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Short Communication

Use of V_H, D and J_H immunoglobulin gene segments in Brazilian patients with Chronic Lymphocytic Leukaemia (CLL)

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Abstract

Chronic lymphocytic leukaemia (CLL) is a haematological malignancy for which reliable prognostic markers are needed in view of its clinical heterogeneity. In approximately 50% of CLL patients, immunoglobulin (*Ig*) rearrangements are modified by somatic hypermutation (SHM), a process that represents a reliable prognostic indicator of favourable progression. In this study, we investigated SHM in 37 Brazilian CLL patients and identified the preferential involvement of specific immunoglobulin gene families and segments through PCR-amplified fragments or subcloned fragments. Forty-one rearrangements were observed and 37 of them were functional. A 98% homology cut-off with germinal sequences showed 18 patients (48.7%) with SHM. Unmutated cases showed a poorer clinical outcome. V_H3 was the most frequent V_H family, followed by V_H4. The V_H4-39 segment was the most frequently used, mainly in unmutated cases, while the V_H3 family was predominant in mutated cases. The D3 and J_H4/J_H6 families were the most frequently observed.

Key-words: chronic lymphocytic leukaemia, immunoglobulin rearrangements, somatic hypermutation.

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Chronic lymphocytic leukaemia (CLL) is the most frequent lymphoproliferative malignancy affecting adults in the western world (Calligaris-Cappio and Hamblin, 1999), characterized by accumulation of $CD5^+$ B cells in peripheral blood (Rozman and Montserrat, 1995). Diagnosis relies on the finding, in peripheral blood, of more than $5x10^9$ B-lymphocytes per litre with a $CD5^+$, $CD19^+$ and $CD23^+$ immunophenotypic profile. CLL is a remarkably heterogeneous disease with respect to prognosis and clinical course, once some patients may survive for prolonged periods without requiring therapy, while in others a prompt fatal outcome occurs, even after aggressive treatment (Damle *et al.*, 1999; Hallek, 2000). Although two different criteria - Rai (Rai *et al.*, 1975) and Binet (Binet *et al.*, 1981) - have been proposed for staging this clinically heterogeneous malignancy, at early stages none of these criteria can accurately distinguish patients with an ensuing indolent course from those with a subsequent poor outcome (Maloum *et al.*, 2000). Other prognostic factors are therefore needed for determining, once the diagnosis is made, the therapy of choice for CLL patients (Dighiero and Hamblin, 2002).

One of the most accurate CLL prognostic factors is the occurrence of somatic hypermutation in *Ig* rearrangements, because patients with mutated *Ig* genes show an indolent clinical course compared to those with unmutated genes (Damle *et al.*, 1999; Hamblin *et al.*, 1999). As some *Ig* rearrangements have been more frequently found in unmutated cases while others are more frequent in mutated ones (Fais *et al.*, 1998; Duke *et al.*, 2003; Guarini *et al.*, 2003), it has been postulated that these differences might be associated to geographic or genetic factors (Stevenson and Calligaris-Cappio, 2004) or to differences of antigen types to which cells might be reactive (Chiorazzi and Ferrarini, 2003; Ghiotto *et al.*, 2004; Kolar and Capra, 2004).

In this paper, we report the first study of *Ig* rearrangements in Brazilian CLL patients and the results concerning the frequency with which V_H, D and J_H segments are involved in *Ig* rearrangements in mutated and unmutated cases.

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Samples of peripheral blood (n = 25) or bone marrow (n = 12) were obtained from 37 patients (19 male and 18 female) with CLL, previously diagnosed by immunophenotyping. Their ages ranged from 38 to 76 years (median = 67 years). Their platelet count ranged from 100,000 to 360,000/mm^3 (median = 215,000/mm^3), haemoglobin levels from 9.0 to 16.1 g/dL (median 13.5 g/dL), and lymphocytes from 5,000 to 58,200/mm^3 (median = 19,663/mm^3). Binet stage (in 24 patients) was: A (n = 16), B (n = 06) and C (n = 02). The median follow-up time for 20 patients with complete clinical data was 23 months, ranging from 6 to 144 months (data from one patient with follow-up shorter than six months - LLC 32 - were excluded from analysis, for a better evaluation of disease progression). At the time of this study, treatment had been established for nine patients, three of which were already deceased.

