Acute Myeloid Leukemia

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Assessment of submicroscopic genetic lesions by single nucleotide polymorphism arrays in a child with acute myeloid leukemia and *FLT3*-internal tandem duplication

The same *FLT3*-internal tandem duplication (ITD) positive clone was detected at diagnosis and relapse, but not at birth, in a child with M1 acute myeloid leukemia. Single nucleotide polymorphism arrays demonstrated that chromosome 13 acquired uniparental disomy, in association with del(9q), represented a progressive event in the course of the disease, and it was responsible for the homozygous *FLT3*-ITD at relapse.

We studied the clonal evolution from birth to diagnosis and relapse in a child with *FLT3*-internal tandem duplication (ITD)-positive acute myeloid leukemia (AML) who experienced two early relapses and for whom we fortuitously had a cord blood sample. *FLT3*-ITD was used as a minimal residual disease marker, and to backtrack the leukemic cells into the cord blood.

The patient was a female diagnosed at 6 years of age with AML-M1. At diagnosis, the patient's bone marrow karyotype was normal; the molecular screening showed *FLT3*-ITD mutation, a wild type *NPM1* sequence and negativity for *CBFB-MYH11* and *CBFA-ETO* fusion genes. She underwent autologous bone marrow transplantation in complete disease remission following induction therapy

