Table 1. Incidence of *PTPN11* mutations (mutated cases/studied cases).

Children						
JMML	*NS-JMML*	*RAEB-T*	*Other MDS*	*AML*	*ALL*	*Ref.*
1/4	–	–	–	–	–	Johan *et al.*[5]
21/62	5/5	5/27	0/23	1/24	–	Tartaglia *et al.*[3]
–	–	–	–	4/69	23/362	Tartaglia *et al.*.[4]
16/49	2/2	–	–	2/95	–	Loh *et al.*[6]
–	–	–	–	11/278	–	Loh *et al.*[7]
3/8	–	–	–	–	–	this study
33% (41/123)	100% (7/7)	18,5% (5/27)	0% (0/23)	4% (18/466)	6,5% (23/362)	Total

Adults					
RAEB-T	*Other MDS*	*AML*	*CMML*	*CML*	*Ref.*
0/2	0/70	1/64	0/35	–	Johan *et al.*[5]
2/7	–	4/38	1/4	0/11	Loh *et al.*[6]
0/15	0/26	0/49	0/1	–	Watkins *et al.*[8]
0/4	0/18	1/16	0/38	–	this study
7% (2/28)	0% (0/114)	3,5% (6/167)	1% (1/87)	0% (0/11)	Total

JMML: juvenile myelomonocytic leukemia; NS-JMML: Noonan's syndrome - JMML; RAEB-T: refractory anemia with excess of blasts in transformation; MDS: myelodysplasia; AML: acute myeloid leukemia; ALL: acute lymphoid leukemia; CMML: chronic myelomonocytic leukemia; CML: chronic myeloid leukemia; –: no case.

7. As previously described by Loh *et al*. in pediatric AML7, monosomy 7 is frequently associated with *PTPN11* mutations. Our results confirm that alteration of *PTPN11* is a rare event in the leukemogenesis of adult myeloid malignancies. Indeed, in published studies[5,6,8] (Table 1), only 1% of CMML, 3.5% of AML and 7% of RAEB-T had somatic mutations of *PTPN11*. Interestingly, *PTPN11* mutations occurred preferentially in leukemia with a monocytic component and/or in the presence of monosomy 7.[4,6,7] Moreover, as in Noonan's syndrome, all somatic mutations described were missense mutations and they were almost exclusively localized in exon 3, rarely in exon 13. As demonstrated by Tartaglia *et al*.,[2] such mutations modify the zone of interaction between N-SH2 and PTPase domains and release enzymatic activity of SHP-2.

Contrary to pediatric myeloid malignancies in which different mechanisms can induce activation of the RAS-MEK-ERK pathway (*RAS* mutations, *NF1* deletions or *PTPN11* mutations), in adult myeloid malignancies this activation only exceptionally involves *PTPN11* mutations and therefore seems related mostly to *RAS* mutations.

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Key words: PTPN11, SHP2, somatic mutation, adult, myeloid malignancies.

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Malignant Lymphomas

The relative levels of cyclin D1a and D1b alternative transcripts in mantle cell lymphoma may depend more on sample origin than on *CCND1* polymorphism

The relative levels of cyclin D1 (*CCND1*) (a) and (b) transcripts were determined by real-time reverse transcription polymerase chain reaction (RT-PCR) and found to vary according to the tissue origin in both control and tumor samples. A five-fold overexpression of both isoforms was observed in 28/38 cases of mantle cell lymphoma (MCL) and of only one isoform in 10/38 MCL. No correlation was observed between expression of cyclin D1 isoforms and *CCND1* genotype at position 870.

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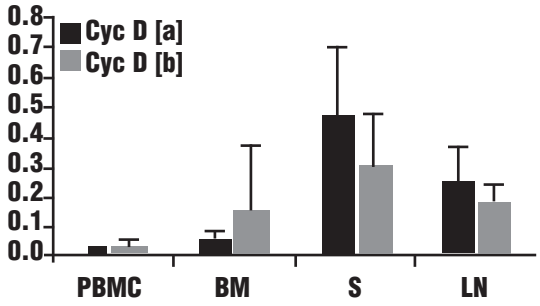

Figure 1. Basal expression of cyclin D1 [a] and [b] transcripts in reactive tissues after normalization with GAPDH. Real-time RT-PCR was performed on peripheral blood lymphocytes (PBMC) (n=6), bone marrow (BM) (n=4), spleen (S) (n=3) and lymph nodes (LN) (n=3). Each sample was tested in duplicate.

