infant’s lymphoma using 2 independent methods: FISH and microsatellite analysis. Procedures were carried out according to the Declaration of Helsinki and with the informed consent of the family. As a result, the infant’s tumor cells were found to be of maternal origin (Figure 1A-B).

Although HLA loss in the tumor tissue is one of the escape mechanisms for evading the immune surveillance system, tumor cells losing HLA-C antigens are recognized and removed by NK cells. Recently, Villabolos et al demonstrated that the uniparental disomy of the HLA-C locus as well as the loss of mismatched HLA-A and -B alleles were associated with leukemic relapse after HLA-haploidentical transplantation. We could not precisely determine whether the tumor tissue lost noninherited maternal HLA alleles in the present study (supplemental Table 1). However, it was crucial that the maternal leukemic cells transmigrated into the paratesticular area, a sanctuary from the immuno-surveillance system, where they were engrafted and proliferated.

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References

To the editor:

Fanconi anemia (FA)–associated 3q gains in leukemic transformation consistently target EVI1, but do not affect low TERC expression in FA

In a recent issue of Blood, Quentin et al1 reported on genomic abnormalities in Fanconi anemia (FA)–associated leukemic transformation. They confirmed 3q gains as an FA-characteristic chromosomal aberration and discussed the potential role of EVI1. Complementing this comprehensive study, we have analyzed gene expression patterns of FA-associated 3q26q29 gains using mononuclear bone marrow cells from backup harvests from 6 FA patients (FA-BM; clinical details in supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online letter), and 2 healthy child-donor marrow harvests as controls. Approval was obtained from the Charité institutional review board for these studies. Informed consent was provided in accordance with the Declaration of Helsinki. We characterized 3q26q29 gains in 4 FA-BMs by high-resolution analysis with 250K SNP array (Affymetrix). Two FA-BM patients did not have 3q gains (Figure 1A). Expression patterns were determined with HG-U133plus2 arrays (Affymetrix) and quantitative real-time PCR (qRT-PCR; supplemental Methods and supplemental Tables 2-3). Transcripts encoded at 3q26q29 are targeted by 491 probesets on the U133plus2arrays, of which 134 were called “absent” in at least 7 of the 8 samples. Present in 2 or more samples, but mean expression levels not different comparing FA-BM with or without 3q gains with normal controls, were 231 probesets. Only 2 probe-sets detected significantly lower transcript levels in FA-BM. Twenty-five probe sets were more highly expressed in FA-BM, irrespective of 3q gains. Changing from absent to present, or significantly more highly expressed in samples with 3q gains, were 99 probesets, of which 32 were also significantly more highly expressed in all FA-BM. The highest fold change of these had 7 of the 8 samples. Present in 2 or more samples, but mean expression levels not different comparing FA-BM with or without 3q gains with normal controls, were 231 probesets. Only 2 probe-sets detected significantly lower transcript levels in FA-BM. Twenty-five probe sets were more highly expressed in FA-BM, irrespective of 3q gains. Changing from absent to present, or significantly more highly expressed in samples with 3q gains, were 99 probesets, of which 32 were also significantly more highly expressed in all FA-BM. The highest fold change of these had probe set 226420_at, which targets EVII. A greater than 10-fold increase in mean EVII transcripts in FA-BM samples was confirmed by qRT-PCR (Figure 1B). U133plus2 arrays do not detect telomerase complex RNA component (TERC), which is encoded in close proximity to EVII. As TERC has an important role in
hematopoiesis, and TERC overexpression is commonly associated with 3q gains, we analyzed TERC expression by qRT-PCR. We detected an up to 10-fold lower TERC level in all FA-BM (Figures 1C; $P_{/H11005} < 0.0005$), irrespective of 3q gains or bone marrow morphology. Validation of expression analysis of other genes in the 3q26q29 region with a role in malignant transformation by qRT-PCR confirmed a small increase in transcript levels for PIK3CA, EIF-4G1, DCUN1D, and FXR1, while PAK2 was also more highly expressed comparing all FA-BM to normal BM. SKIL/SNON expression was not different in FA-BM or affected by 3q gains (Figure 1D-I).

