TET1, a member of a novel protein family, is fused to MLL in acute myeloid leukemia containing the t(10;11)(q22;q23)

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TO THE EDITOR

Chromosomal abnormalities involving the *Mixed Lineage Leukemia* (*MLL*) gene at 11q23 are among the most frequent cytogenetic findings in acute myeloid leukemia (AML).[1] The t(10;11)(q22;q23) has been reported in several cases of AML;[2–5] however, the genes involved in this translocation have not been identified. Here, we have cloned the derivative chromosome 11 breakpoint in a case of AML containing this translocation using long-distance inverse-polymerase chain reaction (LDI-PCR) and

CD65, CD11b (dim), CD4 (dim), and cytoplasmic myeloperoxidase, consistent with acute myelomonocytic leukemia. Chemotherapy was initiated, and the patient has been in durable remission for 24 months. This study was approved by the St Jude Children's Research Hospital Institutional Review Board.

Cytogenetic analysis performed on the patient's leukemic blasts revealed a t(10;11)(q22;q23) as the sole chromosomal abnormality in all 20 metaphases analyzed (Figure 1a). Metaphase FISH analysis using a probe that spans the entire *MLL* locus[6] (Oncor, Gaithersburg, MD, USA) demonstrated three signals, one of which was on the derivative chromosome 10,

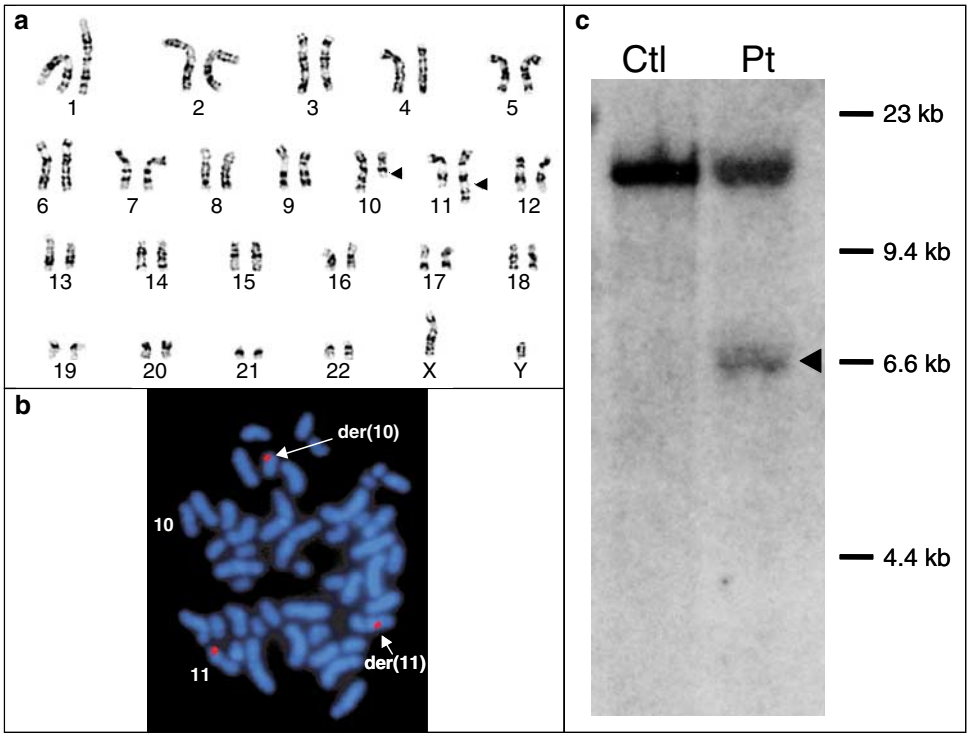

Figure 1 Rearrangement of *MLL* by the t(10;11)(q22;q23) translocation. (a) Cytogenetic analysis performed on bone marrow identified a t(10;11)(q22;q23) as the sole chromosomal abnormality in all metaphases examined. (b) Metaphase FISH performed on leukemic bone marrow cells using a probe that spans the *MLL* locus. (c) Southern blot analysis of HindIII-digested leukemic cell and normal PB leukocyte genomic DNA; the blot was hybridized with an *MLL* probe that spans the MBR. The germline and rearranged bands are 14.5 and 6.8 kb, respectively. The rearranged band in the patient sample is indicated by an arrowhead.

identified the partner gene as *TET1*, which is identical to the recently cloned gene, *LCX*.[5] Importantly, TET1 appears to be a member of a novel, well-conserved protein family of unknown biologic function.

