p-	yes	effective: alemtuzumab, flavopiridol, high-dose steroids; ineffective: fludarabine, rituximab	65-68
11p-	yes	effective: flavopiridol, pentostatin (PCR) not very effective: alemtuzumab	69, 70
+12	(yes)	prognostic power lost after treatment with FC	
Molecular markers			
Mutation			
IgVH	yes	unclear	
MiR genes	yes	unclear	
FCγReceptor IIIA	no	effect of rituximab dependent on genotype	71
Expression			
ZAP70	yes	HSP90 inhibitors effective in vitro, direct inhibitors in development	72
LPL	yes	LPL inhibitor orlistat effective in vitro	73
PI3-Kinase	no	Antiapoptotic effect of Wortmannin, Ly294002 in vitro	74
Fibromodulin	no	Elicits autologous $CD8^+$ T-cell response in vitro	75
MDR1	yes	Multidrug resistance, target for specific agents	76
Dynamic markers			
microarray p53 signature	no	fludarabine response influenced	77
Minimal residual disease	yes	maintenance treatment with alemtuzumab	50,78

tor γIIIA (FCGR3A) generally predict response to rituximab.[71] Rosenwald *et al*. have studied the gene expression pattern of CLL cells after therapy with fludarabine disease *in vivo* and *in vitro* and have shown that fludarabine induced changes result in a p53-related expression signature.[77] The results predict that fludarabine treatment will lead to selection of p53-mutated CLL clones. The phenomenon of multi-drug resistance has recently been investigated.[76]

Conclusions

Genetic markers have already contributed many important insights into the biology of B-CLL. Some genetic markers have been established as routinely used prognostic factors in addition to the traditional staging systems.[82] This is particularly true for cytogenetic aberrations detected by FISH; other markers like ZAP-70 have shown their prognostic power in large patient series, but need to be more thoroughly harmonized for standard use.[63] Markers like IgVH mutational status and minimal residual disease detection by PCR are harmonized, but cannot easily used in daily routine.[48,83] The least developed group, including lipoprotein lipase, activation-induced cytidine deaminase, or micro RNAs, have not reached either one of these stages, but are potentially interesting prognostic markers.

Currently, the use of genetic and molecular markers could be restricted to patients who will receive more than palliative treatment. There is strong evidence that several of the predictive markers will influence treatment decisions for tailored therapy.[84,85] While generalized routine use of these data cannot be recommended to date, we are soon expecting results from randomized trials powered to answer questions related to the rational use of specific therapies subsequent to the identification of molecular genetic markers.

References

1. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood 1975;46:219-34.
2. Binet JL, Lepoprier M, Dighiero G, Charron D, D'Athis P, Vaugier G, et al. A clinical staging system for chronic lymphocytic leukemia: prognostic significance. Cancer 1977;40: 855-64.
3. Rozman C, Montserrat E. Chronic lymphocytic leukemia. N Engl J Med 1995;333:1052-7.
4. Kipps TJ. Chronic lymphocytic leukemia. Curr Opin Hematol 2000;7:223-34.
5. Montserrat E. New prognostic markers in CLL. Hematology. Am Soc Hematol Educ Program 2006;279-84.
6. Mayr C, Speicher MR, Kofler DM, Buhmann R, Strehl J, Busch R, et al. Chromosomal translocations are associated with poor prognosis in chronic lymphocytic leukemia. Blood 2006;107:742-51.
7. Ng D, Toure O, Wei MH, Arthur DC, Abbasi F, Fontaine L, et al. Identification of a novel chromosome region, 13q21.33-q22.2, for susceptibility genes in familial chronic lymphocytic leukaemia. Blood 2007;109:916-25.
8. Goldin LR, Pfeiffer RM, Li X, Hemminki K. Familial risk of lymphoproliferative tumors in families of patients with chronic lymphocytic leukemia: results from the Swedish Family-Cancer Database. Blood 2004;104:1850-4.
9. Caporaso N, Marti GE, Goldin L. Perspectives on familial chronic lymphocytic leukemia: genes and the environment. Semin Hematol 2004;42:201-6.
10. Marti GE, Rawstron AC, Ghia P, Hillmen P, Houlston RS, Kay N, et al. The International Familial CLL Consortium. Diagnostic criteria for monoclonal B-cell lymphocytosis. Br J Haematol 2005;130:325-32.
11. Mauro FR, Giammartini E, Gentile M, Sperduti I, Valle V, Pizzuti A, et al. Clinical features and outcome of familial chronic lymphocytic leukemia. Haematologica 2006; 91: 1117-20.
12. Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, et al. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukaemia. N Engl J Med 2005;352:1667-76.
13. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000;343:1910-6.
14. Catovsky D, Richards S, Matutes E et al. Response to therapy and survival in CLL is influenced by genetic markers. Preliminary analysis from the LRF CLL4 trial. Blood 2004; 104: [Abstract 8a].
15. Dohner H, Stilgenbauer S, James MR, Benner A, Weilguni T, Bentz M, et al. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. Blood 1997;89: 2516-22.
16. Oscier DG, Gardiner AC, Mould SJ, Glide S, Davis ZA, Ibbotson RE, et al. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. Blood 2002;100:1177-84.
17. Haslinger C, Schweifer N, Stilgenbauer S, Dohner H, Lichter P, Kraut N, et al. Microarray gene expression profiling of B-cell chronic lymphocytic leukemia subgroups defined by genomic aberrations and VH mutation status. J Clin Oncol 2004;22:3937-49.
18. Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, et al. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci USA 2002;99:15524-9.
19. Bilban M, Heintel D, Scharl T, Woelfel T, Auer MM, Porpaczy E, et al. German CLL Study Group. Deregulated expression of fat and muscle genes in B-cell chronic lymphocytic leukemia with high lipoprotein lipase expression. Leukemia 2006;20:1080-8.
20. Stratowa C, Loffler G, Lichter P, Stilgenbauer S, Haberl P, Schweifer N, et al. CDNA microarray gene expression analysis of B-cell chronic lymphocytic leukemia proposes potential new prognostic markers involved in lymphocyte trafficking. Int J Cancer 2001;91:474-80.
21. 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; 94:1848-54.
22. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94:1840-7.
23. Hamblin TJ, Orchard JA, Ibbotson RE, Davis Z, Thomas PW, Stevenson FK, et al. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. Blood 2002; 99:1023-9.
24. Krober A, Seiler T, Benner A, Bullinger L, Bruckle E, Lichter P, et al. V(H) mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. Blood 2002;100:1410-6.
25. Vasconcelos Y, Davi F, Levy V, Oppezzo P, Magnac C, Michel A, et al. Binet's staging system and VH genes are independent but complementary prognostic indicators in chronic lymphocytic leukemia. J Clin Oncol 2003;21:3928-32.
26. Thunberg U, Johnson A, Roos G, Thorn I, Tobin G,

Sallstrom J, et al. CD38 expression is a poor predictor for VH gene mutational status and prognosis in chronic lymphocytic leukemia. Blood 2001;97:1892-4.
27. Stilgenbauer S, Bullinger L, Lichter P, Dohner H. German CLL Study Group (GCLLSG). Chronic lymphocytic leukemia. Genetics of chronic lymphocytic leukemia: genomic aberrations and V(H) gene mutation status in pathogenesis and clinical course. Leukemia 2002;16:993-1007.
28. Guarini A, Gaidano G, Mauro FR, Capello D, Mancini F, De Propris MS, et al. Chronic lymphocytic leukemia patients with highly stable and indolent disease show distinctive phenotypic and genotypic features. Blood 2003;102:4497-506.
29. Oscier DG, Gardiner AC, Mould SJ, Glide S, Davis ZA, Ibbotson RE, et al. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. Blood 2002;100:1177-84.
30. Thorselius M, Krober A, Murray F, Thunberg U, Tobin G, Buhler A, et al. Strikingly homologous immunoglobulin gene rearrangements and poor outcome in VH3-21-using chronic lymphocytic leukemia patients independent of geographic origin and mutational status. Blood 2006;107:2889-94.
31. Kienle D, Benner A, Krober A, Winkler D, Mertens D, Buhler A, et al. Distinct gene expression patterns in chronic lymphocytic leukemia defined by usage of specific VH genes. Blood 2006;107:2090-3.
32. Stamatopoulos K, Belessi C, Moreno C, Boudjograh M, Guida G, Smilevska T, et al. Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: pathogenetic implications and clinical correlations. Blood 2007;109:259-70.
33. Durig J, Nuckel H, Cremer M, Fuhrer A, Halfmeyer K, Fandrey J, et al. ZAP-70 expression is a prognostic factor in chronic lymphocytic leukemia. Leukemia 2003;17:2426-34.
34. Stamatopoulos B, Meuleman N, Haibe-Kains B, Duvillier H, Massy M, Martiat P, et al.Quantification of ZAP70 mRNA in B cells by real-time PCR is a powerful prognostic factor in chronic lymphocytic leukemia. Clin Chem 2007;53:1757-66.