Mononuclear cells were isolated from peripheral blood or bone marrow in Ficoll® (Sigma) density gradients. DNA was extracted using DNAzol® (Life Technologies) following the manufacturer's instructions.

Heavy chain *Ig* genes were amplified for clonality analysis as previously reported (Ramasamy *et al.*, 1992; Yamada *et al.*, 1989). Monoclonality was confirmed by the presence of a single DNA band resulting from co-migration of similar-sized fragments in polyacrylamide gels, while the presence of two DNA bands was interpreted as indicative of two different rearrangements within a same clone.

For sequence analysis, six separate PCR reactions were carried out per patient. Each reaction used one of six, family-specific (V_H1-V_H6), sense, leader primer with one antisense J_H-primer, as previously reported (Campbell *et al.*, 1992), and with a V_H1 primer especially designed for co-amplification of V_H7 sequences. Whenever amplifications were unsuccessful, subsequent PCR assays were carried out with FR1 primers (specific for families V_H1, V_H2, V_H3, V_H4a, V_H4b, V_H5 and V_H6) instead of leader primers, with a V_H1 primer especially designed for co-amplification of V_H7, as described by Pritsch *et al.* (1993). Reaction products were run in 1.5% agarose gels, for determining whether the amplifications had been successful and for V_H-family identification. Amplified products were purified in GFX columns (Amersham Biosciences) for direct DNA sequencing or cloning, following the manufacturer's recommendations.

PCR products were cloned in order to (i) label and sequence amplified 5' Ig regions with FR1 primers; (ii) to separate V_H families with two rearrangements; and (iii) to allow sequencing those PCR products whose sequencing was unclear. Cloning was carried out using the pMOS*Blue* (Amersham Biosciences) plasmid vector, as specified by the manufacturer. Plasmids from recombinant colonies were extracted by Miniprep and used for sequencing.

DNA fragments were labelled with the DYEnamic ET Dye Terminator Cycle Sequencing Kit for MegaBACE DNA Analysis Systems® (Amersham Biosciences) according to the manufacturers instructions, and samples were run in a MegaBACE1000 automated DNA sequencer. Two different, non-cloned, PCR products were sequenced in both directions for each patient, and labelling was carried out with the same primers used in the PCR reactions. Whenever sequence differences were detected, one additional sequencing protocol was performed. Sequencing of cloned fragments, in both directions, was carried out with primers M13F e M13R, from at least three colonies per case in cases with a single rearrangement, and from at least six colonies per case in cases with two rearrangements. Sequences were analysed and edited with SeqMan 4.0 software (DNASTAR Inc.).

V_H, D and J_H sequences were submitted to the IMGT, IgBlast and V-base databases, for analysing and identifying rearranged segments with the highest homology to the germinal V_H, D and J_H regions. Conflicting results regarding D segments were analysed according to the criteria of Corbett *et al.* (1997) or of Fais *et al.* (1998). The CDR3 region was identified according to Fais *et al.* (1998).

Mutations were identified by comparisons of rearranged and germinal *Ig* sequences at the DNA and protein levels, although assignment of patients to the unmutated (UM) or mutated (M) group was based only on DNA data. Only the FR1, CDR1, FR2, CDR2 and FR3 regions were analysed, since changes in the CDR3 and FR4 regions could have been originated during the process of junction. A 98% homology cut-off was chosen for recognising somatic hypermutation, thus excluding a component of variance due to polymorphisms.

To analyse our data we used simple standard statistical tests performed on spreadsheets (Microsoft Excel) or commercially available at IFA Services Statistics.

Forty-one rearrangements, from 37 patients, were sequenced. Comparisons with germline V_H, D and J_H segments in databases allowed identification of sequence homologies and mutations. This analysis showed mutated rearrangements in 18 out of the 37 patients (48.7%), indicated by <98% homology with germline sequences, and unmutated rearrangements in 19 patients (51.3%), with ≥98% homology with germline sequences. These results are similar to those previously reported (Damle *et al.*, 1999; Hamblin *et al.*, 1999).