An 8-year-old boy presented with a 2-week history of pallor and petechiae. The bone marrow was hypercellular, and leukemic blasts comprised 59% of nucleated cells. Immunophenotyping revealed that the blasts expressed CD33, CD13, CD15,

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Table 1 Genomic localization of *TET* gene family

Gene	*NCBI accession*	*Celera accession*	*Chromosome*	*Syntenic murine chromosomal region*
Human				
TET1	—	hCP45882	10q22	10
TET2	—	hCP38872	4q24	3
TET3	—	hCP50515	2p12	6
Murine				
TET1	—	mCP1483	10	—
TET2	XP143535	—	3	—
TET3	—	mCP1086	6	—

confirming that the observed t(10;11) involved the *MLL* gene (Figure 1b). Southern blots probed with an *MLL* cDNA probe that spans the major breakpoint region (MBR) revealed a 6.8 kb rearranged band in the leukemic DNA digested with HindIII (Figure 1c).

We utilized LDI-PCR to clone the translocation breakpoint. Genomic DNA from leukemic cells was digested with HindIII, ligated at low concentration, and subjected to two rounds of PCR using nested primers within exon 5 of *MLL* (first round: 5′-TCCAGGAAGTCAAGCAAGCAGGTC-3′, 5′-GGAGTGGTGGC CTGTTTGGATTCA-3′; second round: 5′-GCCTCAGCCACC-TACTACAGGAC-3′, 5′-CTTTCGTGGAGGAGGCTCACTAC-3′). LDI-PCR performed on the leukemic DNA sample yielded a 6.8 kb band, the same size as the rearranged band detected by Southern analysis. Partial sequencing and basic local alignment search tool (BLAST) analysis[7] of this band identified 122 bp of unknown sequence immediately downstream of the 5′ portion of *MLL* intron 6 (Figure 2a), which was 100% identical to intronic sequence within the *KIAA1676* gene on chromosome 10q22. Based on its involvement in a t(10;11)-associated leukemia, we have designated this gene *Ten-Eleven Translocation-1* (*TET1*). Just prior to submission of this manuscript, *TET1* was independently identified as the partner gene in another case of AML containing t(10;11)(q22;q23).[5] In our case, the breakpoint within *MLL* intron 6 was 20 bp upstream of that reported by Ono *et al*,[5] whereas the breakpoint within *TET1* intron 8 was 950 bp downstream of that reported by these investigators.

To independently confirm that *TET1* was the translocation partner, RT-PCR analysis was performed on total RNA extracted from either leukemic cells or control peripheral blood leukocytes (PBL) using 5′ and 3′ primers derived from *MLL* exon 5 (*MLL* 5′-GCCTCAGCCACCTACTACAGGAC-3′) and *TET1* exon 9 (*TET1* 5′-GGAGCTGCTCATC-TTGAGGAATAAC-3′), respectively (Figure 2b). RT-PCR performed on the leukemic sample, but not the control, generated a band of the predicted size. Expression of the reciprocal *TET1-MLL* transcript was not detected in RT-PCR analyses using two separate primer pairs (data not shown). Sequencing of the cloned RT-PCR product revealed, as predicted, a chimeric transcript consisting of *MLL* exon 6 fused in-frame to *TET1* exon 9 (Figure 2c). *TET1* is predicted to encode a protein of 2136 amino acids with a molecular mass of 235.3 kDa. TET1 contains a CXXC domain at position 583–624, a coiled-coil region near the C-terminus (position 2062–2091), and three candidate bipartite nuclear localization signals.[5] The MLL-TET1 fusion protein is predicted to have a molecular mass of 204.4 kDa and to retain the AT hooks, subnuclear localization domains, and the CXXC motif of MLL, as well as the coiled-coil region and

