marrow microenvironment.[7] We plan to carry out a second clinical trial using bortezomib for initial induction therapy as well as incorporating it into the conditioning regimen. Based upon the results of these two studies, the regimen with superior phase II results will be compared to a standard melphalan-based SCT in a randomized phase III study, with the goal of determining whether the addition of bortezomib leads to a higher rate of hematologic and clinical responses, and better progression-free and overall survival.

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Managing individuals with propensity to myeloid malignancies due to germline *RUNX1* deficiency

With great interest we read the recent review on familial myelodysplastic syndromes (MDS) published in this journal by Liew and Owen.[1] Besides telomere disorders and familial monosomy 7, the review focused on familial platelet disorder with propensity to myeloid malignancies (FPDMM) and also surveyed syndromic cases of heterozygous loss of chromosome 21q22. To further highlight the clinical diversity of FPDMM and to discuss the challenges posed by the clinical management of patients with germline *RUNX1* deficiency, we report here on a patient with a constitutional loss of *RUNX1* due to a *de novo* deletion of 21q22.

Due to chronic, idiopathic thrombocytopenia, retrospectively found to have been already present in childhood, mild anemia and neutropenia, cytogenetic investigations were performed in a 19-year old patient and displayed a loss of one *RUNX1* allele in bone marrow cells. There was no evidence of MDS or acute myeloid leukemia (AML). Following genetic counseling, karyotyping and fluorescence *in situ* hybridization of phytohemagglutinin-stimulated peripheral blood cells confirmed a heterozygous deletion in 21q22 (Figure 1A, *Online Supplementary Appendix*). High-resolution array comparative genomic hybridization (aCGH) displayed a 1.6 Mb deletion in the long arm of a chromosome 21 involving among others *RUNX1* (Figure 1B). Breakpoint spanning long distance PCR reconfirmed the deletion in DNA isolated from peripheral blood and a buccal swab (Figure 1C). Mutations of the remaining *RUNX1* allele were excluded by DNA sequencing.

In contrast to the reviewed syndromic cases with deletions in 21q22 that, with the exception of one case,[2] displayed a complex phenotype,[1] our patient did not show any growth or developmental delay, dysmorphic features or other abnormalities. Most of the previously reported patients had been described in early childhood when a complex phenotype probably prompted cytogenetic analyses. However, as demonstrated by our patient, deletions of 21q22 including *RUNX1* do not necessarily lead to a complex phenotype, highlighting again the clinical variability of FPDMM.[1]

In view of the early onset of leukemias in 3 out of 12 patients, Liew and Owen hypothesized that the age of leukemic transformation seems to be earlier in patients

