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Genomic instability at the human CD5 gene promoter

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Background and Objectives. The gene coding for the human CD5 lymphocyte surface receptor maps to the 11q12.2 region, which is in the vicinity of a region commonly affected by multiple somatic mutations in human cancers. The 5'-flanking region of the human CD5 gene includes an evolutionarily conserved (TC)n(CA)n microsatellite (MS) of potential utility as a marker for genome instability. The aim of the present study was to investigate the value of the CD5 MS as a marker for instability in different tumor types, particularly in B-cell leukemia and lymphoma.

Design and Methods. The CD5 MS and a panel of 10 MS markers were analyzed by using a polymerase chain reaction (PCR)-based method and polyacrylamide sequencing gels. This was done in several hematopoietic and non-hematopoietic cell lines, as well as in 28 cases of B-cell chronic lymphocytic leukemia (B-CLL), 19 mantle cell lymphomas (MCL) and 45 head and neck carcinomas (HNC).

Results. The frequency of CD5 MS abnormalities found among HNC was similar to that reported for other well known MS markers at loci near known and suspected cancer genes. However, instability at the CD5 MS was the most frequent MS abnormality among B-CLL and MCL.

Interpretation and Conclusions. The inclusion of MS markers at chromosome 11q may be especially informative for genome instability analyses of certain B-cell leukemias and lymphomas.
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Key words: CD5, human 11q chromosome, microsatellite instability, B-cell chronic lymphocytic leukemia, mantle cell lymphoma, head and neck carcinomas.

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Microsatellites (MS) are short tandem nucleotide repeats ubiquitously distributed throughout eukaryote genomes. Reports of a somehow non-random distribution of MS[1,2] have suggested that the MS are involved in various biological processes such as transcription and chromatin organization.[3,4] MS show pronounced length polymorphism, an attribute that has made them attractive in gene mapping and linkage analyses, and also as markers of the genome instability frequently associated with many types of human cancer.[5] MS instability (MIN) represents a specific type of genome instability that is characterized by generalized alterations in the germ-line size of MS as a result of defects in the DNA mismatch repair function. MIN was initially identified in most hereditary non-polyposis colorectal cancers (HNPCC), but subsequent evidence has documented that MIN represents one of the most common genetic lesions in human solid tumors. It occurs at variable frequencies in a wide variety of human sporadic cancers, including those of the colon, lung, stomach, pancreas, endometrium, kidney, bladder, breast, and head and neck.[5] The replication error phenotype (RER^+) found in these cancers has been attributed to the presence of hereditary and somatic mutations in genes of the mismatch repair machinery, such as *MSH2* and *MLH1*.[6,7]

The involvement of MIN in the pathogenesis and progression of leukemia and lymphoma has not been conclusively demonstrated. In mice, animals homozygous for the human *MSH2*$^{-/-}$, a homolog of the bacterial *MutS* mismatch repair gene, develop lymphoid tumors that show MIN.[8] In humans, some reports indicate that MIN is rare or absent among lymphomas and leukemias,[9-11] while others report the existence of MIN, at least in a subset of

leukemia.[12,13] The reason for such discrepancy remains elusive.

The chromosome 11q13 is a region commonly affected by multiple somatic mutations in human cancers. Amplification of genes at 11q13 (*CCND1, EMS1, INT-2, HST-1, PYGM*) is seen in primary solid tumors, including carcinomas of the head and neck, lung, esophagus, bladder and breast.[14-16] Loss of heterozygosity (LOH) for MS markers at 11q13 has been reported in multiple endocrine neoplasia type 1 (*MEN1*) and sporadic invasive carcinomas of the same tissue types.[17-19] The t(11;14)(q13;q32) translocation is associated with B-cell lymphoproliferative disorders, particularly mantle cell lymphoma (MCL) but also with chronic lymphocytic leukemia (B-CLL).[20,21] Interestingly, B-CLL frequently (20%) present deletions in the nearby 11q22-q23 region,[22] which holds candidate tumor-suppressor genes such as ataxia-telangiectasia mutated (*ATM*) gene[23,24] and radixin (*RDX*), which has homology to the neurofibromatosis-type 2 (*NF2*) tumor-suppressor gene.[25]

