ure 1A). FICH-free survival was not different between pre- and post-ATRA era patients with APL ($p = 0.1363$, Figure 1B). Considering all patients with acute leukemia, there was significantly longer overall survival in patients diagnosed after January 1998 ($p < 0.0001$, Figure 1C) but no difference was found in FICH-free survival despite a plateau in patients diagnosed after January 1998 ($p = 0.2679$, Figure 1D).

Graus *et al.*[9] reported on intracranial hemorrhage in 425 leukemia patients after hematopoietic stem cell transplantation (11 subdural and 5 brain hematomas): these patients differed from our series in terms of common location of hemorrhage, population and procedure-related events. In spite of the improved survival in acute leukemia, achieved through advances in understanding leukemia pathophysiology and the introduction of ATRA treatment for APL,[10] because most FICH (65.9%) occur within seven days of diagnosis and there have been few improvements in treating FICH, a special effort is required to decrease the frequency and mortality of FICH in this early period.

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Malignant Lymphomas

BCL-6 gene mutations in primary cutaneous B-cell lymphomas

We analyzed mutations in the 5' non-coding region of the *BCL-6* gene in 46 cases of primary cutaneous B-cell lymphomas (PCBCL), using a polymerase chain reaction single strand conformation polymorphism (PCR-SSCP) method. The results indicate that PCBCL display a low frequency of mutations and support a marginal zone B-cell origin for most of these neoplasms.

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Primary cutaneous B-cell lymphomas (PCBCL) comprise entities morphologically similar to their nodal counterparts, but with a more favorable clinical behavior and prognosis. According to the EORTC classification, PCBCL generally do not express immunohistochemical markers such as CD5 and CD10 and only rarely show rearrangement of the *C-MYC*, *BCL-1*, *BCL-2* and *BCL-6* genes.[1]

In contrast, more recent studies have reported CD10 expression in the great majority of primary cutaneous follicular lymphomas and the presence of the t(14;18) translocation in about 30% of these cases.[2] The *BCL-6* gene is a proto-oncogene functioning as a transcriptional repressor and mutations of the 5' non-coding region are reported to be a potential mechanism for deregulating *BCL-6* expression, thus playing a role in the pathogenesis of non-Hodgkin's lymphomas (NHL).[3]

We extensively analyzed these molecular alterations in PCBCL. Forty-six cases of PCBCL (32 males and 14 females; age range: 31-86 yrs; mean age: 66 yrs) were examined. According to the EORTC classification there were 11 cases of primary cutaneous immunocytoma/marginal zone B-cell lymphoma (Ic/MZBCL), 31 cases of primary cutaneous follicle center cell lymphoma (FCCL) and 4 cases of large B-cell lymphoma of the leg (LBCL).

BCL-6 gene mutations were detected with non-radioiso-

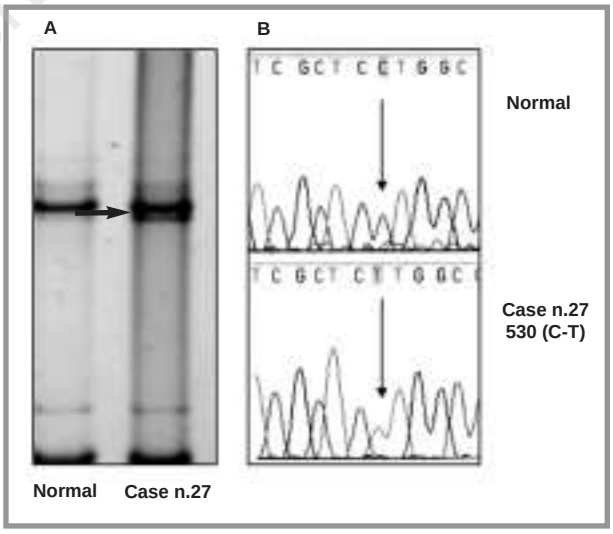

Figure 1. Case #27: A) SSCP analysis showed an anomalous migration pattern in fragment E1.12 of case #27, in comparison to the normal specimen. The arrow indicates the band used for sequencing analysis. B) Sequencing analysis identified a point mutation (530 C-T).

Table 1. Nucleotide substitutions and polymorphisms of the 5' non-coding region of the BCL-6 gene found in 46 primary cutaneous B-cell lymphomas.

