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To the editor:

Imatinib normalizes bone marrow vascularity in patients with chronic myeloid leukemia in first chronic phase

Increased angiogenesis is a feature of many solid tumors but also has been observed in hematological malignancies, including chronic myeloid leukemia (CML), where vessel density is increased approximately 2-fold over normal controls.[1] Elevated plasma concentrations of vascular endothelial growth factor (VEGF) also were demonstrated,[2] and high bone marrow VEGF levels may be associated with a poor prognosis.[3] A recent study showed that *Bcr-Abl* tyrosine kinase activity induces VEGF via a pathway that involves phosphatidyl inositol 3 kinase and mTOR.[4] These findings were corroborated by data that showed dose-dependent down-regulation of VEGF in *BCR-ABL*–positive cell lines upon treatment with imatinib.[5] These results suggest that imatinib treatment of CML patients may normalize bone marrow vascularity, but no data presently are available.

We studied blood vessel density in 18 CML patients in first chronic phase prior to imatinib therapy. All patients were treated within multicenter trials,[6,7] where bone marrow biopsies for follow-up were optional. Of the patients, 12 patients were newly

Table 1. Blood vessel density in CML patients treated with imatinib

Patient no.	Disease phase	Ph positive (%)	Vessels (mean)	Ph positive (%)	Vessels (mean)	Vessels (% control)	Ph positive (%)	Vessels (mean)	Vessels (% control)	Ph positive (%)	Vessels (mean)	Vessels (% control)
		Initial biopsy		3 months			6 months			12 months		
1	CP1, newly diagnosed	100	50	69	ND	ND	63	11	22	ND	ND	ND
2	CP1, newly diagnosed	100	74	16	ND	ND	0	9	12	0	ND	ND
3	CP1, newly diagnosed	100	2	28	ND	ND	4	5	250	0	0	0.00
4	CP1, newly diagnosed	100	9	0	ND	ND	0	5	56	0	ND	ND
5	CP1, newly diagnosed	100	5	ND	ND	ND	0	1	20	0	6	1.20
6	CP1, newly diagnosed	100	9	0	5	56	32	4	44	0	ND	ND
7	CP1, newly diagnosed	100	14	9	19	36	2	9	64	0	ND	ND
8	CP1, newly diagnosed	100	21	100	5	24	60	3	14	68	ND	ND
9	CP1, newly diagnosed	100	24	ND	8	33	16	2	8	0	11	0.46
10	CP1, newly diagnosed	100	40	4	ND	ND	0	ND	ND	0	ND	ND
11	CP1, newly diagnosed	100	13	52	10	77	84	5	38	28	ND	ND
12	CP1, newly diagnosed	100	11	25	11	100	4	ND	ND	0	ND	ND
13	CP1, IFN intolerance	100	41	100	7	17	98	4	10	100	8	0.20
14	CP1, IFN intolerance	88	1	6	2	200	0	0	0	0	1	1.00
15	CP1, IFN intolerance	50	18	0	14	78	3(IP-F)	13	72	ND	9	0.50
16	CP1, IFN intolerance	96	3	96	1	33	68	2	67	100	ND	ND
17	CP1, IFN cytogenetic resistance	100	18	60	7	39	8	1	6	ND	ND	ND
18	CP1, IFN cytogenetic resistance	100	2	96	2	100	100	3	150	68	1	0.50
	Median (range) of all	100 (50-100)	13 (1-74)	40 (0-100)	7 (1-19)	56 (17-200)	3 (0-100)	4 (0-13)	22 (0-250)	0 (0-100)	*	*