The human *CD5* gene was first mapped at chromosome 11q13,[26] and recently it has been more precisely positioned at the 11q12.2 region, 82 kb telomeric from the human *CD6* gene.[27,28] Both the *CD5* and *CD6* genes code for lymphocyte membrane glycoproteins that belong to the scavenger receptor cysteine-rich superfamily (SRCR-SF).[29,30] *CD5* and *CD6* are expressed by most thymocytes and mature T-cells, as well as by a subset of mature B-cells (named B1a cells) which is expanded in certain autoimmune disorders (systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, diabetes mellitus type I, thyroiditis) and B-cell lymphoproliferative diseases (B-CLL, MCL, hairy cell leukemia).[30,31] Our group has previously reported the existence of an evolutionarily conserved $(TC)_n(CA)_n$ MS located at the *CD5* promoter region.[32,33] Here we analyze this MS as a marker of genetic instability in human tumors, particularly in B-CLL and MCL, two lymphoproliferative disorders derived from $CD5^+$ B-cells. We demonstrate the presence of a relatively high frequency of MIN in B-CLL and MCL as compared with that of other well known MS markers located outside the 11q13 region.

Design and Methods

Microsatellite analysis

The sequences of the HUMANSAT3 and 5PSEQ3 primers used to analyze the CD5 MS were 5'-CTCTACATGGAGCTCACACATA-3' and 5'-CATGAATGCTGGGCTTGT GC-3', respectively. The polymerase chain reactions (PCR) were performed with 0.2 μg genomic DNA, 20 mM Tris-HCl pH 8.4, 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM of each dNTP, 25 pmol unlabeled HUMANSAT3 primer, 2.5 pmol [γ-^{32}P] 5'end-labeled 5PSEQ3 primer, and 2.5 units *Taq* DNA polymerase (Gibco-BRL, Eggenstein, Germany) in a total volume of 50 μL. Amplifications were carried out for 25 cycles consisting of 30 s at 95°C, 30 s at 60°C and 30 s at 72°C, with a final extension of 10 min at 72°C. A 3 μL aliquot of each reaction was analyzed on denaturing 6% polyacrylamide sequencing gels, and bands were visualized by autoradiography.[33]

The RER/LOH assay kit (PE Applied Biosystems) for detection of replication error (RER) or loss of heterozygosity (LOH) was used following manufacturer's instructions. This kit includes ten MS markers (D8S254, NM23, D18S35, TP53-DinucI ; D5S346, TP53-Penta, D2S123, D1S2883, D3S1611 and D7S501) located on several (8p22, 17q21, 18q21, 17p13, 5q21, 17p13, 2p16, 1q24, 3p22, and 7q31, respectively) near known and suspected cancer genes. The products resulting from PCR amplification of these MS markers were separated on an AbiPrism 310 DNA sequencer (PE Applied Biosystems) and analyzed using the Genescan Fragment Analysis and the Genotyper software (PE Applied Biosystems).

DNA samples

Paired normal and tumor DNA from 28 cases of B-CLL (4 of which were Richter's syndromes) and 19 MCL were from the Hematopathology Unit of the Hospital Clínic of Barcelona. The criteria used for the diagnosis of these B-cell lymphoproliferative disordes, as well as the clinical characteristics of the patients have been reported elsewhere.[11] Normal and tumor DNA samples from 45 squamous cell head and neck carcinomas (HNC), all of them at the hypopharynx and invasive (T3 and T4 stages), and from 22 laryngeal carcinomas (T1 and T2 stages) were provided by the Oncology Department of Clínica Universitaria of Navarra. DNA from 193 unrelated Caucasian, volunteer blood donors was obtained from the Blood Bank of the Hospital Clínic of Barcelona. The hematopoietic and non-hematopoietic cell lines used in our study were from the American Type Culture Collection (ATCC, Rockville MD, USA).