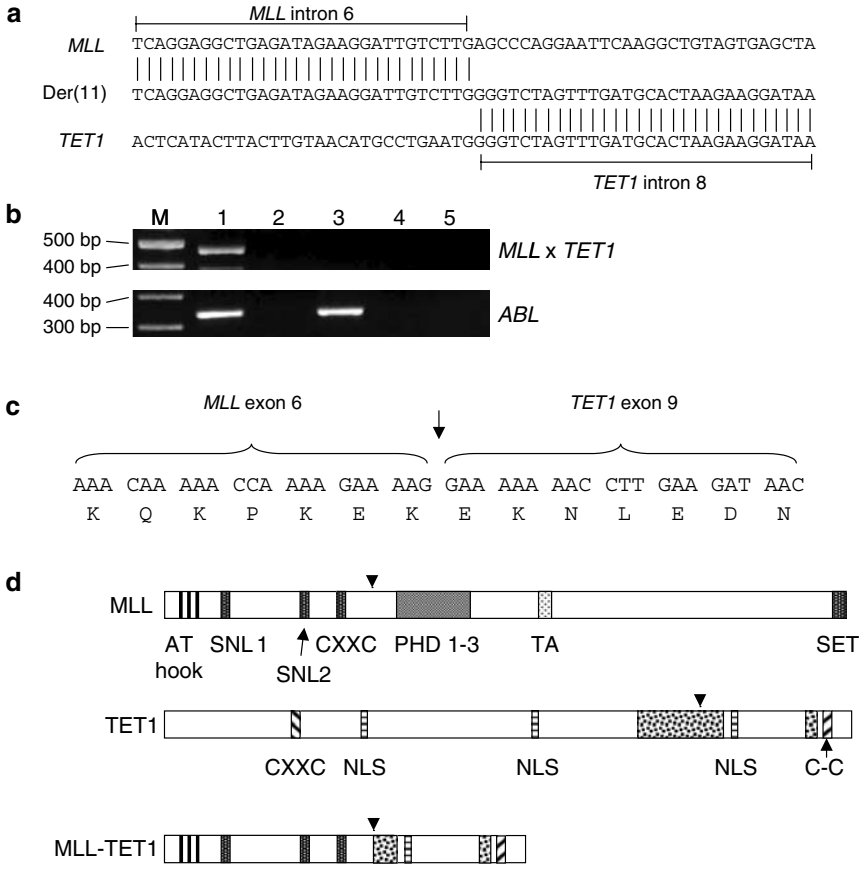

Figure 2 *TET1* is the partner gene of *MLL* in AML with the t(10;11)(q22;q23). (a) The 6.8 kb LDI-PCR product was cloned and partially sequenced, demonstrating fusion of intron 6 of *MLL* with intron 8 of the *TET1* gene. (b) RT-PCR analysis performed on cDNA prepared from leukemic cells (lanes 1 and 2), normal PBL (lanes 3 and 4), and water alone (lane 5) using *MLL* and *TET1*-specific 5′ and 3′ primers with (lanes 1 and 3) and without (lanes 2 and 4) reverse transcription. Control RT-PCR for *ABL* was performed to ensure that comparable amounts of RNA were analyzed. (c) Sequence analysis of the RT-PCR product revealed an in-frame fusion between exon 6 of *MLL* and exon 9 of *TET1*. (d) Schematic of MLL, TET1, and the MLL-TET1 fusion protein. The arrowhead indicates the translocation breakpoint. The stippled boxes in TET1 indicate regions conserved between all human TET proteins. NLS, nuclear localization signal; C-C, coiled-coil motif.

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TET2                                                    TPQKQIIEKDEGPFYTHLGAGPNVAAIREE   30
TET3   LESPLKYLDTPTKSLLDTPAKRAQAEFPTCDCVEQIVEKDEGPYYTHLGSGPTVASIREL   205
TET1   NDYAMNGGTNPTKNLVSITKD---SELPTCSCLDRVIQKDKGPYYTHLGAGPSVAAVREI   1452

TET2   MEERFGQKGKAIRIERVIYTGKEGKSSQGCPIAKWVVRRSSSEEKLLCLVRERAGHTCEA   90
TET3   MEERYGEKGKAIRIEKVIYTGKEGKSSRGCPIAKWVIRRHTLEEKLLCLVRHRAGHHCQN   266
TET1   MENRYGQKGNAIRIEIVVYTGKEGKSSHGCPIAKWVLRRSSDEEKVLCLVRQRTGHHCPT   1512

TET2   AVIVILILVWEGIPLSLADKLYSELTETLRKY-GTLTNRRCALNEERTCACQGLDPETCG   149
TET3   AVIVILILAWEGIPRSLGDTLYQELTDTLRKY-GNPTSRRCGLNDDRTCACQGKDPNTCG   325
TET1   AVMVVLIMVWDGIPLPMADRLYTELTENLKSYNGHPTDRRCTLNENRTCTCQGIDPETCG   1572