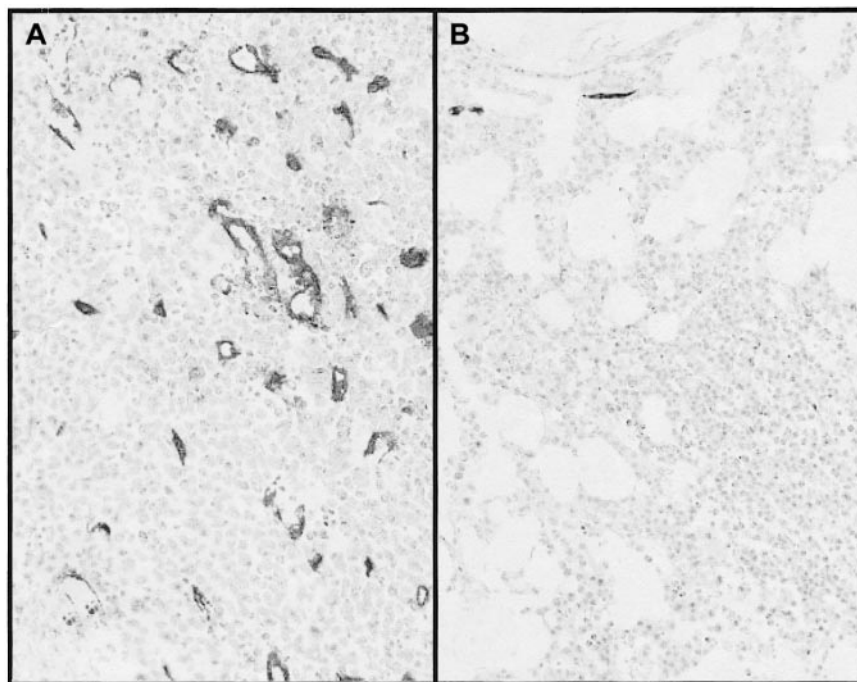ND indicates not determined; IP-F, *

Figure 1. Blood vessels before and after imatinib treatment. Blood vessels in patient no. 2 prior to (A) and after (B) 6 months on imatinib. After decalcification, paraffin-embedded sections were stained with anti-CD34 monoclonal antibody. Note also the marked reduction in cellularity. Magnification × 200.

diagnosed, while 2 were cytogenetically resistant to and 4 intolerant of interferon-alpha. All patients achieved or maintained complete hematological remission on imatinib. Follow-up biopsies after 3, 6, and 12 months of imatinib therapy are available for 12, 16, and 7 patients, respectively. Serving as controls were 19 biopsies without pathological findings. After decalcification, blood vessels in paraffin-embedded sections were stained with anti-CD34 monoclonal antibody (Immunotech, Marseille, France). Values represent the median number of blood vessels in 5 randomly chosen fields (magnification, 200-fold). The Mann-Whitney U test was used to compare median blood vessel numbers between groups of patients and the Wilcoxon test to compare relative changes over time (relative blood vessel density in percent of initial values).

Compared to normal controls (median, 4; range, 0-15), blood vessel density was significantly increased in the CML patients (median, 13; range, 1-74, $P = .001$), confirming earlier studies.[1,2] After 3 months on imatinib, the median blood vessel density had decreased to 7 (range, 1-19) ($P = .036$) vessels per field and the median relative density to 56% (range, 17-200) ($P = .002$) of the initial values (Table 1). After 6 months (Figure 1), there was a further reduction to a median of 4 (range, 0-13) vessels per field ($P = .001$ for comparison with initial values) or 22% (range, 0-250) of initial values ($P = .001$). If only patients with markedly increased median vessel density (> 8 per field) prior to imatinib (which eliminates potential mistakes as a result of small numbers) are considered, then 11 of 11 patients evaluable at 6 months showed a reduction to a median of 22% (range, 6-72) of initial values. Few patients had biopsies at 12 months. However, there was a significant increase in vessel density at 12 months in 2 patients (nos. 5, 9), without evidence for a cytogenetic or hematological relapse. Cytogenetic response and reduction of vascularity were generally not correlated. Although 2 patients (nos. 8, 13) were 100% Ph positive at 3 months, their vessel density was reduced to 24% and 17%, respectively. By contrast, in 2 other patients (nos. 7, 12), blood

vessel density at 3 months had not decreased (136% and 100% of initial values, respectively), but both had entered major cytogenetic response (9% and 25% Ph-positive metaphases, respectively). The finding of a vascular response in the absence of a cytogenetic response can be explained, since down-regulation of VEGF expression by imatinib may occur in the absence of cell death. In addition, vascular effects of imatinib that are mediated by inhibition of the platelet-derived growth factor receptor[8] may play a role. By contrast, the occurrence of a cytogenetic response without vascular response is puzzling. Both patients subsequently achieved complete cytogenetic response at 12 months, but no biopsies are available from this date.

