magnitude of autonomous BFU-E growth greatly varies between individual patients. This is not unexpected and is in line with a large number of in vitro studies using primary cells from patients with other hematologic diseases.[2,3] However, we completely disagree with Spivak and Silver that a lower BFU-E growth in some experiments prevents a meaningful interpretation of the results. Imatinib mesylate completely suppressed autonomous erythroid colony growth in the majority of our patients at a concentration of 10 μM, a dose that did not block colony formation in our 5 healthy controls. Furthermore, imatinib mesylate inhibited BFU-E growth by more than 70% at 1 μM, a concentration of imatinib mesylate that is also achieved in patients.

We agree that additional experiments with purified erythroid progenitor cells are of interest in order to determine a potential involvement of accessory cells in imatinib mesylate–induced suppression of autonomous BFU-E formation. Since spontaneous BFU-E formation can also be observed when using purified progenitor cells from patients with PV,[4,5] we cannot share in speculations that imatinib mesylate–induced inhibition of spontaneous BFU-E growth is solely mediated by a suppression of monocyte accessory cell function. Even if the effect of imatinib mesylate on spontaneous BFU-E growth is partly mediated by modulating accessory cell function, this may be clinically relevant since monocytic cells are also present in the in vivo situation. Moreover, we do not think that the reappearance of erythroid colonies in the presence of exogenous erythropoietin questions the potential usefulness of this drug in PV. The only conclusion that can be drawn from this observation is that the signal transduction pathways, which are activated by erythropoietin and by the unknown target that is blocked by imatinib mesylate, are either completely or at least partly different. C-kit may be one of such targets, but present data are insufficient to consider c-kit hyperexpression as the main pathophysiologic abnormality in PV. If imatinib mesylate blocks the mechanism that renders PV progenitors constitutively activated, exogenous growth factors may still stimulate PV progenitors similar to normal hematopoiesis. PV progenitor cells might thus lose their growth advantage over normal hematopoietic stem cells, which, in turn, may reconstitute normal hematopoiesis upon prolonged treatment.

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To the editor:

The *GATA1* mutation in an adult patient with acute megakaryoblastic leukemia not accompanying Down syndrome

Mutations of the *GATA1* gene, which is located on chromosome X, have been found in almost all cases of transient myeloproliferative disorder (TMD) and acute megakaryoblastic leukemia (AMKL) accompanying Down syndrome (DS).[1-6] No *GATA1* mutations have been detected in patients with AMKL who did not have DS, except in AMKL with acquired trisomy 21.[1,4,6] However, because only 10 cases with AMKL in non-DS have been analyzed for mutations of the *GATA1* gene, it remains unknown whether the *GATA1* mutation is exclusively involved in the development of AMKL with DS. Here, we report for the first time a *GATA1* mutation in AMKL cells from a patient who did not have DS or acquired trisomy 21.

In October 2002, a 48-year-old woman was admitted to Furukawa City Hospital with complaints of shortness of breath and a bleeding tendency. Peripheral blood analysis showed severe anemia, thrombocytopenia, and mild leukocytosis with the appearance of immature cells: red blood cell count, 87×10^{12}/L; hemoglobin, 27 g/L (2.7 g/dL); platelet count, 6×10^{9}/L; and white blood cell count, 10.8×10^{9}/L (neutrophils, 29%; basophils, 1%; lymphocytes, 46%; blasts, 16%; metamyelocytes, 2%; myelocytes, 3%; and promyelocytes, 3%). Pathologic examination of her bone marrow showed a mixture of dominantly proliferated small lymphocytic cells, which were positive for leukocyte common antigen (LCA) and CD79α, and scattered blastic cells, which were negative for myeloperoxidase. Chromosomal analysis revealed 47XX, add(17)(p11), +add(17), add(20)(q13). The patient was diagnosed with acute lymphoblastic leukemia, chemotherapy was given, and complete remission was achieved. In February 2003, the disease relapsed and became refractory to chemotherapy. The morphology of leukemic cells at the relapse resembled that of small megakaryocytes, and they were negative for myeloperoxidase and positive for acid phosphatase. Immunohistochemical examination revealed that the leukemic cells were positive for CD33, factor VIII, and CD41a, and dull-positive for CD4 and CD45. In addition, flow cytometric analysis using an anti–GATA-1 antibody revealed that the leukemic cells were positive for GATA-1.[7] The same chromosomal anomaly as that detected at onset was detected by chromosomal analysis. Based on these results, she was finally diagnosed as having AMKL.