Aware of the limitations resulting from small sample size, our data imply consistent activation of EVI1 expression with FA characteristic 3q gains, which are uncommon in AML, but prognostically very important. As EVI1 expression is highest in BM with complex karyotype aberrations, additional genetic changes in FA-associated leukemic transformation might further contribute to EVI1 overexpression, and implies the possibility that EVI1 overexpression might confer clonal advantage specifically in the presence of an FA defect. Altered telomere maintenance, but not low TERC expression, has been described in FA and might contribute to bone marrow failure in FA. Our finding of up to 10-fold lower TERC

Figure 1. SNP characterization and RT-PCR analysis of 3q gains in FA. (A) SNP array characterization of 3q gains in all FA bone marrow harvests. SNP array data are visualized using CNAG 3.0 software. Analysis of call intensity was conducted using standard Affymetrix analysis software algorithms provided in GCOS and GTYPE 4.0. Chromosomal aberrations were analyzed using Copy Number Analyzer for Affymetrix GeneChip Mapping arrays (CNAG) 3.0 software employing the AsCNAR algorithm. In 4 patients with 3q gain, the extension of aberration is depicted using red bars and the position coordinates are shown on the right side. Position of EVI1 is indicated. (B-I) qRT-PCR analysis of selected genes as indicated (B: EVI1; C: TERC; D: PAK2; E: SKIL; F: PIK3CA; G: EIF-4G1; H: FXR1; I: DCUN1D1) in FA bone marrow samples with 3q-gains (darker-shaded right 4 columns) and without (brighter-shaded left 2 columns). Transcripts levels are shown as 2$^{-\Delta\Delta Ct}$ normalized to normal BM ($^*P_{/H9252} < 0.01$) using $\beta$-actin and SDHA as housekeeper genes. Comparison also between mean transcript levels of FA marrows with and without 3q gains ($t_{/H9004}$ test).
expression in all FA-BM irrespective of chromosomal aberrations or bone marrow morphology implies the possibility of an FA-specific TERC dysregulation.

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To the editor:

Doubts concerning the recently reported human neutrophil lifespan of 5.4 days

Using orally administered deuterium-labeled water to label neutrophils in vivo, Pillay et al measured urinary and blood deuterium-to-proton enrichment ratios at intervals of ~12 days over several weeks before and after termination of intake. From mathematical modeling they obtained a median peripheral blood (PB) human neutrophil lifespan of 5.4 days.

This result is highly surprising in view of the range of neutrophil labeling techniques, including whole blood labeling with DFP-32I; in vivo H-3 labeling followed by blood transfusion; and in vitro labeling of purified neutrophils with Cr-51, In-111, or Tc-99m, which have all previously given a PB lifespan of ~10 hours. It is also starkly at odds with the clinical observations of rapid neutrophil depletion after myeloablative chemotherapy (within 3-5 days) and short-lived normalization of neutrophil counts following therapeutic granulocyte infusion (1-2 days).

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Contribution: S.M. developed the study, designed the research, analyzed microarray data, and wrote the manuscript; C.B. carried out sample preparation and qRT-PCR; S.M., M.W., and S.P. analyzed microarray data; A.D.W. provided scientific input and laboratory facilities and contributed to writing the manuscript; H.H. provided data regarding FA complementation analysis; H.N. and W.E. provided patient material and clinical data and contributed to writing the manuscript; M.W.W. carried out SNP array analysis and contributed to writing the manuscript; and H.T. initiated and developed the study and wrote the manuscript.

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References


We wish to raise several objections to the work of Pillay et al.

1. Bone marrow is a major site of neutrophil destruction as well as development, but there is no discussion on the availability of deuterium-labeled adenosine for salvage that might result in reutilization in newly dividing neutrophil precursors.
2. The authors claim their previous work on human lymphocytes, based on similar sampling intervals, validates their modeling, but lymphocytes had a reported lifespan of >100 days, long enough to be measurable from the sampling interval used.
3. The reported peak (63-day) enrichment in blood (4.3% urine value) is unexplained. Urine enrichment is consistent with the deuterium dose (see supplemental Data, available on the Blood Web site; see the Supplemental Materials link at the top of the online letter), but blood enrichment is inexplicably high. The authors introduced an “amplification factor” between urine and
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