In conclusion, imatinib normalizes bone marrow vascularity in most CML patients in chronic phase, without clear correlation to cytogenetic response. Studies in larger cohorts of patients are needed to clarify whether failure to normalize vascularity on imatinib has prognostic significance. Furthermore, there may be a therapeutic role for angiogenesis inhibitors in CML: a complete cytogenetic response was seen in a CML patient with myeloid blast crisis treated with a monoclonal antibody against VEGF (Bevacizumab) in combination with chemotherapy,[9] and disease stabilization was observed in several blast crisis patients treated with SU5416, a combined VEGF receptor and KIT inhibitor.[10] Given the notorious refractoriness of blast crisis, these results are encouraging enough to justify further studies of angiogenesis inhibitors in CML. Combining imatinib with such agents may hold particular promise for patients with advanced disease or for those who fail to normalize bone marrow vascularity with imatinib alone.

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To the editor:

A monoclonal melanoma-specific T-cell population phenotypically indistinguishable from CD3$^+$ LGL-leukemia

CD3$^+$ T-cell lymphogranular leukemia (TLGL) is classified as a lymphoid neoplasm where the malignant population consists of mature CD3$^+$ T cells that persist in the peripheral circulation over long periods of time.[1,2] TLGL mainly affects elderly individuals and presents generally with a slow progression. Individuals with circulating TLGL populations often have rather modest lymphocytosis and are not necessarily symptomatic.[3] TLGL populations are monoclonal or, less frequently, biclonal. Their common phenotype is CD3$^+$CD8$^+$CD45RA$^+$CD27$^-$(CD57$^{+/-}$), which is virtually indistinguishable from that of normal effector CD8$^+$ T cells.[2,4] Because of these observations, it has been argued whether TLGL clonopathy is truly neoplastic or represents instead the epiphenomenon of an immunoregulatory disorder. In the December 2002 issue of *Blood*, V. Bigouret and colleagues[5] propose that TLGL cells may correspond to CD8$^+$ effector T-cell populations arising as an extreme form of immune response to chronic pathologies. These pathologies, however, along with the antigen specificity of TLGL cells, remain thus far unknown. We have recently described, in melanoma patients, a CD3$^+$CD8$^+$CD45RA$^+$ subset of T cells exerting ex vivo tumor-specific effector functions, including interferon γ IFN-γ secretion and cytolytic activity.[6] In an HLA-A2–expressing patient, these cells accounted for about 5% of the circulating CD8$^+$ T lymphocytes and were composed of a monoclonal population specific for a single antigenic determinant from tyrosinase, a self-antigen expressed by malignant melanoma tumor cells and cells of the melanocytic lineage, but not by other normal cells. This cell population persisted at a stable level over 3 years, including more than one year after the resection of a single metastatic lesion that left the patient free of detectable disease to date. As assessed by using HLA-A2/peptide multimers incorporating peptide tyrosinase 368-376 in addition to CD3, CD8, and CD45RA, these cells expressed CD57, CD69, perforin, and granzyme B, but were CD45RO$^-$, CD27$^-$, CD28$^-$, CLA$^-$, and HLA-DR$^-$.[6] In addition, they expressed several natural killer (NK) receptors including CD94 (but not the inhibitory form associated with NKG2A), p58.2, and ILT2 (D.V., unpublished data, May 2002). Thus, both the prevalence and the phenotype of this population of cells found in a patient with melanoma were undistinguishable from those of TLGL cells. Our data provide strong support to the hypothesis of V. Bigouret and colleagues. Although the frequency of TLGL populations in cancer patients remains to be assessed, it is likely that these populations also include T cells arising as immune responses to chronic diseases, including those caused by pathogens. It has been suggested that in TLGL patients the appearance of clinical symptoms (neutropenia and/or autoimmune phenomena) may occur as a consequence of exposure to antigen.[5,7] This raises questions about the opportunity and the possible outcome of antigen-specific active immunotherapy in these cancer patients. The molecular mechanisms that control the expansion and persistence of single antigen-specific CD8$^+$ effector T-cell populations exhibiting characteristics at the edge between normality and pathology remain unknown. Their elucidation may provide information relevant to the development of novel strategies for both the controlled use of CD8$^+$ T cells in immunotherapy and for the treatment of TLGL-related pathologies.

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