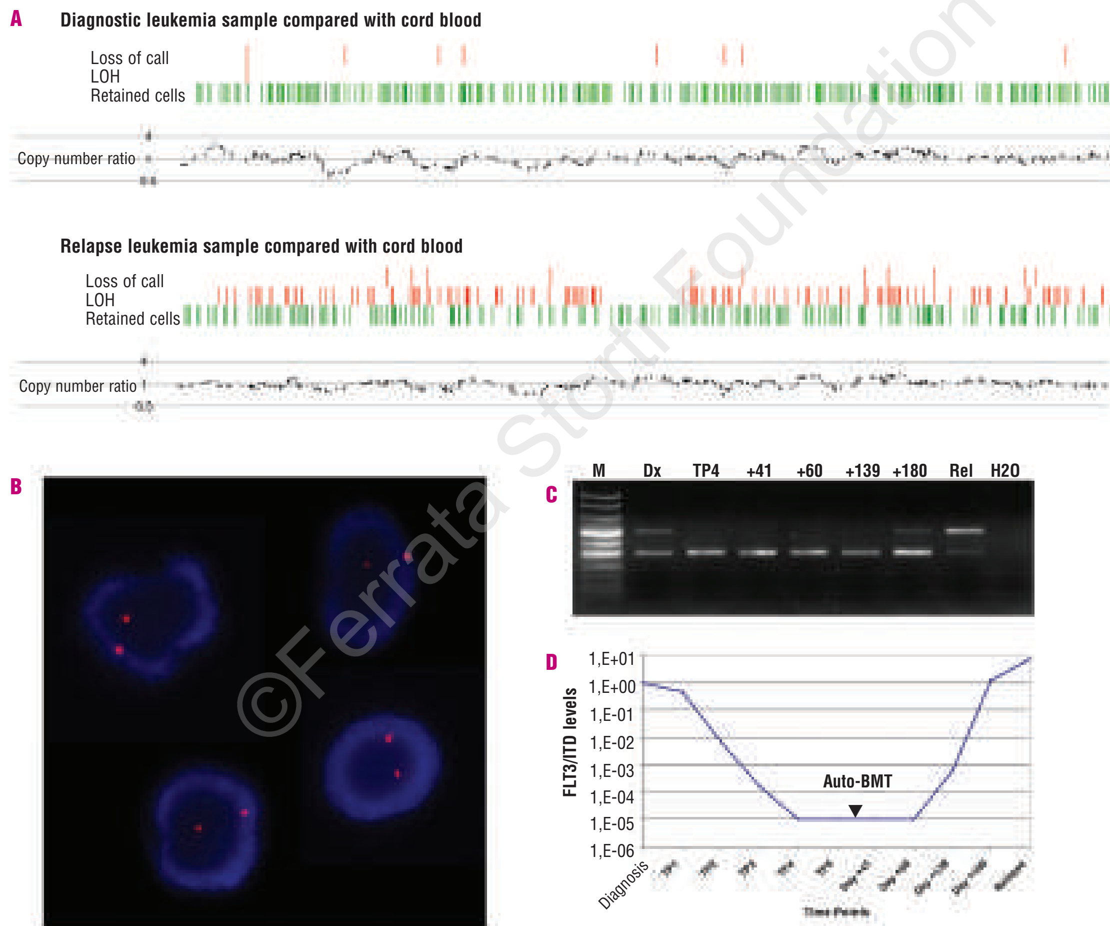

Figure 1. Clonal evolution of chromosome 13 abnormalities. A) A schematic diagram of SNP calls on chromosome 13. Copy number ratio was calculated for each SNP using a ratio of the signals from the germ line (cord blood) and leukemia. For each SNP a mean number of copies in a window of 10 adjacent SNP was used. Green lines represent SNP calls retained in the germ line and the leukemia, with light green representing heterozygous and dark green homozygous SNP. Red lines represent loss of call compared with the germ line. In the relapse sample, there is complete loss of heterozygosity (LOH) without a change in the copy number suggesting UPD. B) FISH analysis with the chromosome 13q14 LSI D13S319 SpectrumOrange probe on the relapse sample shows the presence of two signals, indicating the retention of two alleles on chromosome 13, despite the LOH, thus confirming UPD. C) Agarose gel electrophoresis of PCR-amplified *FLT3* gene indicates the loss of wild type *FLT3* allele at relapse (lower band), together with progressive increase of *FLT3*-ITD (upper band). At diagnosis, both alleles were present. Remission phase samples harboring the wild type allele are shown. The wild type band at relapse might be due to the presence of a low number of non-leukemic cells. D) Quantitative analysis of the *FLT3*-ITD mutation by RQ-PCR confirms the absence of the *FLT3*-ITD positive clone during complete remission, and its reappearance before the relapse. Normalized copy numbers of *FLT3*-ITD with respect to the albumin gene are shown on the Y-axis. TP1=day +15, TP2=day +21; TP3=day +32, TP4= +2 months (end of induction), TP5= +4 months after diagnosis; days after autologous bone marrow transplantation are indicated.