Four of the 37 patients carried two *Ig* rearrangements, but in all of them only one was functional. In three nonfunctional rearrangements, a stop codon was found at their CDR3 junction, while in another rearrangement the lack of function remained unclear, due to our inability to fully amplify this region. It contained three stop codons in its V_H region, probably generated by somatic hypermutation (SHM) without selection (data not shown). Rearrangements are described in Tables 1 and 2.

Comparisons of clinical data (excluding patient LLC32) showed no significant differences in lymphocyte and platelet counts, haemoglobin level, age, sex, and stage

Table 1 - VH-D-JH rearrangements in the 19 unmutated Brazilian CLL patients.

Patient	Closest VH segment	Percentage of homology	Closest D segment	Amino acid sequence in junction	CDR3 length (number of amino acids)	Closest JH segment
LLC12	1-18	99.3	D5-5	CARGLEDTDMAEVYYW	14	Jh4b
LLC04	1-69	99.65	D3-16	CAREGDYVWGSYRYRRLDSVNIW	21	Jh4b
LLC20	1-69	99.2	D3-3	CARGIGDDFWGGYYIYYYYYYGMDVW	24	Jh6b
LLC32	2-26	99.6	D2-2	CARLMWVPPAPGYCSSTSLYRW	20	**N/D**
LLC35	2-70	98.9	D3-16	CARGQSARNYDYVWGWPWGFDYW	21	Jh4b
LLC11	3-23	100	**without D**	CAKVHFGTSGSNNWFDPW	16	Jh5b
LLC10	3-23	100	D3-3	CASRRYYDFWSGYYTSYNWFDPW	21	Jh5b
	3-30	99.7	D2-2	CAKDSIL**YQLLWCGFTGFLLG*GWTFCH CDYYYYYYMDVW	**N/D**	Jh6c
LLC15	3-30	100	D5-18	CAKBAVLGYSYGYWLSPGW	17	**N/D**
LLC25	4-31	99.7	D5-5	CARDWRSYSYGRDYYYYGMDVW	20	Jh6b
LLC19	4-34	100	D3-3	CARGQRRITIFGVVIIFNPDLGYYYGMDVW	28	Jh6b
LLC01	4-39	100	D5-24	CARIPRTRWLQFVTW	13	Jh4b
LLC03	4-39	99.7	**N/D**	**N/D**	**N/D**	**N/D**
LLC05	4-39	98.9	D3-22	CARDRRPTYYYDSSGFDPW	17	Jh5b
LLC30	4-39	100	D3-10	CARLPVLLWFGELPPFGGVILYYFDYW	25	Jh4b
LLC07	4-39	100	D6-19	CASAGQWLEDYYYYYGMDVW	18	Jh6b
LLC06	4-59	99.3	D3-16	CARHGGPGDYDYVWGSYRSYYYYGMDV W	26	Jh6b
	3-23	100	D2-2	CAKFRVVVVPAAILWGL*LLGPGNPGGHR LLR	**N/D**	Jh4b
LLC23	4-59	100	D3-22	CARNRRDYYDSSGYIHYYYGMDVW	22	Jh6b
	4-59	100	D6-13	CARRGRV*QQLALLVLRSLGPW	**N/D**	Jh2
LLC14	5-51	100	D5-5	CARRKVEYSYEIDYYYYYGMDVW	21	Jh6b
LLC26	5-51	100	**N/D**	**N/D**	**N/D**	**N/D**

* = stop codon; N/D = not determined.

status between the UM and M cases (data not shown). When comparing treatment inclusion and outcome, non-significant but relatively small probability test values (p values = 0.070 and 0.058, respectively, in Fisher's exact test) were found, indicating that a similar investigation should be performed on a larger number of subjects to assess the true nature of the observed differences.

Although complete clinical data were not available for every patient, the M group showed a better outcome than the UM group, in agreement with previous reports (Damle *et al.*, 1999; Hamblin *et al.*, 1999; Maloum *et al.*, 2000). This provided further evidence of the importance of determining the mutational status of CLL patients in order to make a prognosis, although the small number of patients analysed in this study should be taken into account.