Samples of tumor and non-tumor control genomic DNA were purified by using QIAamp kits (Qiagen, Inc., CA, USA), following the manufacturer's instructions. Frozen or formalin-fixed, paraffin-embedded specimens were used for MCL or HNC

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analysis. For B-CLL analysis, blood samples were subjected to centrifugation on Ficoll/Hypaque (Seromed, Berlin, Germany) gradient, and peripheral blood mononuclear cells used as a source of tumor DNA and peripheral blood granulocytes for normal control DNA.

Results

Nucleotide sequence analysis of the 5'-flanking region of the human CD5 gene reveals the existence of evolutionarily conserved transcription regulatory elements.[32] Among them, there is a conserved $(TC)_n(CA)_n$ MS present at orthologous positions in the human and mouse *CD5* gene promoter.[33] Here we have extended our studies on the polymorphic nature of this MS by assessing its allele frequencies in our population and by investigating its potential as a marker for genome instability studies in human cancers.

A PCR-based method[33] allowed us to define 9 alleles in a population sample of 193 unrelated Caucasian individuals, but the size of the smallest and largest observed alleles suggest that a further allele may exist. Accordingly, we designated the alleles 1-10, with allele 10 being the smallest. The sizes and proportions (of the total) of each allele, assuming no null alleles, were as follows: allele 1 (177bp), 3.34%; allele 2 (175 bp), 14.77%; allele 3 (173 bp), 41.97%; allele 4 (171 bp), 23.84%; allele 5 (169 bp), 11.92%; allele 6 (167 bp), 1.82%; allele 7 (165 bp), 1.82%; allele 8 (163 bp), 0.26%; allele 9 (161 bp), not seen; allele 10 (159 bp), 0.26%. Allele 3 (173 bp) was the most common allele observed in our sample (42% of alleles) and the genotype of individuals was dominated by 3/3 homozygous and heterozygous forms containing allele 3. The most common genotypes observed were: 3/4 (28.5%), 3/3 (14.5%), 3/5 (11.4%), 2/3 (9.3%), 2/4 (6.7%), and 4/5 (5.2%). The observed total heterozygosity was 77.7%, which is above the average found for human MS.[34]

Since genetic instability is an integral component of human neoplasia, we examined a panel of tumor-derived human cell lines available in our laboratory for stability of the CD5 promoter MS (CD5Prom) by our PCR-based method.[33] The cell lines were from hematopoietic and non-hematopoietic origin, and included epithelial (Hela, Hep-2), lymphoid T (HSB2, 8402, HPB-ALL, HUT-78, Molt-4, Jurkat, CEM, Kit225,) lymphoid B (Namalwa, Ramos Raji, RPMI, Nalm-6, TE-29), and myelomonocytic (HL60, K562, U937, THP-1, KM-3) cells. While most cell lines were either homozygous or heterozygous for the above reported alleles, four of them (CEM, Molt-4, HSB2 and Jurkat), all of lymphoid T-cell origin, showed a band pattern compatible with MIN (Figure 1). By using a further set of 5 MS markers (D1S2883, D2S123, D5S346, D7S501, TP53-Dinucl) from the Microsatellite RER/LOH Assay (PE Applied Biosystems), MIN could be demonstrated at other loci near known and suspected cancer genes (Table 1). LOH phenomena could not be documented due to the lack of availability of non-tumor counterpart DNA samples. Confirming the results obtained for CD5Prom, MIN