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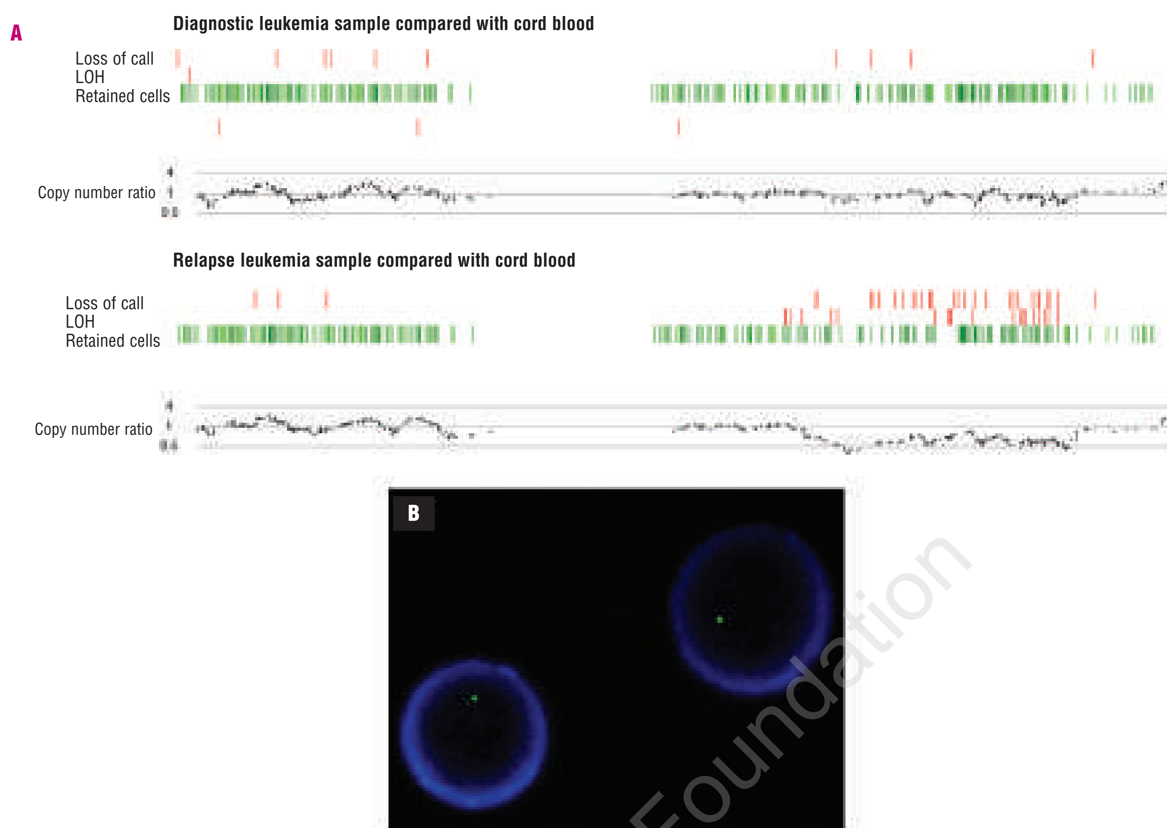

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Figure 2. Analysis of chromosome 9 deletion on a relapse sample. A. A schematic diagram of SNP calls on chromosome 9. Copy number ratio was calculated for each SNP using a ratio of the signals from the germ line (cord blood) and leukemia. For each SNP a mean number of copies in a window of 10 adjacent SNP was used. Green lines represent SNP calls retained in the germ line and the leukemia, with light green representing heterozygous and dark green homozygous SNP. Red lines represent loss of call compared with the germ line. In the relapse sample, there is complete LOH with decrease in the copy number on chromosome 9q21.32-q33.2, suggesting interstitial deletion. B. FISH analysis with BAC probe RP11-402M4 shows only one signal, validating SNP array results on 9q deletion.

(AIEOP-02 AML protocol). However, 3.5 months later the patient relapsed. Four months after an allogeneic bone marrow transplant, she had a second relapse and died of disease progression.

FLT3-ITD monitoring from diagnosis to relapse was performed by a patient-specific highly sensitive (10^{-4}) real-time quantitative (RQ)-polymerase chain reaction (PCR),[1] showing a progressive decrease of minimal residual disease (Figure 1, panel D). The same *FLT3*-ITD clone re-emerged at relapse, suggesting that the leukemic clone responsible for the first diagnosis was stable and could be used for backtracking the leukemia into the cord blood.

When tested by highly sensitive RQ-PCR, the cord blood DNA resulted negative for the *FLT3*-ITD mutation, consistent with the current hypothesis that *FLT3*-ITD is a secondary event in leukemogenesis.[2] This is the first direct report on post-natal occurrence of *FLT3*-ITD.

