response to both induction and relapse chemotherapy. IIMM patients are routinely monitored by measuring their serum immunoglobulin and urine light chains – a logical step would be to supplement, or replace, the urine assays with serum FLC measurements, as has already happened in many centres.

Thus, the conclusion of Tate *et al* (2005) that larger prospective studies are needed before FLC measurement is 'routinely applied to the monitoring of patients with IIMM', is a generalization, drawn outside the context of the data they have presented. Serial FLC measurement is being undertaken in all patients in the current Medical Research Council Myeloma IX Study, which should provide further information on patients treated in a systematic way. Future studies may investigate clinical benefit further, perhaps with modification or change in treatment determined by FLC measurement.

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The co-expression of *PML/RARα* and *AML1/ETO* fusion genes is associated with ATRA resistance

Acute promyelocytic leukemia (APL), associated with the t(15;17)/*PML/RARα*, and acute myeloid leukaemia (AML), with t(8;21)/*AML1/ETO*, are distinct subtypes of AML with characteristic cytomorphological, genetic and clinical features. We report the simultaneous expression of *PML/RARα* and *AML1/ETO* fusion genes in a 47-year-old female patient with AML, who was referred to our University Hospital presenting with petechiae, ecchymoses and pallor of 1-month duration. She was previously healthy and there was no evidence of exposure to drugs, chemicals or any potential mutagen. There were no relevant findings in her family history. Adenopathy and splenomegaly were absent. The peripheral blood (PB) counts showed haemoglobin level: 6 g/dl; platelet count: 14×10^9/l and leucocyte counts: $10{\cdot}2 \times 10^9$/l, with 22% blasts. The coagulation profile was normal. Bone marrow (BM) was hypercellular, infiltrated by 45% aberrant myeloid precursors. Two leukaemic cell subpopulations were detected in PB and BM, one with a moderate amount of cytoplasm with few myeloperoxidase-positive granules, accounting for 8% and 21% of PB and BM cells respectively. The other subset morphologically resembled promyelocytes, with round nuclei, abundant cytoplasmatic granules, well-defined perinuclear Golgi area, and accounted for 14% and 24% PB and BM cells respectively (Fig 1A,B). Auer rods were absent. Morphological features of myelodysplasia were apparent, with granulocytes displaying reduced granulation, abnormal nuclear segmentation and pseudo-Pelger-Hüet anomaly (Fig 1B). The immunophenotypic analysis identified two cell subsets: one $CD33^+$ $CD13^+$ HLA-DR^- $CD34^-$ $CD15^{+low}$ with high sideward scatter (SSC), and $CD33^+$ $CD13^+$ HLA-DR^+ $CD34^+$ $CD15^-$ with low SSC, accounting for 25% and 13% of the BM cells respectively. Conventional cytogenetics and spectral karyotyping (SKY) were performed on BM aspirates. By G-banding, the karyotype was: 46X, iso(X)(q11), t(8;21)(q22;q12) in 18/18 metaphases (Fig 1C). SKY analysis further revealed a cryptic insertion of chromosome 15 material into chromosome 17 (Fig 1D) in 5/5 metaphases. *AML1/ETO* and *PML/RARα* transcripts were demonstrated by nested