35. Oppezzo P, Vasconcelos Y, Settegrana C, Geannel D, Vuillier F, Legarff-Tavernier M, et al. The LPL/ADAM29 expression ratio is a novel prognosis indicator in chronic lymphocytic leukaemia. French Cooperative Group on CLL. Blood 2005; 106:650-7.
36. Heintel D, Kienle D, Shehata M, Kröber A, Kroemer E, Schwarzinger I, et al. High expression of lipoprotein lipase in poor risk B-cell chronic lymphocytic leukaemia. CLL Study Group. Leukemia 2005;19:1216-23.
37. van't Veer MB, Brooijmans AM, Langerak AW, Verhaaf B, Goudswaard CS, Graveland WJ, et al. The predictive value of lipoprotein lipase for survival in chronic lymphocytic leukemia. Haematologica 2006;91:56-63.
38. Nückel H, Huttmann A, Klein-Hitpass L, Schroers R, Führer A, Sellmann L, et al. Lipoprotein lipase expression is a novel prognostic factor in B-cell chronic lymphocytic leukemia. Leuk Lymphoma 2006;47:1053-61.
39. Van Bockstaele F, Pede V, Janssens A, Callewaert F, Offner F, Verhasselt B, et al. Lipoprotein lipase mRNA in whole blood is a prognostic marker in B cell chronic lymphocytic leukaemia. Clin Chem 2007;53:204-12.
40. Nikitin EA, Malakho SG, Biderman BV, Baranova AV, Lorie YY, Shevelev AY, et al. Expression level of lipoprotein lipase and dystrophin genes predict survival in B-cell chronic lymphocytic leukemia. Leuk Lymphoma 2007;48:912-22.
41. Kainz B, Shehata M, Bilban M, Kienle D, Heintel D, Krömer-Holzinger E, et al. Overexpression of the paternally expressed gene 10 (PEG10) from the imprinted locus on chromosome 7q21 in high-risk B-cell chronic lymphocytic leukemia.Int J Cancer 2007;121:1984-93.
42. Oppezzo P, Vuillier F, Vasconcelos Y, Dumas G, Magnac C, Payelle-Brogard B, et al. Chronic lymphocytic leukemia B cells expressing AID display a dissociation between class switch recombination and somatic hypermutation. Blood 2003;101:4029-32.
43. McCarthy H, Wierda WG, Barron LL, Cromwell CC, Wang J, Coombes KR, et al. High expression of activation-induced cytidine deaminase (AID) and splice variants is a distinctive feature of poor prognosis chronic lymphocytic leukemia. Blood 2003;101:4903-8.
44. Albesiano E, Messmer BT, Damle RN, Allen SL, Rai KR, Chiorazzi N. Activation-induced cytidine deaminase in chronic lymphocytic leukemia B cells: expression as multiple forms in a dynamic, variably sized fraction of the clone. Blood 2003;102:3333-9.
45. Heintel D, Kroemer E, Kienle D, Schwarzinger I, Gleiss A, Schwarzmeier J, et al. High expression of activation-induced cytidine deaminase (AID) mRNA is associated with unmutated IGVH gene status and unfavourable cytogenetic aberrations in patients with chronic lymphocytic leukaemia. German CLL Study Group. Leukemia 2004;18:756-62.
46. Tchirkov A, Chaleteix C, Magnac C, Vasconcelos Y, Davi F, Michel A, et al. hTERT expression and prognosis in B-chronic lymphocytic leukemia. Ann Oncol 2004;15:1476-80.
47. Buhl AM, Jurlander J, Geisler CH, Pedersen LB, Andersen MK, Josefsson P, et al. CLLU1 expression levels predict time to initiation of therapy and overall survival in chronic lymphocytic leukemia. Eur J Haematol 2006;76:455-64.
48. Bottcher S, Ritgen M, Pott C, Bruggemann M, Raff T, Stilgenbauer S, et al. Comparative analysis of minimal residual disease detection using four-color flow cytometry, consensus IgH-PCR, and quantitative IgH PCR in CLL after allogeneic and autologous stem cell transplantation. Leukemia 2004;18:1637-45.
49. Nabhan C, Coutre S, Hillmen P. Minimal residual disease in chronic lymphocytic leukaemia: is it ready for primetime? Br J Haematol 2007;136:379-92.