In this study, V_H3 was the most frequently used V_H family (37.8%), followed by V_H4 (35.2%), V_H1 (10.8%), V_H5 (8.1%), V_H2 (5.4%) and V_H6 (2.7%). When comparing M and UM cases with regard to families, 61.1% of M cases were represented by the V_H3 family and 47.3% of UM cases by the V_H4 family.

V_H4-39 was the most frequently used segment (in seven rearrangements), followed by V_H3-23, V_H3-33, V_H4-34, and V_H5-51 (three rearrangements each), V_H1-69, V_H3-7, V_H3-11, V_H3-53, and V_H4-59 (two rearrangements each), and V_H1-18, V_H1-8, V_H2-26, V_H2-70, V_H3-30, V_H3-72, V_H4-31, and V_H6-1 (one rearrangement each).

Comparing the use of V_H segments in UM and M cases, V_H4-39 was the most frequently used in UM cases (26.3%), followed by V_H4-59, V_H1-69 and V_H5-51 (10.5% each); in M cases, V_H3 segments were more frequently used, except for V_H3-23 that was more frequent in UM cases.

Although we found that the V_H3 family was the most frequently involved in functional rearrangements, followed by V_H4 and V_H1, in agreement with Pasqualucci *et al.* (2000), several reports have shown V_H3 followed by V_H1 and V_H4 (Fais *et al.*, 1998; Pritsch *et al.*, 1999; Sakai *et al.*, 2000; Duke *et al.*, 2003; Vilpo *et al.*, 2003) or $V_H4 > V_H3 > V_H1$ profiles (Hashimoto *et al.*, 1995). The occurrence of V_H1 as the third most frequent family coincided with the profile of normal cells (Rassenti *et al.*, 1995; Matsuda *et al.*, 1998), although, in most CLL studies, it was found only

Table 2 - VH-D-JH rearrangements observed in the 18 mutated Brazilian CLL patients.

Patient	Closest VH segment	Percentage of homology	Closest D segment	Amino acid sequence in junction	CDR3 length (number of amino acids)	Closest JH segment
LLC22	1-8	91.9	D2-8	CARSCSNGICNGRVAGNYFDPW	20	Jh5b
LLC09	3-7	93.5	D3-10	CMRDPRDYSGSDIDNW	14	Jh4b
LLC13	3-7	91.5	D3-10	CARYVRGGDKYYVDYW	14	Jh4b
LLC17	3-11	94.2	D5-12	CAREWWLQDSYNGVDVW	15	Jh6b
LLC31	3-11	93.5	**N/D**	**N/D**	**N/D**	**N/D**
LLC18	3-23	89.4	D2-21	CAKEQCGSDCYRGFDYW	15	Jh4b
LLC24	3-33	96.6	**without D**	CARDQSLTVESLDYW	13	Jh4b
LLC34	3-33	97.6	D6-6	CARGCIAARNCGEFFKHW	16	Jh1
LLC27	3-33	90.65	**N/D**	**N/D**	**N/D**	**N/D**
LLC21	3-53	90.9	**N/D**	**N/D**	**N/D**	**N/D**
LLC29	3-53	96.2	D3-3	CASQYYDFWSGRTW	12	**N/D**
LLC28	3-72	94.6	D5-5	CVASRYSYGPFDYW	12	Jh4d
LLC37	4-34	91	D6-13	CARGPPAASNAVLLPLDYW	17	Jh4b
LLC33	4-34	95.2	D5-5	CARGYPDTPVVKRYYYYGMDVW	20	Jh6a
	3-30	78.7	**N/D**	**N/D**	**N/D**	**N/D**
LLC02	4-39	90.6	D1-1	CVGHTPEPTYGTYFSAIDYW	18	Jh4b
LLC16	4-39	94.2	D1-26	CGRLYYYYYYMDVW	12	Jh6c
LLC08	5-51	94.2	D6-19	CARHYYSTAWDRYNWFDPW	17	Jh5b
LLC36	6-1	96.4	**N/D**	**N/D**	**N/D**	**N/D**

N/D = not determined.

when the V_H1-69 segment was excluded from the analyses (Oscier *et al.*, 1997; Duke *et al.*, 2003). The findings of only two V_H1-69 segments in our patients represented a relevant difference with respect to other reports. This, however, might be due to the small number of patients analysed or, alternatively, to individual variations in number of the germline V_H1-69 segment in our population (Stewart *et al.*, 1993; Cook and Tomlinson, 1995).