TET2   ASFSFGCSWSMYYNGCKFARSKIPRKFKLLGDDPKEEEKLESHLQNLSTLMAPTYKKLAP   209
TET3   ASFSFGCSWSMYFNGCKYARSKTPRKFRLAGDNPKEEEVLRKSFQDLATEVAPLYKRLAP   385
TET1   ASFSFGCSWSMYFNGCKFGRSPSPRRFRIDPSSPLHEKNLEDNLQSLATRLAPIYKQYAP   1632

TET2   DAYNNQIEYEHRAPECRLGLKEGRPFSGVTACLDFCAHAHRDLHNMQNGSTLVCTLTRED   269
TET3   QAYQNQVTNEEIAIDCRLGLKEGRPFAGVTACMDFCAHAHKDQHNLYNGCTVVCTLTKED   445
TET1   VAYQNQVEYENVARECRLGSKEGRPFSGVTACLDFCAHPHRDIHNMNNGSTVVCTLTRED   1692

TET2   NREFGGKPEDEQLHVLPLYKVSDVDEFGSVEAQEEKKRSGAIQVLSSFRRKVRMLAEPVK   329
TET3   NRCVGKIPEDEQLHVLPLYKMANTDEFGSEENQNAKVGSGAIQVLTAFPREVRRLPEPAK   505
TET1   NRSLGVIPQDEQLHVLPLYKLSDTDEFGSKEGMEAKIKSGAIEVLAPRRKKRTCFTQPVP   1752

TET2   TCRQRKLEAKKAAAEKLSSLENSSNKNEKEK----------SAP------SRTKQTENAS   373
TET3   SCRQRQLEARKAAAEKKKIQKEKLSTPEKIKQEALELAGITSDPGLSLKGGLSQQGLKPS   565
TET1   RS------GKKRAAMMTEVLAHKIRAVEKKP--------------------IPRIKRKNN   1786

TET2   QAKQLAESVNSYSASG----STNPYMRRPNPVSPYPN----SSHTSDIYGSTSPMN-FYS   424
TET3   LKVEPQNHFSSFKYSGNAVVESYSVLGNCRPSDPYSMNSVYSYHSYYAQPSLTSVNGFHS   625
TET1   STTTNNSKPSSLPTLG----SNTETVQPEVKSETEPHFILKSSDNTKTY-SLMPSAPHPV   1841

TET2   TSSQAAGSYLN--SSNPMNP----YPGLLNQNTQYPSYQCNGNLSVDN--CSPYLG----   472
TET3   KYALPSFSYYGFPSSNPVFPSQFLGPGAWGHSGSSGSFEKKPDLHALHNSLSPAYGGAEF   685
TET1   KEASPGFSWSPKTASATPAP-------LKNDATASCGFSER--------------------   1875

TET2   -SYSPQSQPMDLYR----------YP----------------SQDP---------------   491
TET3   AELPSQAVPTDAHHPTPHHQQPAYPGPKEYLLPKAPLLHSVSRDPSPFAQSSNCYNRSIK   745
TET1   ----------------------------------------SSTP---------------   1879

TET2   ----------------LSKLSLPPIHTLYQP-------RFGNSQSFTSKYLG---YGNQN   525
TET3   QEPVDPLTQAEPVPRDAGKMGKTPLSEVSQNGGPSHLWGQYSGGPSMSPKRTNGVGGSWG   805
TET1   ----------------------------------------HCTMPSGRLS---GANA   1893

TET2   MQGDGFSSCTIRPNVHHVGKLPPYPTHEMDGHF-------MGATSRLPPNLS-NPNMDYK   577
TET3   VFSSGESPAIVPDKLSSFGASCLAPSHFTDGQWGLFPGEGQQAASHSGGRLRGKPWSPCK   865
TET1   AAADG-------PGISQLGEVAPLPT---------------LSAPVMEPLINSEPSTGVT   1931

TET2   NGEHHS----PSHIIHNYSAAPGMFNSSLHALH---------LQNKENDMLSHTANGLS-   623
TET3   FGNSTSALAGPSLTEKPWALGAGDFNSALKGSPGFQDKLWNPMKGEEGRIPAAGASQLDR   925
TET1   ----------EPLTPHQPNHQPSFLTSPQ-------------------DLASS-------   1955