50. Wendtner CM, Ritgen M, Schweighofer CD, Fingerle-Rowson G, Campe H, Jager G, et al. Consolidation with alemtuzumab in patients with chronic lymphocytic leukemia (CLL) in first remission - experience on safety and efficacy within a randomized multicenter phase III trial of the German CLL Study Group (GCLLSG). The German CLL Study Group (GCLLSG). Leukemia 2004;18:1093-101.
51. Moreton P, Kennedy B, Lucas G, Leach M, Rassam SM, Haynes A, et al. Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. J Clin Oncol 2005;23:2971-9.
52. Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 2001;194:1639-47.
53. Klein U, Tu Y, Stolovitzky GA, Mattioli M, Cattoretti G, Husson H, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J Exp Med 2001;194:1625-38.
54. Vasconcelos Y, De Vos J, Vallat L, Reme T, Lalanne AI, Wanherdrick K, et al. Gene expression profiling of chronic lymphocytic leukemia can discriminate cases with stable disease and mutated Ig genes from those with progressive disease and unmutated Ig genes. The French Cooperative Group on CLL. Leukemia 2005;19:2002-5.
55. Haferlach C, Dicker F, Schnittger S, Kern W, Haferlach T. Comprehensive genetic characterization of CLL: a study on 506 cases analysed with chromosome banding analysis, interphase FISH, IgV(H) status and immunophenotyping. Leukemia 2007[Epub ahead of print].
56. Fernàndez V, Jares P, Salaverria I, Giné E, Beà S, Aymerich M, et al. Gene expression profile and genomic changes in disease progression of early-stage chronic lymphocytic leukaemia. Haematologica 2007; 93:132-6.
57. Chen L, Widhopf G, Huynh L, Rassenti L, Rai KR, Weiss A, Kipps TJ. Expression of ZAP-70 is associated with increased B-cell receptor signaling in chronic lymphocytic leukemia. Blood 2002;100:4609-14.
58. Wiestner A, Rosenwald A, Barry TS, Wright G, Davis RE, Henrickson SE, et al. ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood 2003;101:4944-51.
59. Crespo M, Bosch F, Villamor N, Bellosillo B, Colomer D, Rozman M, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. N Engl J Med 2003;348:1764-75.
60. Rai KR, Chiorazzi N. Determining the clinical course and outcome in chronic lymphocytic leukemia. N Engl J Med 2003;348:1797-9.
61. Orchard JA, Ibbotson RE, Davis Z, Wiestner A, Rosenwald A, Thomas PW, et al. ZAP-70 expression and prognosis in

chronic lymphocytic leukaemia. Lancet 2004;363:105-11.
62. Rassenti LZ Huynh L, Toy TL, Chen L, Keating MJ, Gribben JG, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. N Engl J Med 2004; 351:893-901.
63. Letestu R, Rawstron A, Ghia P, Villamor N, Leuven NB, Boettcher S, et al. Evaluation of ZAP-70 expression by flow cytometry in chronic lymphocytic leukemia: A multicentric international harmonization process. Cytometry B Clin Cytom 2006;70:309-14.
64. Krober A, Bloehdorn J, Hafner S, Buhler A, Seiler T, Kienle D, et al. Additional genetic high-risk features such as 11q deletion, 17p deletion, and V3-21 usage characterize discordance of ZAP-70 and VH mutation status in chronic lymphocytic leukemia. J Clin Oncol 2006;24:969-75.
65. Stilgenbauer S, Dohner H. Campath-1H-induced complete remission of chronic lymphocytic leukemia despite p53 gene mutation and resistance to chemotherapy. N Engl J Med 2002;347:452-3
66. Lozanski G, Heerema NA, Flinn IW, Smith L, Harbison J, Webb J, et al. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood 2004;103:3278-81.
67. Pettitt AR, Matutes E, Oscier D. Alemtuzumab in combination with high-dose methylprednisolone is a logical, feasible and highly active therapeutic regimen in chronic lymphocytic leukaemia patients with p53 defects. Leukemia 2006; 20:1441-5.
68. Giné E, Crespo M, Muntanola A, Calpe E, Joao Gomes M, Villamor N, Montserrat E, Bosch F. Induction of Histone H1.2 cytosolic release in chronic lymphocytic leukemia cells after genotoxic and non-genotoxic treatment. Haematologica 2007; 93:75-82.
69. Byrd JC, Lin TS, Dalton JT, Wu D, Phelps MA, Fischer B, et al. Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukaemia. Blood 2007;109:399-404.
70. Kay NE, Geyer SM, Call TG, Shanafelt TD, Zent CS, Jelinek DF, et al. Combination chemoimmunotherapy with pentostatin, cyclophosphamide, and rituximab shows significant clinical activity with low accompanying toxicity in previously untreated B chronic lymphocytic leukemia. Blood 2007; 109:405-11.