We did not find any rearrangement involving the V_H3-21 segment, a very frequent occurrence in CLL (Duke *et al.*, 2003) and usually related to a very poor prognosis in mutated cases (Tobin *et al.*, 2002; Dighiero, 2004), although the relation of mutational status and the molecular profile of other V_H segments were coincident with other reports (Duke *et al.*, 2003; Guarini *et al.*, 2003; Stevenson and Calligaris-Cappio, 2004).

D segments were not completely resolved by sequencing in six rearrangements, due to loss of the initial J_H region (the annealing region) when sequencing. D5-5 was the most frequently used D segment (five rearrangements), followed by D3-3 (four rearrangements), D3-10 and D3-16 (three rearrangements each), D3-22 and D6-19 (two rearrangements each), and D1-1, D1-26, D2-2, D2-8, D2-21, D5-12, D5-18, D5-24, D6-6 and D6-13 (one rearrangement each). Two rearrangements did not contain a D segment. The use of D segments differed between UM and M patients; in UM cases, the most frequent segments were D3-3, D3-16, D3-22 and D5-5, while in M cases an equal distribution of D segments was observed.

D5-5 was the most frequent D segment in productive rearrangements, unlike previous reports pointing to D3-3 as the most frequent segment involved in CLL rearrangements (Vilpo *et al.*, 2003), mainly in UM cases (Hamblin *et al.*, 1999; Duke *et al.*, 2003). In M cases, we found D5-5 and D3-10 to be the most frequent segments, unlike another report in which D3-22 was the most frequent (Duke *et al.*, 2003). Interestingly, in our patients, both rearrangements involving D3-22 segments were found in UM cases.

The J_H family was not completely resolved by sequencing in six rearrangements, due to loss of the initial J_H region (the annealing region) when sequencing. J_H4 was the most frequently used J_H family (12 rearrangements), followed by J_H6 (10 rearrangements), J_H5 (five rearrangements), and J_H1 (one rearrangement). The J_H6 family was more frequently used in UM cases, while J_H4 was more frequently used in M cases. Our findings regarding J_H families were coincident with previous studies, with J_H4 being more frequently represented in M rearrangements and J_H6 in UM rearrangements (Fais *et al.*, 1998; Pritsch *et al.*, 1999; Duke *et al.*, 2003; Vilpo *et al.*, 2003).

The length of the CDR3 protein region, determined in 31 functional rearrangements, ranged from 12 to 28 amino acids. UM cases showed a longer CDR3 region (from 13 to 28 amino acids; median = 21) than M cases (from 12 to 20

amino acids; median = 15). This difference was statistically significant ($p < 0.001$, by both Student's *t*-test and the Wilcoxon test), in agreement with previous data in the literature (Opezzo *et al.*, 2002; Duke *et al.*, 2003). Here again, we do not know if these findings reflect genetic and/or environmental factors or result from the small number of patients analysed by us. It is noteworthy that the genetic background of the Brazilian population is markedly different from the European and North American populations in ancestry and miscegenation. Moreover, environmental factors might be responsible for the presence of different types of antigens, accounting for specific antigenic stimulations (Ghiotto *et al.*, 2004; Kolar and Capra, 2004) and leading to the occurrence of different rearrangements in different geographic regions (Stevenson and Calligaris-Cappio, 2004). To elucidate this point, a much larger Brazilian population study should be conducted. Moreover, the lack of data on the incidence or prevalence of CLL in Brazil makes it impossible to know whether these epidemiological parameters are similar to those in other western countries, a fact that might explain the difficulties in finding larger cohorts for confirming our results.