TET2   --------------KMLPALNHDRTACVQGGLHKLSDANGQEKQPLALVQGVASGAEDNDE   670
TET3   AWQSFGLPLGSSEKLFGALKSEEKLWDPFSLEEGPAEEPPSKGAVKEEKGGGGAEEEEEE   985
TET1   ----------------PMEEDE---QHSEADEPPSDEPLSDDPLSP---AEEKLPHIDE   1992

TET2   VWSDSEQSFLDPDIGGVAVAPTHGSILIECAKRELHATTPLKNPNRNHPTRISLVFYQHK   730
TET3   LWSDSEHNFLDENIGGVAVAPAHGSILIECARRELHATTPLKKPNRCHPTRISLVFYQHK   1045
TET1   YWSDSEHIFLDANIGGVAIAPAHGSVLIECARRELHATTPVEHPNRNHPTRLSLVFYQHK   2052

TET2   SMNEPKHGLALWEAKM---AEKAREKEEECEKYGPDYVPQKSHGKKVKREPAEPHETSEP   787
TET3   NLNQPNHGLALWEAKMKQLAERARARQEEAARLGLGQQEAKLYGKKRKWGGTVVAEPQQK   1105
TET1`  NLNKPQHGFELNKIKF--EAKEAKNKK-----------MKASEQKDQAANEGPEQSSEV   2098

TET2   TYLRFIKSLAERTMSVTTDSTVTTSPYAFTRVTGPYNRYI   827
TET3   EKKGVVP--TRQALAVPTDSAVTVSSYAYTKVTGPYSRWI   1143
TET1   NELNQIP--SHKALTLTHDNVVTVSPYALTHVAGPYNHWV   2136
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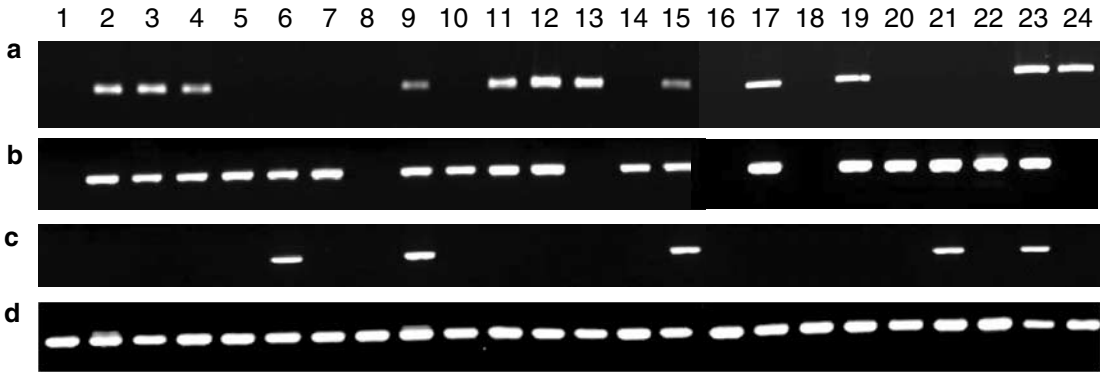
Figure 3 TET1 is the member of a novel family of proteins. Amino-acid sequences of the human TET family members were aligned using a ClustalW algorithm.[8] Residues identical in all TET proteins are underlined and bold. Two regions (corresponding to residues 1431–1737 and 1991–2061 of TET1) are highly conserved among all three proteins.



1. Brain
2. Heart
3. Kidney
4. Spleen
5. Liver
6. Colon
7. Lung
8. Small intestine
9. Muscle
10. Stomach
11. Testis
12. Placenta
13. Salivary gland
14. Thyroid
15. Adrenal
16. Pancreas
17. Ovary
18. Uterus
19. Prostate
20. Skin
21. PBL
22. Bone marrow
23. Fetal brain
24. Fetal liver

Figure 4 Differential expression of *TET* family members in adult and fetal tissues. PCR was performed on a human cDNA panel using gene-specific primers for *TET1* (a), *TET2* (b), or *TET3* (c). A control PCR reaction for *β-actin* was performed to confirm the presence of amplifiable cDNA (d). PCR products were analyzed by agarose gel electrophoresis. Lane designation is indicated in the figure.

the most C-terminal nuclear localization domain of TET1 (Figure 2d).