Case n.	*Gender/Age*	*Site of presentation*	*EORTC*	*Nucleotide substitutions and Polymorphisms*	*Follow-up*	*Status*
1	M/66	Gluteal	FCCL		47	ned
2	F/64	Back	FCCL	397(G-C)	58	ned
3	M/72	Back	FCCL		10	ned
4	M/54	Back	FCCL		64	awd
5	M/59	Trunk	FCCL	397(G-C); 423(C-T); 426(T-G); ΔT 520	144	awd
6	F/64	Back	FCCL	ΔT 520	168	ned
7	M/58	Leg	FCCL	ΔT 520	95	ned
8	F/64	Back	FCCL	ΔT 520	14	ned
9	M/52	Back	FCCL	ΔT 520	10	ned
10	F/80	Leg	LBCL	ΔT 520	25	dod
11	M/60	Back	FCCL	ΔT 520	14	ned
12	F/77	Back	FCCL		13	ned
13	M/35	Trunk	FCCL		16	ned
14	M/37	Back	FCCL	397(G-C)	34	ned
15	M/60	Back	FCCL		46	ned
16	M/32	Head	FCCL	396(C-T); ΔT 520	52	ned
17	F/80	Head	FCCL		94	ned
18	F/63	Arm	IC/MZBCL	397(G-C)	68	ned
19	F/80	Leg	LBCL		21	awd
20	F/50	Trunk	IC/MZBCL	692(C-G)	57	awd
21	M/35	Arms	FCCL	ΔT 520	44	awd
22	M/31	Back	IC/MZBCL		83	awd
23	M/46	Back	FCCL		74	awd
24	F/60	Abdomen	FCCL	397(G-C)	48	awd
25	F/50	Back	IC/MZBCL	ΔT 520	26	awd
26	M/52	Back	FCCL	88(C-G); 101(T-G); 369(G-C)	74	awd
27	M/58	Back	FCCL	397(G-C); 530(C-T)	118	ned
28	M/58	Back	FCCL	397(G-C); ΔT 520	33	ned
29	F/62	Back	FCCL		57	awd
30	M/54	Neck	FCCL	397(G-C)	20	ned
31	M/64	Arm	IC/MZBCL		58	awd
32	M/68	Back	FCCL	ΔT 520	70	ned
33	F/44	Leg	IC/MZBCL	397(G-C)	87	ned
34	M/60	Back	FCCL		21	ned
35	M/33	Back	FCCL	ΔT 520	37	awd
36	M/36	Back	FCCL	556(T-A); 595(C-G)	34	ned
37	M/39	Neck	FCCL	416(G-T)	35	awd
38	M/61	Back	IC/MZBCL	ΔT 520	28	ned
39	F/59	Leg	LBCL	ΔT 520	30	ned
40	M/33	Arm	FCCL	397(G-C)	13	ned
41	M/86	Leg	LBCL	ΔT 520; 615(A-G); 710(G-A); 743(T-A)	16	awd
42	M/53	Scalp	IC/MZBCL		18	ned
43	M/49	Leg	IC/MZBCL	ΔT 520	10	awd
44	M/54	Back	FCCL		14	ned
45	M/64	Neck	IC/MZBCL	ΔT 520	13	ned
46	M/66	Neck	IC/MZBCL	397(G-C)	32	ned

FCCL: follicle center cell lymphoma; LBCL: large B-cell lymphoma of the leg; IC/MZBCL: primary cutaneous immunocytoma/marginal zone B-cell lymphoma; ned: no evidence of disease; awd: alive with disease; dod: dead of disease.

topic polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), as previously described[4] and confirmed with fluorescent cycle sequencing. Mutations were confirmed twice. The primers used for the mutational analysis of *BCL-6* exon1-intron1 boundary region (fragments E1.10, E1.11, and E1.12) have been previously reported.[3] In addition, we investigated two single nucleotide polymorphisms (SNP), 397 (G or C) and ΔT520, previously identified in the 5' non-coding regulatory region of the *BCL-6* gene.[5] As negative controls for each fragment analyzed we examined thirty-five blood samples from healthy donors.