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References

Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, Vaugier G, Potron G, Colona P, Oberling F, *et al.* (1981) A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 48:198-206.

Calligaris-Cappio F and Hamblin TJ (1999) B-cell chronic lymphocytic leukemia: A bird of a different feather. J Clin Oncol 17:399-408.

Campbell MJ, Zelenetz AD, Levy S and Levy R (1992) Use of family specific leader region primers for PCR amplification of the human heavy chain variable region gene repertoire. Mol Immunol 29:193-203.

Chiorazzi N and Ferrarini M (2003) B cell chronic lymphocytic leukemia: Lessons learned from studies of the B cell antigen receptor. Annu Rev Immunol 21:841-894.

Cook GP and Tomlinson IM (1995) The human immunoglobulin VH repertoire. Immunol Today 16:237-242.

Corbett SJ, Tomlinson IM, Sonnhammer ELL, Buck D and Winter G (1997) Sequence of the human immunoglobulin diversity (D) segment locus: A systematic analysis provides no evidence for the use of DIR segments, inverted D segments, "minor" D segments or D-D recombination. J Mol Biol 270:587-597.

Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K, Kolitz J, *et al.* (1999) Ig V gene status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 94:1840-1847.

Dighiero G (2004) Unsolved issues in CLL. Biology and management. Leukemia 17:2385-2391.

Dighiero G and Hamblin T (2002) Is chronic lymphocytic leukemia one disease? Haematologica 87:1233-1241.

Duke VM, Gandini D, Sherrington PD, Lin K, Heelan B, Amlot P, Mehta AB, Hoffbrand AV and Foroni L (2003) VH gene usage differs in germline and mutated B cell chronic lymphocytic leukemia. Haematologica 88:1259-1271.

Fais F, Ghiotto F, Hashimoto S, Sellars B, Valetto A, Allen SL, Schulman P, Vinciguerra VP, Rai K, Rassenti LZ, *et al.* (1998) Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest 102:1515-1525.

Ghiotto F, Fais F, Valetto A, Albesiano E, Hashimoto S, Dono M, Ikematsu H, Allen SL, Kolitz J, Rai KR, *et al.* (2004) Remarkably similar antigen receptors among a subset of patients with chronic lymphocytic leukemia. J Clin Invest 113:1008-1016.

Guarini A, Gaidano G, Mauro FR, Capello D, Mancini F, De Propis MS, Mancini M, Orsini E, Gentile M, Breccia M, *et al.* (2003) Chronic lymphocytic leukemia patients with highly stable and indolent disease show distinctive phenotypic and genotypic features. Blood 102:1035-1041.

Hallek M (2000) New concepts in the pathogenesis, diagnosis, prognostic factors and clinical presentation of chronic lymphocytic leukemia. Rev Clin Exp Hematol 4:103-117.

Hamblin TJ, Davis Z, Gardiner A, Oscier DG and Stevenson FK (1999) Unmutated Ig VH genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 94:1848-1854.

Hashimoto S, Dono M, Wakai M, Allen SL, Lichtman SM, Schulman P, Vinciguerra VP, Ferrarini M, Silver J and Chiorazzi N (1995) Somatic diversification and selection of immunoglobulin heavy and light chain variable region genes in IgG+ CD5+ chronic lymphocytic leukemia B cells. J Exp Med 181:1507-1517.

Kolar GR and Capra D (2004) IgV region restrictions in human chronic lymphocytic leukemia suggest some cases have a common origin. J Clin Invest 113:952-954.

Maloum K, Davi F, Merle-Béral H, Pritsch O, Magnac C, Vuillier F, Troussard X, Mauro FF and Bénichou J (2000) Expression of unmutated VH genes is a detrimental prognostic factor in chronic lymphocytic leukemia. Blood 96:377-379.

Matsuda F, Ishii K, Bourvagnet P, Kuma K, Hayashida H, Miyata T and Honjo T (1998) The complete nucleotide sequence of the human immunoglobulin heavy chain variable region locus. J Exp Med 188:2151-2162.

