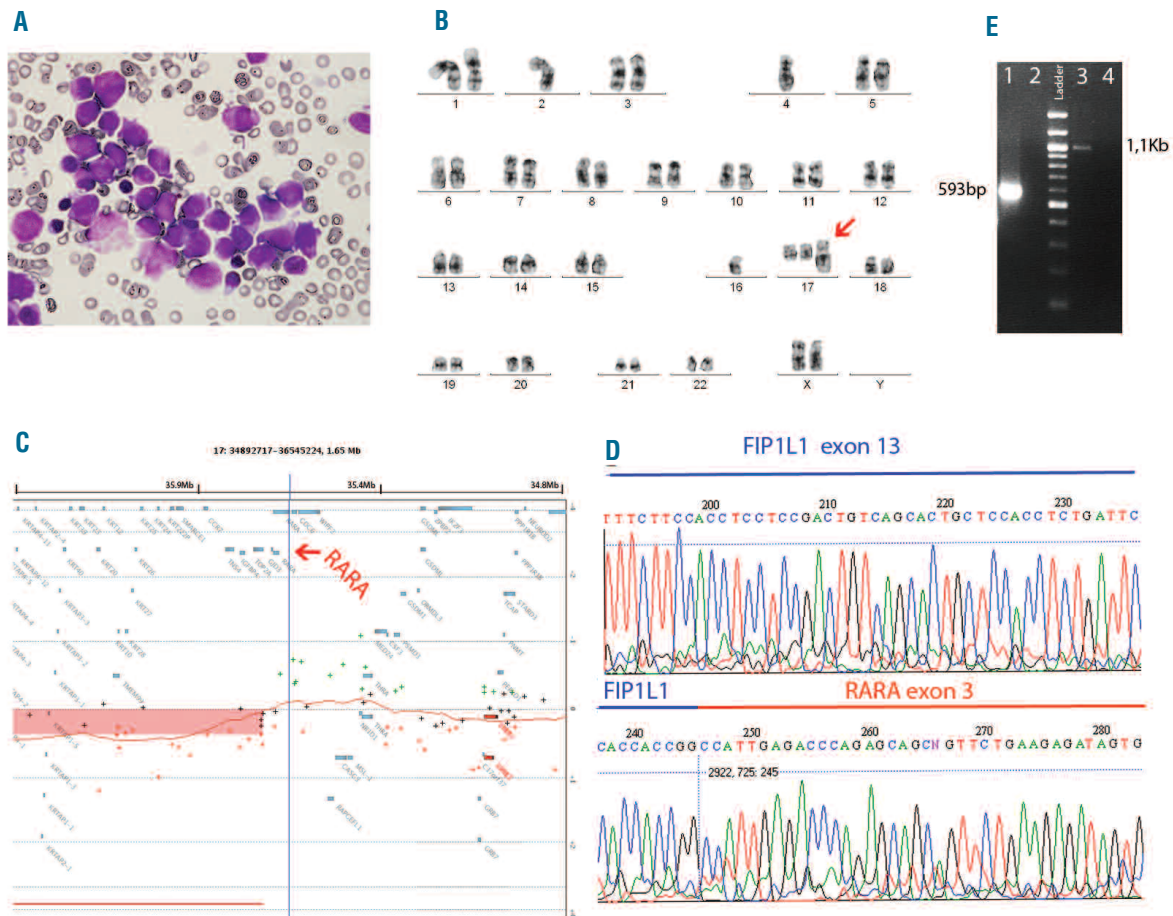


### **FIP1L1/RARA with breakpoint at FIP1L1 intron 13: a variant translocation in acute promyelocytic leukemia**

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia that is characterized by three distinct features: i) accumulation in the bone marrow (BM) of tumor cells with promyelocytic phenotype; ii) association with specific translocations which involve chromosome 17 at the retinoic acid receptor alpha (*RARA*) locus; iii) and the sensitivity of APL blasts to the differentiating action of retinoic acid (RA).<sup>1</sup> The *RARA* locus was first demonstrated to be involved in the t(15;17)(q22;q21) that fuses the *RARA* and the *PML* genes. While the *PML/RARA* fusion transcript is present in over 95% of APL cases, variant rearrangements have been identified involving *RARA*, and at a lower frequency (>3%) the *PLZF*, or more rarely the *NPM1*, *NUMA*, *STAT5b*, *PRKAR1A* and *FIP1L1* as partner genes.<sup>2,3</sup> The nature of

the fusion partner has an important role on the disease biology particularly regarding RA sensitivity, with *PLZF-RARA* patients characterized by RA resistance.<sup>4</sup> Following the description published in the journal *Haematologica* in 2008,<sup>5</sup> we describe here the second case of *FIP1L1/RARA* fusion gene in an APL patient.