71. Kim DH, Jung HD, Kim JG, Lee JJ, Yang DH, Park YH, et al. FCGR3A gene polymorphisms may correlate with response to frontline R-CHOP therapy for diffuse large B-cell lymphoma. Blood 2006;108:2720-5.
72. Castro JE, Prada CE, Loria O, Kamal A, Chen L, Burrows FJ, et al. ZAP-70 is a novel conditional heat shock protein 90 (Hsp90) client: inhibition of Hsp90 leads to ZAP-70 degradation, apoptosis, and impaired signaling in chronic lymphocytic leukemia. Blood 2005;106:2506-12.
73. Pallasch, Schwamb J, Schulz A, Debey S, Kofler D, Schultze Hallek M, et al. Overexpression of lipases enables specific cytotoxicity by the lipase inhibitor orlistat in chronic lymphocytic leukemia cells. ASH Annual Meeting Abstracts. Blood 2006;108:[abstract 2800].
74. Shehata M, Schnabl S, Demirtas D, Schwarzmeier JD, Hilgarth M, Duechler M, et al. Lymphoid microenvironment inhibits apoptosis in B-CLL cells: Involvement of PI3-K/AKT Pathway and PTEN. ASH Annual Meeting Abstracts. Blood 2006; 108:[abstract 1441].
75. Mayr C, Bund D, Schlee M, Moosmann A, Kofler DM, Hallek M, et al. Fibromodulin as a novel tumor-associated antigen (TAA) in chronic lymphocytic leukemia (CLL), which allows expansion of specific $CD8^+$ autologous T lymphocytes. Blood 2005;105:1566-73.
76. Matthews C, Catherwood MA, Larkin AM, Clynes M, Morris TC, Alexander HD. MDR-1, but not MDR-3 gene expression, is associated with unmutated IgVH genes and poor prognosis chromosomal aberrations in chronic lymphocytic leukemia. Leuk Lymphoma 2006;47:2308-13.
77. Rosenwald A, Chuang EY, Davis RE, Wiestner A, Alizadeh AA, Arthur DC, et al. Fludarabine treatment of patients with chronic lymphocytic leukemia induces a p53-dependent gene expression response. Blood 2004;104:1428-34.
78. Montillo M, Tedeschi A, Miqueleiz S, Veronese S, Cairoli R, Intropido L, et al. Alemtuzumab as consolidation after a response to fludarabine is effective in purging residual disease in patients with chronic lymphocytic leukemia. J Clin Oncol 2006;24:2337-42.
79. Montserrat E. Treatment of chronic lymphocytic leukemia: achieving minimal residual disease-negative status as a goal. J Clin Oncol 2005;23:2884-5.
80. Ritgen M, Stilgenbauer S, von Neuhoff N, Humpe A, Bruggemann M, Pott C, et al. Graft-versus-leukemia activity may overcome therapeutic resistance of chronic lymphocytic leukemia with unmutated immunoglobulin variable heavy-chain gene status: implications of minimal residual disease measurement with quantitative PCR. Blood 2004; 104:2600-2.
81. Moreno C, Villamor N, Colomer D, Esteve J, Martino R, Nomdedeu J, et al. Allogeneic stem-cell transplantation may overcome the adverse prognosis of unmutated VH gene in patients with chronic lymphocytic leukemia. J Clin Oncol 2005;23:3433-8.
82. Binet JL, Caligaris-Cappio F, Catovsky D, Cheson B, Davis T, Dighiero G, et al. International Workshop on Chronic Lymphocytic Leukemia (IWCLL). Perspectives on the use of new diagnostic tools in the treatment of chronic lymphocytic leukemia. Blood 2006;107:859-61.
83. Ghia P, Stamatopoulos K, Belessi C, Moreno C, Stilgenbauer S, Stevenson F, et al. European Research Initiative on CLL. RRIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia. Leukemia 2007; 21:1-3.
84. Gowda A, Byrd JC. Use of prognostic factors in risk stratification at diagnosis and time of treatment of patients with chronic lymphocytic leukemia. Curr Opin Hematol 2006; 13:266-72.
85. Byrd JC, Gribben JG, Peterson BL, Grever MR, Lozanski G, Lucas DM, et al. Select high-risk genetic features predict earlier progression following chemoimmunotherapy with fludarabine and rituximab in chronic lymphocytic leukemia: justification for risk-adapted therapy. J Clin Oncol 2006;24: 437-43.