BLAST analysis of the NCBI and Celera databases identified two human proteins with significant homology to TET1, which we have designated as TET2 and TET3 (Celera accession numbers hCP38872 and hCP50515). They are 827 and 1143 amino-acid residues long, respectively, and may represent incomplete protein products. Multiple alignment analysis revealed two regions that were highly conserved among all human TET family members (Figure 3). The first consists of a 307 amino-acid residue segment that shows 63% identity and 80% similarity between all three proteins. The second region located near the carboxy terminus consists of a 70 amino-acid residue segment (corresponding to residues 1991–2061 of TET1) and is 74% identical between all TET family members. Analysis of mouse and fruitfly databases identified three murine proteins (Celera accession numbers mCP1086 and mCP1483) and the *Drosophila* CG2083 gene product (NCBI accession number AAF47691) as sharing significant sequence homology with TET1. Genomic information for the human and murine *TET* genes is summarized in Table 1.

To analyze the expression pattern of the *TET* genes, we performed PCR on a cDNA panel prepared from 24 adult and fetal human tissues (OriGene Technologies, Rockville, MD, USA) using primers specific for each of the TET genes (*TET1* 5′-CCTCCTTCCTCACCTCTCCTCAAGAC-3′, 5′-AGCCTTCAGAC CCAATGGTTATAG-3′; *TET2* 5′-GCACACGCTGGAGGAGAAG CTACT-3′, 5′-CGAGGTGTCTTGCTCCGAGCATAC-3′; or *TET3* 5′-GGAGCTTACCGAGACGCTGAGGAA-3′, 5′-AGACGGCAC TCTGGTGCTCTGTGT-3′). As shown in Figure 4, *TET2* was widely expressed, whereas the other *TET* genes had more limited expression. *TET2* was most frequently coexpressed with either *TET1* or *TET3*. Expression of all three *TET* genes was observed only in muscle, adrenal gland, and fetal brain. Several organs expressed only *TET2*; however, expression of *TET1* alone was seen only in salivary gland and fetal liver; expression of *TET3* alone was not detected in any of the 24 tissues surveyed. Expression of *TET1* within the hematolymphoid system was limited to spleen, with no detectable expression in bone marrow or PBL. *TET1* was expressed in fetal liver, but not in its adult counterpart. Within the hematolymphoid system, *TET3* expression was detected only in PBL. Taken together, these findings indicate that the expression of the *TET* family members is differentially regulated.

Six cases of AML have been described that harbor the t(10;11)(q22;q23), including the current case, and all, but one, have had a myelomonocytic or monoblastic immunophenotype.[2–5] Of the five patients with available clinical information, one patient was pediatric and the remainder were adults, with mean and median ages of 29.8 and 35 years, respectively. Four of these patients relapsed and subsequently died, two because of persistent leukemia and two because of infectious complications. Although definitive conclusions regarding prognosis cannot be made given the small number of patients, it appears that the presence of the t(10;11)(q22;q23), like several other *MLL* translocations, confers a poor prognosis.

In summary, we have demonstrated that *TET1* is fused to *MLL* in a case of pediatric AML containing the t(10;11)(q22;q23). TET1 is a member of a novel protein family, the biologic function of which is unknown. Given that TET1 was independently identified as an MLL fusion partner in another leukemia harboring the t(10;11)(q22;q23),[5] these data confirm that *TET1* likely represents the sole recurrent chromosomal target in AML containing this translocation.

