

**Table 3. Haplotype analysis**

IL-1 $\beta$ -31	IL-1RN 86VNTR	Low grade, %e	High grade, %	All, %	Controls, %	E I, %	E II-IV, %
C	1	20.4	15.7	17.5	17.6	19.5	17.0
C	2	18.1	15.8	16.5	16.5	20.2	15.9
C	3	0.0	1.6	1.3	0.0	0.0	1.7
T	1	48.9	58.4	55.2	55.0	48.2	57.3
T	2	10.8	7.1	8.0	8.8	9.2	7.0
T	3	1.9	1.4	1.5	1.9	2.9	1.1

Haplotype analysis of the proinflammatory haplotype IL-1 $\beta$  -31/IL-1RN 86 VNTR. Analysis was conducted as described in the notes for Table 2.

disease stages E II to E IV, 20.6% were homozygous for IL-1 $\beta$  -31 allele C, compared to only 10.2% of patients with disease stage E I (Pearson  $\chi^2 P = .112$ , OR 2.27, CI 95% 0.81-6.46). IL-1 $\beta$  +3954 CC was found in 9.4% of patients with disease stages E II to E IV, compared to 2.8% of patients with stage E I (Pearson  $\chi^2 P = .109$ , OR 3.57, CI 95% 0.69-18.72) (Table 2). Haplotype analysis of the IL-1 cluster and especially the proinflammatory haplotype IL-1 $\beta$  -31 C/IL-1RN 2 did not show any significant associations with histological grade or disease progression (Table 3).

In conclusion we could not confirm the results of Rollinson et al.<sup>3</sup> One reason may be that Rollinson et al extracted DNA to investigate germ-line mutations from biopsy specimen and surgical blocks of lymphoma tissue and not from peripheral blood. A contamination with tumor material cannot be excluded. The admixture of somatic DNA may have obscured their analysis. To our knowledge, there is no mechanistic evidence so far that the proinflammatory effect of IL-1 $\beta$  contributes to development of primary gastric B-cell lymphoma. In contrast, recombinant IL-1 $\beta$  exerted a marked antilymphoma activity, reflected by significantly improved survival of treated mice after inoculation of BCL-1

cells.<sup>5</sup> The genetic susceptibility of patients with chronic *H pylori* infection to develop primary gastric B-cell lymphoma, especially of the MALT-type, appears to remain unclear.

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## To the editor:

### The PRV-1 gene expression in essential thrombocythemia

Recently, Temerinac and colleagues<sup>1</sup> found that the polycythemia rubra vera receptor-1 (PRV-1) m-RNA is increased in granulocytes of patients with polycythemia vera (PV) and in some cases of essential thrombocythemia (ET). We have previously described a sensitive qualitative method for PRV-1 investigation, based on PRV-1 m-RNA amplification by reverse transcriptase-polymerase chain reaction (RT-PCR).<sup>2</sup> By using this approach, we found that PRV-1 m-RNA is increased in 95% of patients with PV and in all patients with ET, while it is undetectable in secondary erythrocythosis (SE) and thrombocythosis (ST).<sup>2</sup> In other published studies, the PRV-1 m-RNA has been quantitatively evaluated,<sup>3-8</sup> and the percentage of PRV-1-positive ET patients varies widely among the mentioned studies, ranging from 16%<sup>3</sup> to 100%.<sup>7</sup> Up to date we have investigated by RT-PCR 75 patients with ET, 27 patients with ST, and 20 healthy individuals. Clinical and laboratory data of patients are shown in Table 1. Our results show that 71 of 75 ET patients are PRV-1 positive (95%), while all ST and healthy subjects are PRV-1 negative. These findings are in contrast with other reports and may result from the use of different methodological procedures in both granulocyte purification and PRV-1 evaluation (RT-PCR instead of real-time PCR). In a recent technical

report, Palmqvist et al<sup>9</sup> compared the quantification of PRV-1 m-RNA in whole-blood leukocytes and in selected granulocytes. The authors clearly demonstrated that the detection of PRV-1-positive patients in ET increases by examining purified granulocytes (26% and 46% in unfractionated cells and granulocytes, respectively).<sup>9</sup> Thus, the low percentage of PRV-1-positive patients reported in some studies can be explained by the evaluation of RNA extracted by unfractionated nuclear cell population. Moreover, Jelinek et al<sup>10</sup> reported that PRV-1 m-RNA is more rapidly degraded than control m-RNAs when blood samples are stored for several hours before using. In our assay, granulocyte separation is performed within 2 hours of blood collection; the hypotonic lysis of red cells, reported in other studies,<sup>1,5</sup> is not required; and granulocyte pellets are stored in TRIZOL (Invitrogen, Paisley, Scotland) at -80°C until PRV-1 analysis. In this way, the PRV-1 m-RNA integrity is assured.

Finally, it has been demonstrated that treatment with interferon can induce a significant decrease of PRV-1 m-RNA at real-time PCR in PV patients.<sup>11</sup> In our series, only a small proportion (28%) of patients received cytoreductive therapy at the time of examination. Interestingly, a high percentage of patients evaluated by Liu et al<sup>3</sup>

**Table 1. Characteristics of patients and healthy individuals**

Characteristics	Patients with ET	Patients with ST	Healthy individuals
<b>No.</b>	75	27	20
<b>Age</b>			
Mean, y	50	48.8	40
Median, y	47	54	39
Range, y	24-83	2-80	22-51
Sex, no. male/no. female	24/51	17/10	13/7
<b>Hemoglobin level</b>			
Mean, g/dL	13.4	11	13.7
Median, g/dL	13.5	10.5	13.7
Range, g/dL	7.6-16.2	7.4-22	12-15.6
<b>Hematocrit level (%)</b>			
Mean	41.4	33.5	42
Median	41.6	31.6	42
Range	24.5-50.7	22-43	37-47
<b>No. leukocytes</b>			
Mean, $\times 10^9/L$	8.9	11.9	6.6
Median, $\times 10^9/L$	8.4	10.6	6.7
Range, $\times 10^9/L$	4.8-24.5	6-32.4	4-9.8
<b>No. platelets</b>			
Mean, $\times 10^9/L$	808	717	239
Median, $\times 10^9/L$	721	656	223
Range, $\times 10^9/L$	419-3500	605-1500	152-447
<b>No. subjects with splenomegaly</b>	27	0	0
<b>Treatment</b>			
None, no. subjects	54	27	0
HU, no. subjects	13	0	0
IFN, no. subjects	6	0	0
Anagrelide, no. subjects	2	0	0

ET denotes essential thrombocythemia; ST, secondary thrombocytosis; HU, hydroxyurea; and IFN, interferon.

received chemotherapy at the time of PRV-1 examination (58%), and the PRV-1 positivity reported is very low (16%). In contrast, in the study of Florensa et al<sup>6</sup> the percentage of treated patients is low (29%), and PRV-1 m-RNA is increased in 59% of cases. So, further studies are necessary to assess whether in ET patients the cytoreductive treatment could affect the PRV-1 expression level.

In conclusion, we retain that a qualitative evaluation of PRV-1 can be a feasible diagnostic tool for ET.

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