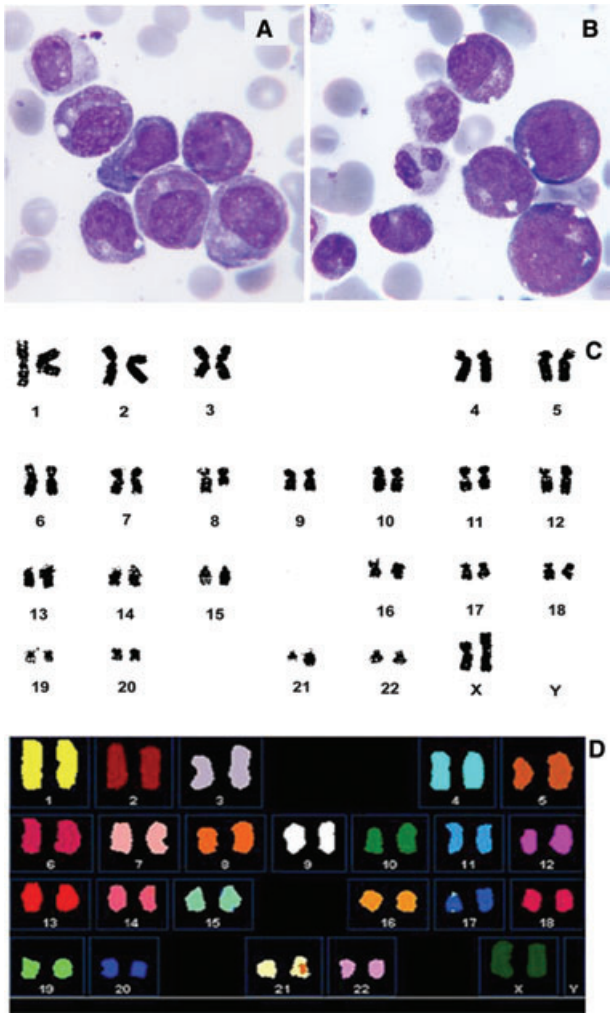

Fig 1. Morphological findings in PB (A) and BM (B) smears (Leishman stain, original magnification: 100×) showing the presence of hypergranular promyelocytes and myeloblasts. Pseudo-Pelger-Hüet anomaly is shown in (B). Conventional cytogenetics (C) of BM aspirates showing 46X, iso(X)(q11), t(8;21)(q22;q12). Spectral karyotyping (D) showing t(8;21) and the cryptic insertion of chromosome 15 material into chromosome 17.

reverse transcription polymerase chain reaction (RT-PCR), performed as described in van Dongen *et al* (1999). Finally, RARα rearrangements were detected by Southern blot analysis performed on genomic DNA digested with HindIII or EcoRI and hybridized with H18 and K3 RARα genomic probes as described by Biondi *et al* (1999). Immunofluorescence staining of BM cytospin preparations using the PG-M3 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) revealed that PML was delocalized from the nuclear bodies, a feature suggestive of APL.

Based on the presence of cytomorphological and molecular characteristics of both APL and AML associated with t(8;21)/*AML1/ETO*, we treated the patient with all *trans* retinoic acid (ATRA), 45 mg/m^2/d, in combination with the standard daunorubicin + cytarabine (so called '3 + 7') AML induction regimen. The patient had not achieved remission after 30 d of continuous ATRA treatment, so the ATRA dose was increased to 90 mg/m^2/d and a second identical course of chemotherapy was administered from day +35. On day +63, the patient was pancytopenic and the BM was hypocellular with <5% of blasts/promyelocytes, however the *PML/RARα* and *AML1/ETO* transcripts were still detectable by RT-PCR. The patient died of sepsis on day +67.

In the present case, the co-expression of AML1/ETO and PML/RARα was demonstrated by SKY in a small number of cells, therefore we cannot exclude the presence of multiple clones. In fact, the immunophenotypic analysis suggested that at least two cell populations were present. In previous reports of AML1/ETO and PML/RARα co-expression was detected either by G-banding (Charin *et al*, 1992; Movafagh *et al*, 1996) or by fluorescence *in situ* hybridization analysis (Varella-Garcia *et al*, 1999) and there was evidence of the presence of two (Varella-Garcia *et al*, 1999) or three (Movafagh *et al*, 1996) distinct leukaemic clones harbouring either or both chromosomal translocations. In the cases reported by Sttavroyianni *et al* (2003) and Bonomi *et al* (1998), the expression of PML/RARα and AML1/ETO was determined by RT-PCR at diagnosis, whereas by conventional cytogenetics only the t(15;17) was detectable. Only the presence of t(8;21)/AML1/ETO was detected by G-banding and RT-PCR at relapse, suggesting that one of the clones was selected during treatment (Bonomi *et al*, 1998; Sttavroyianni *et al*, 2003).

Despite the prolonged use of therapeutic doses of ATRA + chemotherapy, the patient did not achieve remission, in contrast to the observed in the majority of APL and AML patients with t(8;21). As both PML/RARα and AML1/ETO oncoproteins affect transcription by forming repressor complexes containing histone deacetylases, it is formally possible that the expression of both oncoproteins could lead to irreversible chromatin remodelling. The results reported here suggest that PML/RARα and AML1/ETO fusion proteins may mutually affect their pathogenic mechanisms, rendering the cells resistant to treatment.

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