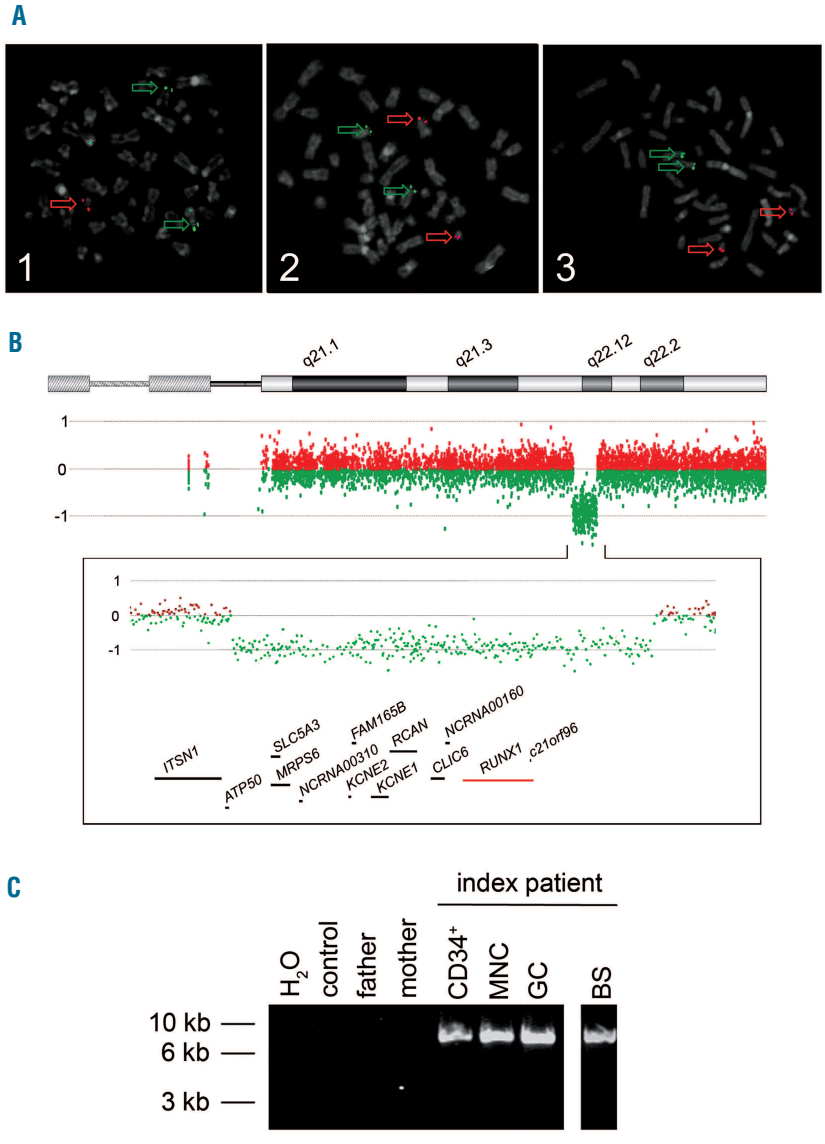

Figure 1. (A) *ETV6/RUNX1* metaphase fluorescence *in situ* hybridization (FISH). Metaphase plates of PHA-stimulated peripheral blood cells of the index patient (1) and his parents (2, 3) were investigated using specific probes for RUNX1 (LSI AML1, 21q22, red) and *ETV6* (LSI TEL, 12p13, green) (Vysis, Abott, LSI ETV6(TEL)/RUNX1(AML1) ES dual color translocation probe set, Wiesbaden, Germany). While metaphases of the index patient (1) displayed one red and two green signals indicative of a loss of one *RUNX1* allele, the metaphases of the parents (2, 3) showed two red and two green signals. Interphase FISH analysis of 100 nuclei displayed the submicroscopic loss of one *RUNX1* allele in 93% of cell nuclei investigated. (B) High resolution oligo array comparative genomic hybridization (aCGH) of the index patient. DNA of peripheral blood cells was analyzed using a 400k oligo array following the manufacturer's instructions (Agilent Technologies, Boeblingen, Germany). In comparison to a healthy control sample, aCGH displayed a 1.6 Mb deletion in the long arm of a chromosome 21 of the index patient which is probably due to a *de novo* rearrangement: arr 21q22.11q22.12(35,304,856-36,864,010)x1 dn. Below the ideogram of chromosome 21 from 21pter to 21qter, the aberration states based on normalized log2 transformed fluorescent intensity ratios are shown indicating the heterozygous interstitial deletion. Further on, an enlarged view of the aberrant region is given (chromosome 21: 34,912,522-37,106,477; 2.19Mb) displaying genes located within the region lost as well as some of the neighboring genes. The *RUNX1* locus is highlighted in red. (C) Breakpoint spanning long-distance PCR. Using primers located within the last aCGH probes before and after the deletion detected, a breakpoint spanning PCR product of approximately 9 kb was generated in the index patient (lanes 5-8) while no product was seen in peripheral blood DNA samples of his parents (lane 3, 4) and a healthy control (lane 2).

Analyses of DNA of several peripheral blood cell populations of the index patient displayed the deleted allele in $CD34^+$ cells ($CD34^+$, lane 5), cells of a mononuclear (MNC, lane 6), and a granulocytic cell fraction (GC, lane 7). Finally, in DNA of a buccal swab of the patient, the PCR product could also be amplified indicating the germline origin of the submicroscopic deletion in 21q (BS, lane 8). The identified *de novo* deletion is probably the result of a recombination between two L1PA2 long interspersed nuclear elements (LINE) that lie in the breakpoint region and display a sequence identity of 97%.

with deletions in 21q22 than in classical FPDMM.[1] Despite detailed morphological and cytogenetic investigations, there was no evidence of MDS or AML in our 19-year old patient, although he carries a deletion similar to that seen in the case[2] of non-syndromic thrombocytopenia with myelodysplasia. Moreover, we recently reported on a patient with a *RUNX1* mutation showing a malignant transformation by the age of 13 years, indicating that in *RUNX1* mutation carriers early onset of leukemia is also possible.[3] It is known that secondary genetic alterations are required for the development of leukemia in FPDMM.[3,4] Possibly, the age of onset depends more on the time and nature of additionally acquired alterations than on the presence of a deletion or mutation of *RUNX1*.

As pointed out by Liew and Owen,[1] there is fortunately an increased awareness of FPDMM. In addition, the report on the association of *RUNX1* mutation with thrombocytopenia, bone marrow blasts, and poor overall survival in patients with MDS[5] will further increase the number of patients with germline *RUNX1* deficiency. While there is an estimated risk of up to 60% of developing MDS or AML in patients with FPDMM, no surveillance guidelines are available for these patients and their families. In cases presenting with overt leukemia, the clinical management is dictated by the disease and it is broadly accepted that bone marrow transplantations by donors carrying the familial mutation have to be avoided.[6] But what can we offer otherwise healthy individuals, e.g. the patient reported herein or individuals identified during screening as potential donors for a bone marrow transplant? We provided genetic counseling for the patient and his parents before we proceeded with further analyses and would recommend that this be the

standard procedure whenever possible. In addition, we recommended peripheral blood cell counts and clinical evaluation of the patient every six months. So far, regular investigations of bone marrow aspirates have not been scheduled. In view of the association of *KCNE2*, *KCNE1*, *RCAN1* and *CLIC6*, also affected by the deletion (Figure 1B), with cardiac malformations and arrhythmias, the index patient was referred to a cardiologist.[7,8] However, is that enough? As far as the increased awareness of individuals with germline *RUNX1* deficiency is concerned, efforts for the development of broadly accepted surveillance programs and clinical management guidelines for FPDMM are the next critical step. These are urgently needed to ensure that the increased awareness of this disease is translated into a clinically useful management approach towards the affected individuals and their families.

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