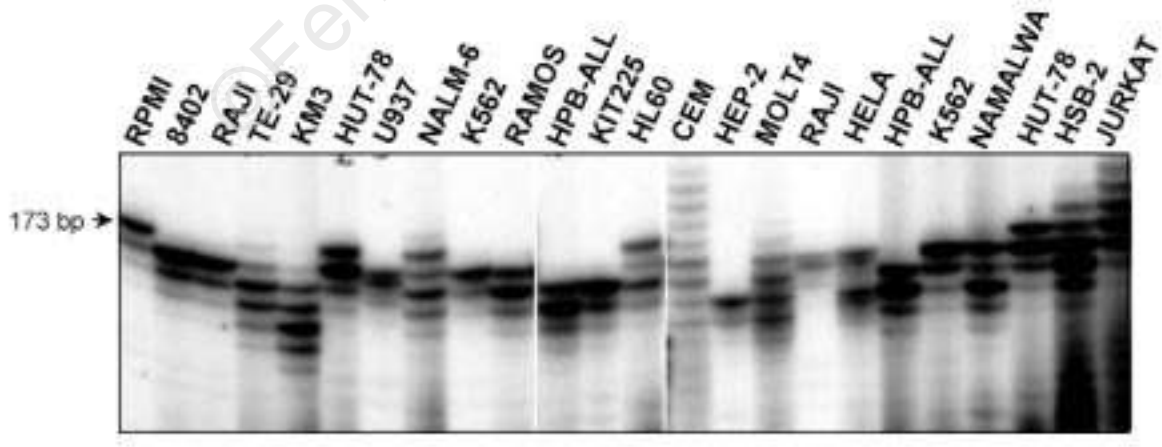

Figure 1. Analysis of the CD5Prom microsatellite in a panel of tumor-derived human cell lines. The primers used were HUMANSAT3 and 5PSEQ3. Amplifications were carried out for 25 cycles: 30 s at 95°C, 15 s at 60°C and 30 s at 72°C, with a final extension of 10 min at 72°C. Each reaction was analyzed on a denaturing 6% polyacrylamide sequencing gel. The autoradiograph illustrates the existence of microsatellite instability in some but not all cell lines. The position of allele 3 (173 bp) is indicated by an arrow.

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could not be demonstrated in Raji cells at any of the MS markers analyzed (Figure 1 and Table 1).

In order to test the potential usefulness of the CD5Prom MS in genome instability analyses several hematopoietic and non-hematopoietic human tumors were analyzed. The CD5Prom was first studied in conjunction with 10 other well known MS markers (D8S254, NM23, D18S35, TP53-Dinucl; D5S346, TP53-Penta, D2S123, D1S2883, D3S1611 and D7S501; Microsatellite RER/LOH Assay, PE Applied Biosystems) in normal and tumor DNA from 28 and 19 individuals diagnosed as having B-CLL and MCL, respectively.[11] We found abnormalities, at least in one of the MS markers used, in 21% of both B-CLL (6 cases) and MCL (4 cases), with CD5Prom MIN being the most frequent alteration (Table 2). In fact, the inclusion of CD5Prom in the analysis dramatically increased the percentage of observed MS abnormalities (from 10.7% to 21% for B-CLL, and from 0% to 21% for MCL).[11] Interestingly, no alterations were observed in any of the 4 analyzed cases of B-CLL transformed into large-cell lymphoma (Richter's syndrome). This supports a lack of correlation between MIN and histologic or clinical disease progression.

A parallel analysis was performed in a series of 45 squamous cell head and neck carcinomas (HNC), all of them at the hypopharynx and invasive (T3 and T4 stages). This type of tumor shows frequent structural abnormalities of many chromosome regions, including the 11q13 region.[35] By using the Microsatellite RER/LOH Assay, we detected abnormalities, at least in one MS marker, in 29 cases (64.4%). When we included the CD5Prom in the analysis, this percentage increased only up to 66.6%. The frequency of MS abnormalities detected at CD5Prom was 22.2% (15.5% MIN; 6.7% LOH), which was similar to the frequency found for the most unstable D1S2883 (20%) and D18S35 (17.7%) MS markers (Table 3). This indicates that CD5Prom is a good informative marker for genome instability. Once again, the grade of informativeness did not depend on the stage of tumor progression since the analysis of 22 laryngeal carcinomas at stages T1 and T2 also showed MS abnormalities at CD5Prom in 31.8% of cases, with MIN representing 22.7% (5 cases) and LOH 9.1% (2 cases). In this series, the paucity of the tumor samples prevented us from extending the analysis to the other 10 MS markers used above.

Discussion

In this study we analyzed the potential of the CD5Prom MS as a useful genetic tool, not only in population studies but also in genome instability analyses of human tumors. The latter was exemplified by the analysis of hematopoietic (B-CLL and MCL) and epithelial (squamous cell laryngeal and hypopharyngeal) malignancies in which the CD5Prom MS highlighted the presence of MS abnormalities with similar, if not better, efficiency than other well known MS markers. This was especially true for B-CLL and MCL, two lymphoproliferative disorders in which abnormal B-cells express the surface CD5 antigen. The inclusion of the CD5Prom MS raised the frequency of MS abnormalities detected in both B-CLL (from 10.7% to 21%), and MCL (from 0% to 21%).