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References

1 Ayton PM, Cleary ML. Molecular mechanisms of leukemogenesis mediated by MLL fusion proteins. *Oncogene* 2001; **20:** 5695–5707.
2 Aventin A, La Starza R, Martinez C, Wlodarska I, Boogaerts M, Van den Berghe H *et al*. Involvement of MLL gene in a t(10;11)(q22;q23) and a t(8;11)(q24;q23) identified by fluorescence *in situ* hybridization. *Cancer Genet Cytogenet* 1999; **108:** 48–52.
3 Harrison CJ, Cuneo A, Clark R, Johansson B, Lafage-Pochitaloff M, Mugneret F *et al*. Ten novel 11q23 chromosomal partner sites. European 11q23 Workshop participants. *Leukemia* 1998; **12:** 811–822.
4 Thirman MJ, Gill HJ, Burnett RC, Mbangkollo D, McCabe NR, Kobayashi H *et al*. Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. *N Engl J Med* 1993; **329:** 909–914.
5 Ono R, Taki T, Taketani T, Taniwaki M, Kobayashi H, Hayashi Y. LCX, leukemia-associated protein with a CXXC domain, is fused to MLL in acute myeloid leukemia with trilineage dysplasia having t(10;11)(q22;q23). *Cancer Res* 2002; **62:** 4075–4080.
6 Mathew S, Behm F, Dalton J, Raimondi S. Comparison of cytogenetics Southern blotting and fluorescence *in situ* hybridization as methods for detecting MLL gene rearrangements in children with acute leukemia and with 11q23 abnormalities. *Leukemia* 1999; **13:** 1713–1720.
7 Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990; **215**: 403–410.
8 Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994; **22**: 4673–4680.

VLA-4 affinity correlates with peripheral blood white cell count and DNA content in patients with precursor B-ALL

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TO THE EDITOR

We have recently completed a study that suggests affinity state measurements may be a useful tool in studies of acute leukemia pathogenesis. Cellular adhesion molecules such as VLA-4 (very late antigen-4; CD49d/CD29) play an important role in the survival of normal and leukemic B cells.[1,2] mAb to VLA-4 inhibits lymphopoiesis,[1] and the binding of VLA-4 on precursor B-ALL cells to its ligand vascular cell adhesion molecule-1 (VCAM-1) on BM stromal cells is required for leukemic cell survival.[2,3] Defective VLA-4-dependent adhesion has also been identified in a subset of precursor B-ALL cases.[3] Yet, the clinical and biologic significance, if any, to defective VLA-4-mediated adhesion on precursor B-ALL cells is unknown.

VLA-4-mediated adhesion is regulated through affinity changes of VLA-4 for VCAM-1. VLA-4 has several affinity states that are due to conformational changes. We have recently developed a means of detecting these affinity changes, which correspond to the affinity for its natural ligands VCAM-1 and fibronectin, using an FITC-labeled VLA-4-specific peptidomimetic.[4] This technology rests on the observation that when these small molecules are used at a concentration that approximates its affinity constant for the high-affinity state, it will bind to the high-affinity state but not the lower affinity state. The rapid on and off rates of peptidomimetics in contrast to mAbs allow them to be used to detect changes in affinity state in real time.[4] $MnCl_2$ and TS2/16 are two agents that activate VLA-4 to higher affinity states. TS2/16 binds to VLA-4, inducing a higher affinity state, not dependent on cellular signaling.

In this study, we took advantage of this novel technology to examine how alterations in affinity regulation may affect the clinical or biologic features of precursor B-ALL cases. We first assessed VLA-4 affinity changes on a group of 36 diagnostic samples from patients with precursor B-ALL. These samples were obtained from children diagnosed with precursor B-ALL, and selected from precursor B Phase III trials (COG 9400). The cases consisted of 18 males, four of eight standard risk who achieved CCR and five of ten poor risk who achieved CCR; and 18 females, four of seven standard risk and six of 11 poor risk who achieved CCR. The assays were conducted in a blinded fashion, and the results forwarded to the central data center for statistical analysis. The methods used to quantify monoclonal antibody binding and receptor number peptidomimetic binding have been previously described.[4] After all the results were obtained, we received clinical, laboratory, and immunophenotypic data for each patient from the POG tumor bank. We correlated these data using Kruskal–Wallis and Spearman correlation coefficients to determine r and P values, respectively.

Significant differences in VLA-4 expression between CCR and relapse cases were not observed (Table 1). All patient samples expressed VLA-4; however, a striking range of VLA-4 sites/cell was observed (3015–25 526 sites/cell) with a mean of 7402 sites/cell. In contrast, we have previously shown that normal peripheral blood B cells have approximately 4000 sites/cell with a narrow variation among donors.[4] Site number did not change with Mn or TS2/16 treatment (data not shown). In each patient sample, we next examined the VLA-4 affinity state obtained at rest and after stimulation with $MnCl_2$ and TS2/16. Fluorescence intensity (MESF) is a measure of the affinity state of VLA-4. A statistically significant association between VLA-4 expression and VLA-4 affinity state measurements was not observed among cases ($P = 0.09$). There were no significant differences among the CCR and relapse groups in

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