A 77-year old female patient presented with a progressive history of asthenia which lasted for several weeks. Initial laboratory evaluation of peripheral blood revealed a white blood cell count of  $59 \times 10^9/L$  with 84% of abnormal promyelocyte cells, hemoglobin level of 9.2 g/dL, and a platelet count of  $109 \times 10^9/L$ . The coagulation function in this patient was normal and lactate dehydrogenase was 1,938 U/L. The BM aspirate showed a hypercellular marrow replaced by promyelocyte blasts with intense azurophilic granule and prominent nucleoli accounting for 93% of all nucleated cells, suggestive of APL (Figure 1A). Immunophenotype was: CD45<sup>+</sup>, CD45RA<sup>+</sup>, CD13<sup>+</sup>, CD15<sup>low</sup>, CD33<sup>+</sup>, CD133<sup>low</sup>, HLA-DR<sup>+</sup>, CD11c<sup>low</sup>, CD65<sup>low</sup>, CD71<sup>low</sup>, CD117<sup>low</sup> and CD38<sup>low</sup>. The molecular analysis identified the FLT3-ITD muta-



**Figure 1.** Characterization of APL cells. (A) Morphology of the leukemia cells shows hypergranular promyelocytes with Auer rods in BM (100X). (B) A representative G-banded karyotype of the aberrant clone. The arrow indicates the derivative chromosome 17. (C) The panel shows the representative ideogram of the gain (40.8Mb) in chromosome 17q21.2q25.3, which results in the partial gain of the *RARA* locus and a possible rearrangement of this gene (arrow). (D) The sequence analysis of the identified fusion gene from the reverse sequence of *RARA* exon 3 identified *FIP1L1* at exon 13 as the fusion partner gene. (E) Confirmation of the presence of the two reciprocal fusion transcripts by RT-PCR. Line 1: *FIP1L1-RARA* detection with a forward primer on *FIP1L1* exon 10 (5'-ACAGCAGGGGAAGAACTGGAA-3'), and a reverse primer on *RARA* exon 3 (5'-CCCCATAGTGGTAGCCTGAG). Line 3: *RARA-FIP1L1* detection with a forward primer on *RARA* exon 1 (5'-ACACACCTGAGCAGCATCAC-3'), and a reverse primer on *FIP1L1* exon 18 (5'-GTGTAGCTTCGGTCTCTCC-3'). Lanes 2 and 4 are negative controls.

tion, being negative for the presence of *FLT3-D835Y*, *CEBPA* and *NPM1* mutations, as well as for the presence of *PML/RARA*, *AML1/ETO* and *CBFβ/MYH11* fusion genes. The patient was treated according to PETHEMA APL 2005 protocol. Unfortunately, she died after ten days of treatment, probably due to RA syndrome, and response to RA treatment could not be assessed in this case due to early death.

Cytogenetics revealed, in addition to normal metaphases, a complex karyotype with the presence of a der(17) in 50% of the metaphases with the following formula: 44,X,der(X)t(X;?) (p?;?),-2,-4,-16,+der(17)t(17;?) (q21;?) [cp10] (Figure 1B). FISH analysis with the *PML-RARA* dual-fusion translocation probe (Kreatech Diagnostics, Amsterdam, The Netherlands) identified no dual fusion signal but the presence of two copies of *PML* and three signals of *RARA* gene, indicating a possible variant rearrangement of this gene. The presence of DNA copy number changes was analyzed by array CGH with Agilent 44K platform (Agilent Technologies, Palo Alto, CA, USA). We found a mono-allelic gain of 40.8Mb in the long arm of chromosome 17 (Figure 1C). The duplicated region started within the *locus* of *RARA* gene, also indicating its possible rearrangement. To identify the 5'-fusion partner of *RARA*, we assayed the 5'-RACE method (SMARTer RACE cDNA, Clontech, Mountain View, CA, USA) designing a reverse primer complementary to exon 3 of the *RARA* gene. *FIP1L1* was identified as the fusion partner of *RARA*. The rearrangement fused the *RARA* exon 3 with exon 13 of the *FIP1L1* gene (Figure 1D). Direct sequencing of the reverse-transcriptase PCR products revealed that the *FIP1L1/RARA* and the *RARA/FIP1L1* fusion transcripts were both in frame fusions (Figure 1E).

*FIP1L1* is an integral subunit of cleavage and polyadenylation specificity factor and interacts with poly(A)polymerase to stimulate polyadenylation.<sup>6</sup> This gene is recurrently fused to *PDGFRA* in patients with eosinophilia-associated myeloproliferative neoplasms (Eos-MPNs), with clinical response to imatinib.<sup>7,8</sup> Although a *FIP1L1/RARA* fusion was described in a case of juvenile myelomonocytic leukemia,<sup>9</sup> Kondo *et al.* described, for the first time, the *FIP1L1/RARA* fusion gene in APL.<sup>5</sup> In both cases, the fusion gene was generated juxtaposing exons 15 and 3 of *FIP1L1* and *RARA*, respectively. In the molecular pathogenesis of APL, fusion gene products must form homodimers to repress RA-responsive transcriptional activity.<sup>10,11</sup> In fact, homodimerization, which seems to be dependent on the *FIP1L1* portion, was demonstrated for the isoforms identified in the previous patient with the *FIP1L1/RARA* fusion that retained *FIP1L1* exon 15 and responded to RA treatment.<sup>5</sup> Conversely in the *FIP1L1/PDGFR*A fusion in Eos-MPNs, the breakpoint in *FIP1L1* is variable and spreads from exon 10 to exon 13 (exons 7 to 9, according to Cools *J et al.*<sup>7</sup>), lacking the ability to form homodimers in a mouse pro-B cell line.<sup>12</sup> It still remains to be clarified whether the RA syndrome observed in our case could be due to the abnormal homodimerization predicted by the breakpoint in *FIP1L1*, similar to that observed in Eos-MPNs.

In conclusion, we report the second occurrence of t(4;17)(q12;q21), with the reciprocal *FIP1L1/RARA* transcripts, in a very aggressive case of APL. Our results confirm *FIP1L1* as a recurrent partner of *RARA* gene with breakpoint at intron 13 that was associated with the RA syndrome. We, therefore, propose the inclusion of the *FIP1L1/RARA* variant fusion gene in the screening in *PML/RARA*-negative APL patients in order to indicate alternatives therapies.

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