We analyzed the *GATA1* mutation in her bone marrow samples. Written informed consent was obtained from the patient. Genomic

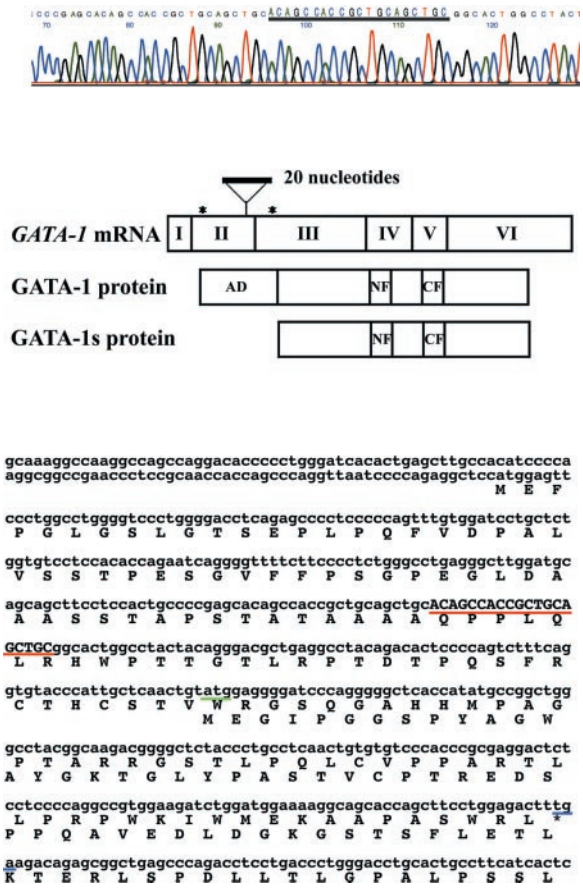

Figure 1. *GATA1* mutation in an adult patient with AMKL not accompanying DS. Direct sequence analysis of cDNA and genomic DNA from AMKL blast cells of the adult patient who did not have accompanying DS showed that 20 nucleotides of the duplicated sequence were inserted in exon 2 of the *GATA1* gene. The mutation resulted in the introduction of a premature stop codon in the gene sequence encoding the N-terminal activation domain. The predicted protein from a downstream initiation site, GATA-1s, lacks the transactivation domain.

DNA was extracted and cDNA was constructed, and then polymerase chain reaction was performed and the products were sequenced directly, as described previously.[6] An insertion of 20 nucleotides corresponding to a sequence in exon 2 of the *GATA1* gene was detected, resulting in the introduction of a premature stop codon in the gene sequence encoding the N-terminal activation domain (Figure 1).

Our results suggest that *GATA1* mutations play an important role in the development of AMKL in non-DS individuals. Our discovery warrants further investigation in a larger series of non-DS patients with AMKL.

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To the editor:

*REL*evant gene amplification in B-cell lymphomas?

The *REL* proto-oncogene encodes a transcription factor in the nuclear factor κB (NF-κB) family, and the activation of the REL protein can be controlled by subcellular localization.[1] The *REL* locus, located at chromosomal position 2p16, is amplified in many human B-cell lymphomas,[2] and overexpression of REL can transform chicken lymphoid cells in vitro.[3]

Houldsworth et al[4] recently reported on REL protein expression in a panel of diffuse large B-cell lymphomas (DLBCLs) with and without *REL* amplification. Using indirect immunofluorescence to assess subcellular localization of REL, these authors determined that DLBCLs with *REL* gene amplification did not have increased nuclear accumulation of REL protein compared with DLBCLs without *REL* gene amplification. This led these authors to conclude that REL protein activity is not involved in the development of DLBCLs with *REL* amplification, and that *REL* may not be the relevant oncogene in DLCBLs with amplifications of chromosomal region 2p16.

Based on the large amount of research that has been conducted on in vitro transformation of chicken lymphoid cells by v-Rel or more recently by human REL, we believe this is a faulty conclusion. First, by indirect immunofluorescence, v-Rel and human REL are largely cytoplasmic proteins in transformed chicken lymphoid cells.[3,5] However, in electrophoretic mobility shift assays using nuclear extracts, there is clearly nuclear Rel DNA-binding activity in v-Rel– and REL-transformed chicken lymphoid cells,[3,6] and v-Rel induces the expression of several κB site–containing target genes.[7] In addition, in these transformed cells, v-Rel and REL are continually shuttling through the nucleus,[3,8] and one cannot detect this movement by a static immunofluorescence image. Moreover, this nuclear shuttling appears to be important for oncogenic activity, because if the nuclear shuttling of v-Rel is decreased by the addition of a strong nuclear export signal onto v-Rel, its transforming activity is reduced.[9] Thus, it is our contention that the REL immunofluorescence data of Houldsworth