We then applied a 10K single nucleotide polymorphism (SNP) array approach (Affymetrix, Inc., Santa Clara, CA, USA) to search for genome-wide loss of heterozygosity and/or copy number changes of genes involved in tumor evolution,[3] comparing cord blood, diagnostic and relapse samples. SNP arrays on the relapse sample showed chromosome 13 uniparental disomy (UPD) (Figure 1, panel A), confirmed by fluorescent *in situ* hybridization (FISH) with a 13q14.3 LSI D13S319 SpectrumOrange probe (Vysis, Abbott GmbH, Germany) (Figure 1, panel B). We assume that, through the evolution of the disease from diagnosis to relapse, chromosome 13 harboring the *FLT3*-ITD had undergone non-disjunction. The leukemic cells with chromosome 13 UPD and *FLT3*-ITD homozygosity may have acquired a clonal advantage over those with the wild type *FLT3* allele. This finding was also confirmed by qualitative PCR, as shown in Figure 1, panel C. Agarose gel electrophoresis of *FLT3*-ITD PCR products indicated the increase of the ITD PCR amplification over the wild type band. Acquired UPD has been recently demonstrated in approximately 20% of AML[4] and UPD on chromosome 13 was detected by SNP arrays in three out of five relapse samples from *FLT3*-ITD positive patients.[5] In addition to UPD13, an interstitial deletion was found by SNP arrays on chromosome 9q21.32-q33.2 (Figure 2, panel A), confirmed by FISH with BAC-derived probe RP11-402M4 located on 9q31.1 (Invitrogen, Paisley, UK) (Figure 2, panel B). Unfortunately, no information about the karyotype was available on the relapse sample to exclude the presence of structural chromosomal rearrangements. Del(9q) has been reported in about 2% of AML cases, frequently associated with t(8;21) and rarely with t(15;17).[6] The presence of tumor suppressor genes potentially involved in disease progression on the 9q deleted chromosomal fragment must be further explored.

We hypothesize that a still unknown primary event, such as a mutation in a proto-oncogene, hit the patient's

leukemia stem cell and that the *FLT3*-ITD occurred as a further step, affecting the same stem cell population. Subsequently, the cell harboring *FLT3*-ITD accumulated both chromosome 13 UPD and 9q deletion, giving rise to the subsequent relapses.

In summary, by using a combined SNP array and RQ-PCR approach we have been able to indicate the post-natal origin of the *FLT3*-ITD mutation observed at diagnosis in this AML patient, and to demonstrate the occurrence of loss of heterozygosity as a mechanism of disease progression, which may be associated with both copy number changes -del(9q)- or UPD (*FLT3*-ITD homozygosis). We are aware that the primary event(s) giving rise to leukemia in association with *FLT3*-ITD mutation may be represented by aberrations not detected by SNP array analysis, and affect genes involved in differentiation pathways, as expected from the model of co-operative mutations.[7] Other methods must be applied in order to find these class II putative mutations.

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References

1. Beretta C, Gaipa G, Rossi V, Bernasconi S, Spinelli O, Dell'Oro MG, et al. Development of a quantitative-PCR method for specific FLT3/ITD monitoring in acute myeloid leukemia. Leukemia 2004;18:1441-4.
2. Stirewalt DL, Radich JP. The role of FLT3 in haematopoietic malignancies. Nat Rev Cancer 2003;3:650-65.
3. Huang J, Wei W, Zhang J, Liu G, Bignell GR, Stratton MR, et al. Whole genome DNA copy number changes identified by high density oligonucleotide arrays. Hum Genomics 2004;1:287-99
4. Raghavan M, Lillington DM, Skoulakis S, Debernardi S, Chaplin T, Foot NJ, et al. Genome-wide single nucleotide polymorphism analysis reveals frequent partial uniparental disomy due to somatic recombination in acute myeloid leukemias. Cancer Res 2005;65:375-8
5. Fitzgibbon J, Smith L-L, Raghavan M, Smith ML, Debernardi S, Skoulakis S, et al Association between Acquired Uniparental Disomy and Homozygous Gene Mutation in Acute Myeloid Leukemias. Cancer Res 2005; 65:9152-54.
6. Pui CH, Schrappe M, Ribeiro RC, Niemeyer CM. Childhood and adolescent lymphoid and myeloid leukemia. Hematology (Am Soc Hematol Educ Program) 2004:118-45.
7. Gilliland DG, Jordan CT, Felix CA. The molecular basis of leukemia. Hematology (Am Soc Hematol Educ Program). 2004;80-97.

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