Our findings confirm the general relative paucity of MS abnormalities in leukemias and lymphomas[9–12] compared to in carcinomas (21% vs. 66.6% abnormalities in at least one MS analyzed, in our study). This paucity, however, seems to be lower than

Table 1. Microsatellite analysis of four human lymphoblastoid cell lines.

MS marker	*CEM*	*Jurkat*	*HSB2*	*Raji*
D1S2883 (1q24)	Hetero	Hetero	MIN	Hetero
D2S123 (2p16)	MIN	Homo	MIN	Homo
D5S346 (5q21)	Hetero	Hetero	MIN	Hetero
D7S501 (7q31)	Hetero	MIN	MIN	Hetero
TP53-Dinucl (17p13)	MIN	MIN	MIN	Homo
CD5Prom (11q12.2)	MIN	MIN	MIN	Homo

MIN: microsatellite instability; Hetero: heterozygous; Homo: homozygous.

Table 2. Summary of the genomic instabilities found in 28 cases of B-CLL and 19 MCL.

	MS abnormalities				
Case	*CD5Pro mMIN*	*D1S2883 MIN*	*TP53-Dinucl MIN*	*D2S123 MIN*	*NM23 LOH*
B-CLL.1	+	–	–	–	–
B-CLL.2	–	+	–	–	–
B-CLL.18	–	–	+	–	+
B-CLL.20	–	+	–	+	+
B-CLL.5	+	–	–	–	–
B-CLL.6	+	–	–	–	–
MCL.1	+	–	–	–	–
MCL.2	+	–	–	–	–
MCL.3	+	–	–	–	–
MCL.4	+	–	–	–	–
Total:	8	2	1	1	2

MIN, microsatellite instability; LOH: loss of heterozygosity.

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Table 3. Summary of the microsatellite instabilities found in 45 invasive (T3 and T4 stages) carcinomas of the hypopharynx.

	MS abnormalities											
	CaseCD5Prom		*TP53-Penta*	*TP53-Dinucl*	*D2S123*	*NM23*	*D18S35*	*D1S2883*	*D3S1611*	*D8S254*	*D5S346*	*D7S501*
	MIN	*LOH*	*MIN*	*MIN*	*MIN*	*MIN*	*MIN*	*MIN*	*MIN*	*MIN*	*MIN*	*MIN*
HNC1	+	–	–	–	–	–	–	–	–	–	–	–
HNC2	–	+	–	–	–	–	+	+	–	–	–	–
HNC3	–	–	–	+	+	–	+	–	–	–	–	–
HNC4	–	–	–	+	–	–	–	+	–	–	–	–
HNC5	–	–	–	–	+	–	–	–	–	+	–	–
HNC6	+	–	+	–	–	–	+	–	–	–	+	–
HNC7	+	–	-–	–	+	–	–	–	–	–	–	–
HNC8	–	–	–	+	–	–	–	+	–	–	–	–
HNC9	–	–	–	–	–	–	–	–	+	–	–	–
HNC10	–	–	–	+	–	–	+	–	–	–	–	–
HNC11	–	–	–	+	–	–	–	–	–	+	–	–
HNC12	–	–	–	–	+	–	–	–	–	+	+	–
HNC13	–	–	+	–	–	–	–	+	–	–	–	+
HNC14	–	+	–	–	–	–	+	–	–	–	–	–
HNC15	–	–	–	–	+	–	–	–	+	–	–	–
HNC16	+	–	–	–	–	–	-–	–	–	+	–	–
HNC17	–	–	–	–	–	–	–	+	–	–	+	–
HNC18	–	–	+	+	–	+	+	–	–	–	–	–
HNC19	+	–	–	-–	–	–	–	+	–	–	–	–
HNC20	–	–	+	–	–	–	–	–	+	+	–	–
HNC21	–	–	–	–	+	–	–	–	–	–	+	–
HNC22	–	–	–	–	–	–	–	+	–	–	–	+
HNC24	+	–	–	–	–	–	–	–	–	–	–	+
HNC25	–	–	-–	–	+	+	–	–	–	–	–	–
HNC26	+	–	–	+	–	–	–	–	–	–	–	–
HNC27	–	+	–	+	–	–	–	–	–	–	–	–
HNC28	–	–	–	–	–	–	–	+	–	–	+	–
HNC29	–	–	+	–	+	–	+	–	–	–	–	–
HNC30	–	–	–	–	–	+	–	+	–	–	–	–
Total:	7	3	5	8	8	3	8	9	3	5	5	3