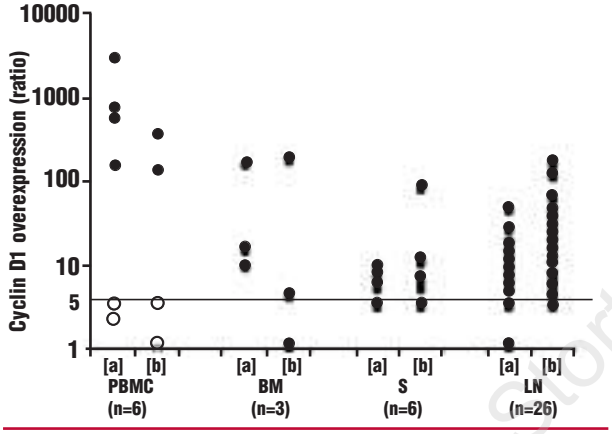

Figure 2. Overexpression rates of cyclin D1 [a] and [b] transcripts as determined by real-time RT-PCR in 39 samples of mantle cell lymphoma (MCL) (●) and 2 of chronic lymphocytic lymphoma (○). A 5-fold threshold was specific for MCL samples. Each sample was tested in duplicate.

Mantle cell lymphoma (MCL), an aggressive form of B-cell non-Hodgkin's lymphoma, is associated with t(11;14)(q13;q32) leading to cyclin D1 overexpression. The original cDNA called (a) (or D1a) includes the 5 exons of the cyclin D1 gene (*CCND1*).[1] Two major transcripts have been shown to differ by the length of their 3' untranslated region (UTR). Overexpression of shorter cyclin D1 transcripts without 3'UTR was shown to be associated with both a proliferation signature and shorter survival of patients with MCL.[2] An alternative isoform called (b) cDNA (or D1b) results from an absence of splicing at the exon 4/intron4 boundary, lacks sequences encoded by exon 5 and contains a short specific sequence of intron 4.[3] The alternative splicing is at least modulated by an A/G polymorphism within the final codon of exon 4 at position 870 corresponding to the splice donor site. However, the relative amounts of both isoforms may also depend on the tumor cell type.[3-5] The relationship between *CCND1* genotype, expression of cyclin D1a or D1b isoforms and aggressiveness of various cancers is still under debate.[6,7]

Using a classical RT-PCR, Howe *et al*. showed that both transcripts were detected in normal peripheral blood and in B-cell chronic lymphocytic leukemia (B-CLL).[8] We have developed an original RQ-PCR assay to quantify both cyclin D1a and D1b transcripts (sequences of primers and probes available on request). The relative amount of each isoform was normalized and expressed as a ratio to GAPDH value. Among several housekeeping genes, GAPDH expression was initially found constant irrespective of the tissue source and the proportion of malignant cells allowing the comparative study of heterogeneous tissues (*data not shown*). Normalization to a B-cell specific transcript such as CD19 mRNA has been used for cyclin D1 mRNA quantification.[9] However, the expression of CD19 varies between normal and lymphoma cells and has been found to be low in 31% of MCL cases.[10] Indeed, different cyclin D1a and D1b transcripts levels were observed in control tissues such as peripheral blood mononuclear cells (PBMC), lymph nodes, bone marrow and spleen (Figure 1). Moreover, cyclin D1 a+b levels were significantly lower in control PBMC than in other tissues ($p<0.01$) with a predominant expression of cyclin D1b isoform ($p<0.05$). Thereafter, a mean basal expression of each transcript was established for each type of tissue. In tumor samples, overexpression of cyclin D1 was determined as a ratio calculated over the basal expression rate in the same tissue type. Two CLL blood samples used as negative controls showed an overexpression rate below a 5-fold, which was chosen as a cut-off value (Figure 2). Among the 38 MCL samples, at least 5-fold overexpression of both (a) and (b) transcripts was observed in 28 samples (72%), of only (a) transcripts in 7 samples (18%) and of only (b) transcripts in 3 samples (7.5%). The highest overexpression rates in patients with MCL were detected in peripheral blood as compared with other tissue types ($p<1.10^{-5}$).

We further investigated the role of the A/G single nucleotide polymorphism (A870G) of the *CCND1* gene on the transcript level of cyclin D1 isoforms in MCL samples. A low percentage of cyclin D1a transcripts was observed in patients with a G/G genotype but there was no statistical correlation between genotype and expression of either cyclin D1 isoform. Moreover, the relative levels of cyclin D1a and D1b overexpression were different between paired tumor samples of different tissue origin in the same patients (n=3). Our real-time RQ-PCR assay and the competitive D1/D2+D3 reverse transcription polymerase chain reaction described by Uchimaru *et al*.[11] provided identical results, showing cyclin D1 overexpression in all MCL cases whatever their tissue origin.