Opezzo P, Magnac C, Bianchi S, Vuillier F, Tiscornia A, Dumas G, Payelle-Brogard B, Ajchenbaum-Cymbalista F, Dighiero G and Pritsch O (2002) Do CLL B cells correspond to naïve or memory B-lymphocytes? Evidence for an active Ig switch unrelated to phenotype expression and Ig mutational pattern in B-CLL cells. Leukemia 16:2438-2446.

Oscier DG, Thompset A, Zhu D and Stevenson F (1997) Differential rates of somatic hypermutation in VH genes among subsets of chronic lymphocytic leukemia defined by chromosomal abnormalities. Blood 89:4153-4160.

Pasqualucci L, Neri A, Baldini L, Dalla-Favera R and Migliazza A (2000) BCL-6 mutations are associated with immunoglobulin variable heavy chain mutations in B cell chronic lymphocytic leukemia. Cancer Res 60:5644-5648.

Pritsch O, Magnac C, Dumas G, Egile C and Dighiero G (1993) V gene usage by seven hybrids derived from CD5$^+$ B-cell chronic lymphocytic leukemia and displaying autoantibody activity. Blood 82:3103-3112.

Pritsch O, Troussard X, Magnac C, Mauro FR, Davi F, Payelle-Brogard B, Dumas G, Pulik M, Clerget F, Mandelli F, *et al.* (1999) VH gene usage by family members affected with chronic lymphocytic leukaemia. Br J Haematol 107:616-624.

Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN and Pasternack BS (1975) Clinical staging of chronic lymphocytic leukemia. Blood 46:219-234.

Ramasamy I, Brisco M and Morley AA (1992) Improved PCR method for detecting monoclonal immunoglobulin heavy chain rearrangement in B cell neoplasms. J Clin Pathol 45:770-775.

Rassenti LZ, Kohsaka H and Kipps TJ (1995) Analysis of immunoglobulin VH genes repertoire by an anchored PCR-ELISA. Ann NY Acad Sci 764:463-473.

Rozman C and Montserrat E (1995) Chronic lymphocytic leukemia. N Engl J Med 333:1052-1057.

Sakai A, Marti GE, Caporaso N, Pittaluga S, Touchman JW, Fend F and Raffeld M (2000) Analysis of expressed immunoglobulin heavy chain genes in familial B-BLL. Blood 95:1413-1419.

Stevenson FK and Calligaris-Cappio F (2004) Chronic lymphocytic leukemia: Revelations from B cell receptor. Blood 103:4389-4395.

Stewart AK, Huang C, Stolar DD and Schwartz RS (1993) High frequency representation of a single VH gene in the expressed human B cell repertoire. J Exp Med 177:409-418.

Tobin G, Thunberg U, Johnson A, Thorn I, Soderberg O, Hultdin M, Botling J, Enblad G, Salstrom J, Sundstrom C, *et al.* (2002) Somatically mutated Ig Vh3-21 genes characterize a new subset of chronic lymphocytic leukemia. Blood 99:2262-2264.

Vilpo J, Tobin G, Hulkkonen J, Hurme M, Thunberg U, Sundstrom C, Vilpo L and Rosenquist R (2003) Surface antigen expression and correlation with variable heavy-chain gene mutation status in chronic lymphocytic leukemia. Eur J Haematol 70:53-59.

Yamada M, Hudson S, Tournay O, Bittenbender S, Shane S, Lange B, Tsujimoto Y, Caton AJ and Rovera G (1989) Detection of minimal disease in hematopoietic malignancies of the B-cell lineage by using third-complementarity-determining region (CDR-III)-specific probes. Proc Natl Acad Sci USA 86:5123-5127.

Internet Resources

IFA Services Statistics. www.fon.hum.uva.nl/Service/Statistics.html (February, 2005).

IMGT®, the International ImMunoGeneTics information system® http://imgt.cines.fr (April, 2004).

MRC Centre for protein engineering. V-Base: http://www.mrc-cpe.cam.ac.uk (April, 2004).

National Center for Biotechnology Information. IgBLast: http://www.ncbi.nlm.nih.gov/igblast (April, 2004).

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