MIN, microsatellite instability; LOH: loss of heterozygosity.

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expected as revealed by the enhancing effects of including a MS marker located at the 11q13 region, such as the CD5Prom MS. In this regard, it is worth noting that most studies in leukaemias and lymphomas reported so far looked at well-defined loci involved in solid tumors, but did not include markers at the 11q region.[9-12] Interestingly, a study reporting a relatively high frequency of MS abnormalities in adult leukemia employed MS markers linked to chromosomal breakpoint regions, which included several markers at the 11q region.[13] Thus, it seems that the 11q region may be of special informative relevance when analyzing genome instability in leukemias and lymphomas, at least in B-CLL and MCL.

The 11q chromosome, as well as the syntenic murine region,[35] is commonly targeted by multiple somatic mutations in malignancies (deletions, amplifications, chromosomal breakages and translocations), and contains growth regulatory and tumor-suppressor genes (*ATM, RDX*) supposedly implicated in tumorigenesis.[21] Therefore, it seems appropriate to reassess the incidence of genome instability in leukemias and lymphomas by carefully looking at informative loci such as the 11q region where the CD5Prom locates. The analysis of other MS markers placed at the 11q chromosome is needed to support this assumption. Similarly, the investigation of deleterious somatic mutations at the ATM gene, as well as at other local tumor-suppressor genes of the 11q region, would also be informative.

Several studies have suggested that MIN may participate in the progression of different types of tumors.[36,37] In hematologic malignancies, it has been

suggested that MIN in combination with other cytogenetic changes could be associated with progression of chronic to acute myeloid leukemias.[38,39] In this study we did not detect MS abnormalities in the 4 analyzed cases of Richter's syndrome, which results from the transformation of B-CLL into aggressive large cell-lymphoma. Additionally, we observed a similar incidence of CD5Prom MS abnormalities among non-invasive (T1 and T2 stages) and invasive (T3 and T4 stages) HNC. Therefore, while our findings do not exclude a role of MIN-related events in the pathogenesis of certain cases of leukemia and lymphoma as well as of carcinoma, they do not support the concept that such events participate in tumor progression.

Contributions and Acknowledgments

ALI, JC and LP participated in the design of the study and carried out the experimental work on CD5 analysis of DNA samples from normal donors, cell lines and patients suffering from B-CLL, MCL, HNC malignancies. ALI and JC analyzed the data and wrote the first draft of the manuscript. LLSV, DC and EC provided DNA samples and analyzed RER/LOH genotype of B-CLL and MCL, and revised the paper. JGF provided DNA samples and analyzed RER/LOH genotype of HNC, and revised the paper. FL was the principal author responsible for interpretation of the experimental work, and wrote the final version of the manuscript. JV contributed to design the study and gave final approval to the manuscript. The order of the authors reflects their contribution to this study in their own center, except EC, JV and FL who are the senior authors and/or heads of the departments in which the major part of study was performed.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Peer Review Outcomes

Manuscript processing

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What is already known on this topic

Microsatellite instability has been found in certain solid tumors and appears unfrequently associated with leukemia and lymphoma.

What this study adds

By analysing the CD5 gene promoter, the authors establish microsatellite instability at this region as the most frequent one presently known in B-BLL and in mantle cell lymphoma

Potential implications for clinical practice

There are no clinical implications in the short term. Further studies might better verify whether this instability has something to do with clinical progression.

Francesco Lo Coco, Deputy Editor

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