Our results suggest that the expression of cyclin D1a and D1b isoforms is strongly repressed in normal PBMC whereas the basal levels of these isoforms vary in different cell or tissue types. Blood PBMC are likely to represent the best target for meaningful cyclin D1 quantification at diagnosis and during follow-up because the basal expression of both isoforms is very low in healthy subjects.

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Malignant Lymphomas

Rituximab monotherapy for splenic marginal zone lymphoma

In this retrospective study, rituximab was found to be effective therapy in 10 of 11 patients with splenic marginal zone lymphoma, inducing prompt reduction in splenomegaly, improvement in blood counts in 9 patients and clearance of a pleural effusion in 1 patient. Median response duration was 21 months (range 4 to 37 months). Two patients who relapsed at 21 and 23 months responded to retreatment. Rituximab should be considered in patients who are poor candidates for splenectomy.

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Splenic marginal zone lymphoma (SMZL) is accepted within the WHO classification as an indolent disease with distinct clinical, morphological and immunophenotypic features.[1,2] Patients typically have splenomegaly, inconspicuous lymphadenopathy, and circulating neoplastic cells characterized by unipolar cytoplasmic projections, round or oval nuclei and clumped chromatin. The lymphoma cells express CD19, CD20, and CD22 but not usually CD5, CD10, CD23, CD25, CD43, CD103 or cyclin D1.[3] No treatment may be necessary at the outset. In initially untreated patients the 5-year survival rate has been reported to be 88%.[4] Although no standard therapy has yet been established, splenectomy is considered the treatment of choice giving a significant survival advantage over chemotherapy.[5]

We treated 11 patients with the anti-CD20 chimeric monoclonal antibody rituximab at a dose of 375 mg/m^2 once a week for 4 consecutive weeks and retrospectively reviewed these patients' records.

The diagnosis of SMZL was based on clinical features, cell morphology and an immunofluorescent phenotype which excluded other types of lymphoid malignancy (Table 1). Ten patients had enlarged spleens palpable between 6 and 21 cm below the costal margin and were treated because of cytopenias and symptomatic splenomegaly. One patient (#9) had had a splenectomy three months previously and was treated because of a persistent symptomatic exudative pleural effusion. Nine patients had had previous chemotherapy with prednisone, chlorambucil, fludarabine, or cyclophosphamide. Rituximab was chosen because the patients were considered poor candidates for surgery or had refused surgery. A complete hematologic response was defined as the absence of a palpable spleen, disappearance of villous lymphocytes from the peripheral blood and normalization of the complete blood count. A partial response was defined as at least a 50% decrease in spleen size and improvement in blood counts. Bone marrow was not re-examined. All patients tolerated rituximab infusions without serious side effects such as tumor lysis syndrome. Ten of the 11 responded. Nine of the 10 patients with splenomegaly had an initial rapid reduction in spleen size followed by a slower decrease, resulting in an impalpable or barely palpable spleen in 2 to 34 weeks (median 16 weeks) accompanied by improvement in blood counts and disappearance of villous lymphocytes. The pleural effusion in patient #9 cleared completely leaving minimal blunting of the left costal margin within 3 months. Eight of the 10 responding patients had complete resolution of their cytopenias (Table 2). Two patients had persistent mild thrombocytopenia, although their spleens were barely palpable. Patient #5 did not respond and later underwent splenectomy. She developed a post-operative pancreatic fistula which took some months to heal, but now remains well with improved blood counts.

In 8 of the 10 responders there has been no evidence of disease progression after a median follow-up of 21 months (range 4-37). Patient #1 was successfully retreated with a second course of rituximab at 21 months because of recurrent progressive splenomegaly and anemia. She was then aged 93 and tolerated the therapy very well. Patient #7 was also successfully retreated at 23 months because of anemia, splenomegaly and night sweats.

As the neoplastic cells in SMZL express CD20 strongly, response to rituximab seemed likely. Indeed, a gratifying response was obtained to rituximab given as a single agent in this series, even in a nonagenarian who could tolerate few other treatment modalities. A previous abstract reported a comparable experience with rituximab in 14 patients described as having splenic lymphoma with villous lymphocytes or marginal zone lym-