To the editor:

Prenatal origin of childhood acute myeloid leukemias harboring chromosomal rearrangements t(15;17) and inv(16)

A prenatal origin of translocations associated with pediatric leukemia has been demonstrated for *MLL-AF4*[1] in infant leukemia, *TEL-AML1*[2,3] in common acute lymphocytic leukemia (cALL), and *AML1-ETO*[4] in acute myeloid leukemia (AML). We investigated whether AML-associated translocations *PML-RARA* and *CBFB-MYHII* could arise before birth. *PML-RARA* arises from the t(15;17) translocation,[5] characteristic of acute promyelocytic leukemia (APL)[6] (AML FAB subtype M3), while *CBFB-MYHII* arises from inv(16)(p13q22)[7] and t(16;16).[8]

Diagnostic samples from 2 t(15;17) and 2 inv(16) cases were obtained with informed consent and ethics committee approval from patients enrolled in the Northern California Childhood Leukemia Study (NCCLS) and from the Children's Oncology Group AML cell bank. Corresponding Guthrie cards (neonatal blood spots) for patients were obtained from a central repository maintained by the Genetic Diseases Branch of the California Department of Health Sciences. We obtained genomic breakpoints from patients by multiplex long-distance polymerase chain reaction (PCR) using eLONGase DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA), according to manufacturers' instructions, and sequenced the translocation junctions. For each *PML-RARA* case, 10 multiplex reactions were set up, containing 2 *PML* primers from bcr3 (intron 3; 1447 bp) or bcr1 (intron 6; 1056 bp), in combination with 1 of 5 *RARA* primers. For each *CBFB-MYHII* sample, 6 individual PCR reactions were set up using 1 of 6 *CBFB* primers (targeted to intron 5; 16 359 bp) in combination with the single *MYHII* primer (targeted to intron 11; 370 bp). Primer sequences are available on request.

We established nested or seminested PCR assays for each clonotypic sequence prior to analysis of the corresponding Guthrie cards for the presence of these sequences. Assay specificity was confirmed by testing patient-specific DNA as well as nonpatient DNA. Assay sensitivity was determined using serial dilutions of patient-specific DNA. Amplification was performed with Ampdirect buffers (Rockland Immunochemicals, Gilbertsville, PA) and eLONGase enzyme, according to the manufacturer's protocol. Prior to translocation-specific PCR, each Guthrie card was tested for capacity to support PCR amplification of a normal gene, NAD(P)H:quinone oxidoreductase (*NQO1*), as previously described.[2]

One of the t(15;17) cases (no. P1), aged 10.7 years, generated a positive PCR result in 2 separate assays (bands observed in 1 segment of 8 and 3 segments of 6 analyzed) (Figure 1). Of the 2 inv(16) cases examined, one (no. P5), aged 9.4 years, gave a positive result, in one segment of 14 analyzed in 2 separate assays (Figure 1). For both positive cases, PCR products were sequenced and confirmed as identical to the sequences obtained from the respective patient samples at diagnosis, verifying the in utero origination of the leukemic clonal translocations. The postnatal latencies observed in this study (10.7 and 9.4 years) are among the most protracted demonstrated by backtracking AML to birth, the longest such latency (12 years) having been reported for an AML case with t(8;21) and an *AML1-ETO* fusion gene.[4] The latency period may reflect postnatal persistence of translocation-positive, quiescent multipotential cells, which, upon later recruitment into the myeloid differentiation pathway, acquire additional secondary changes necessary for leukemia. Animal models of *PML-RARA* and *CBFB-MYHII* support a multistep progression from fusion gene acquisition to development of these leukemias.[9,10]

A prenatal origin has therefore been demonstrated for 2 more translocations, *PML-RARA* and *CBFB-MYHII*, associated with childhood AML, suggesting that a wide diversity of childhood leukemias originate prenatally.

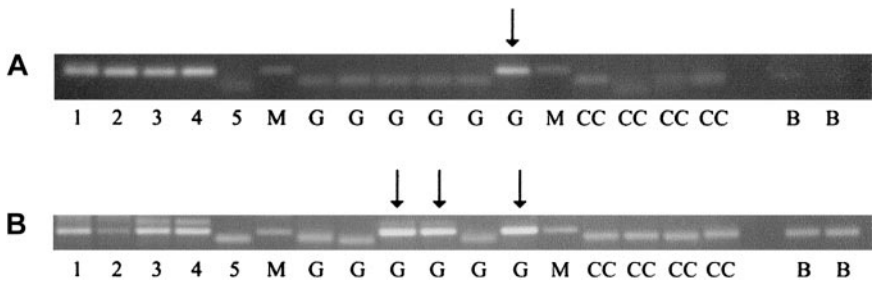

Figure 1. PCR analysis of clonotypic genomic inv(16) (*CBFB-MYHII*) and t(15;17) (*PML-RARA*) sequences in neonatal Guthrie cards of leukemic patients. For analysis of Guthrie cards, 1/16 pieces were placed directly in first-round PCR reactions (50 μL), and for each individual, we tested at least 8 such segments (1/2 of a 1.5-cm spot). Second-round amplification (25 μL) was performed with 1 μL from the first-round PCR reactions. PCR conditions included 2 preincubation steps of 80°C for 15 minutes and 94°C for 4 minutes, followed by 40 amplification cycles of 94°C for 30 seconds, 58°C for 1 minute, 72°C for 1 minute, and, finally, a 72°C extension step for 7 minutes. Results shown are from the second round of nested PCR analysis. Lane markers: M indicates molecular weight marker. Lanes 1-5 show 1:10 dilutions of patient diagnostic DNA with lane 1 being 10 ng/μL and lane 5, 1 pg/μL DNA. G indicates patient Guthrie card samples; CC, control Guthrie card samples (from a healthy nonaffected individual); B, blank (no DNA sample). Arrows indicate PCR products from patient Guthrie segments. (A) *CBFB-MYHII* case no. P5: a single patient Guthrie segment yielded a PCR product from 6 analyzed. (B) *PML-RARA* case no. P1: 3 patient Guthrie segments yielded positive PCR products from 6 analyzed.

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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

		Initial biopsy		3 months			6 months			12 months		
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)
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, *