Eight of the 46 cases (17%) of PCBCL had mutations. We observed 1 mutated case among the Ic/MZBCL (1/11=9%), 6 mutated cases in the FCCL category (6/31=19%) and 1 mutated case among the LBCL (1/4=25%). The percentage of mutations was lower in the different subtypes of PCBCL than in their nodal or extranodal counterpart. In fact, these entities are reported in the literature to carry mutations of the *BCL-6* gene in 13-33% of the marginal zone, in 37-60% of follicular and in 48-73% of diffuse large B-cell lymphomas. *BCL-6* mutations have been considered as a genetic marker of B-cell transit through the GC and NHL with mutations have been postulated to originate from GC derived B-cells. Therefore the low frequency of *BCL-6* mutations found in our series of PCBCL (most of them classified as FCCL) could support the opinion, expressed by some authors, that not only Ic/MZBCL, but most FCCL, as defined by the EORTC, should be considered as derived from marginal zone B cells.[6-8] Furthermore, two recently reported studies identified *BCL-6* gene mutations in 2/4 cases (50%) of FCCL and in 4/5 cases (80%) of LBCL. Both studies were conducted with a direct sequencing method and therefore the results are only partially comparable to those of our study; however, their findings of a high frequency of mutations in cases showing a well documented GC origin do support our results. In fact all cases reported by Franco *et al.*[9] expressed the GC markers bcl-6 and CD10, whereas in the study by Paulli *et al.*[10] all the cases of LBCL expressed the GC marker bcl-6 and carried hypermutation of the Ig genes, as reported to occur in GC-derived lymphomas.The distribution

and nature of *BCL-6* mutations found in PBL did not show striking differences to those of other extranodal lymphomas. In our series, 14 mutational events were identifiable in the 8 mutated cases, with 50% of the mutated cases harboring more than one mutation. All the mutations were single base-pair substitutions: 5 transitions (36%) and 9 transversions (64%). The most frequent substitutions corresponded to C-T transition (3/14=21%) and to C-G transversion (3/14=21%). The *BCL-6* mutations found in PCBCL are detailed in Table 1.

We also investigated the genotypic prevalence and allele frequency of two previously described polymorphisms 397 (G or C) and ΔT520. In accordance with literature data, we found the 397C and G polymorphic alleles in, respectively, 11/46 (24%) and 35/46 (76%) cases and the 520DT polymorphic allele in 18/46 (39%) cases.

In conclusion, the low frequency of mutations of the 5' non-coding region of the *BCL-6* gene found in PCBCL reinforces the observation of heterogeneity in the FCCL category as defined by the EORTC, and supports a marginal zone B-cell origin for most of these cases.

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Key words: cutaneous lymphoma, extranodal non-Hodgkin's lymphoma, primary cutaneous B-cell lymphomas, BCL-6 gene, BCL-6 mutations.

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Monoclonal Gammopathies

Effect of statins, smoking and obesity on progression of monoclonal gammopathy of undetermined significance: a case-control study

Interleukin-6 (IL-6) and C-reactive protein (CRP, a surrogate marker for IL-6) are important in monoclonal gammopathy of undetermined significance (MGUS) and myeloma. Smoking and obesity may elevate CRP levels, while statins decrease CRP levels. A case-control study in 200 MGUS patients found that statin use, smoking history and obesity do not affect MGUS progression.

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Monoclonal gammopathy of undetermined significance (MGUS) occurs in approximately 3% of persons older than 70 years.[1] The risk of MGUS progressing to multiple myeloma (MM) or a related disorder is approximately 1% per year.[2] C-reactive protein (CRP) is a surrogate indicator of interleukin-6 (IL-6) level.[3] Elevated CRP and IL-6 levels are adverse prognostic factors in MM. Smoking and obesity are positively associated with elevated CRP levels.[4,5] Conversely, hydroxymethylglutaryl-CoA reductase inhibitors (statins) are known to decrease CRP. Simvastatin induced death in MM cell lines,[6] and recent data suggest that statin use is associated with a reduced risk of some forms of cancer.[7] Studies using the Radl myeloma model also support the potential benefit from statins.[8] Durie and Mundy studied 409 myeloma patients, 34 of whom took a statin prior to developing myeloma.[8] There was a trend toward less severe bone disease (lytic lesions and/or osteoporosis) in patients receiving a statin, with only a 14% incidence of severe bone disease in patients on statins for more than five years.

So far, no study has evaluated the effect of statins, smoking, or obesity on progression of MGUS to MM or related disorders. We hypothesized that statin use may have protective effects on MGUS progression and that smoking and obesity might result in increased MGUS progression.

Following approval by our Institution Review Board, we conducted a case-control study in the 100 most recent patients with MGUS seen at Mayo Clinic Rochester who had progression to MM (or related malignancy) at least one year after the diagnosis of MGUS (Table 1). Progression was defined as the diagnosis of any of the following: MM, plasmacytoma, macroglobulinemia, osteosclerotic myeloma, primary amyloidosis (AL), IgM heavy chain with lymphoma, lymphoproliferative disorder with IgM-type protein, or chronic lymphocytic leukemia (CLL) with IgM-type protein. Additionally, 100 controls with MGUS who did not progress were matched by age, gender, and year of MGUS diagnosis. Statin use prior to the date of diagnosis of MGUS progression for each case and the