(diagnosed as FA) we observed a significant (p<0.001) accumulation of cells in G2 phase in the following experimental conditions: PHA, PHA+melphalan 0.01, 0.05, 0.1, 0.5 µg/mL (Table 2). Results yielded with melphalan 0.1, 0.05 and 0.01 µg/mL discriminated well between controls and FA patients; establishing a G2% cut-off of 21%; the sensitivity and specificity of the test performed with melphalan 0.1 µg/mL was 100%. The percentage of G2 cells did not correlate with the presence of pancytopenia or the severity of malformations. No significant difference in the percentage of cells in G2 phase was observed between controls and non-FA cytopenias, nor between controls and FA parents (heterozygous). We observed a normalization of G2 phase when patient 7 developed anemia, 2 MDS, 2 acquired erythroblastopenia, 5 acquired anemia, 2 Diamond-Blackfan anemia, 10 acquired neutropenia, 2 MDS, 2 acquired thrombocytopenia, and FA parents. Flow cytometry analysis was performed after exposure to PHA alone or in the presence of 0.01, 0.05, 0.1, 0.5, 1, 2 µg/mL melphalan.

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<td>8±4</td>
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<tr>
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Key words
Fanconi’s anemia, cell cycle analysis, DEB test.

Contributions and Acknowledgments
FT and UR conceived and designed the study. FT wrote the paper and, with NC, carried out the colony assays and cyt fluorimetric analyses. LL discussed and analyzed data. GP performed the DEB tests. UR, PS and FT are the clinicians involved in following the patients. We thank Prof. Giuseppe Basso for his help in flow cytometric analysis.

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References

Near-tetraploid acute myeloid leukemia after allogeneic bone marrow transplantation

Tetraploidy and near-tetraploidy are infrequently observed in acute myeloid leukemia (AML). Several cases have been reported in patients treated for other cancers or after autologous bone marrow trans-
plantation (ABMT). We report the first case of near-tetraploid AML with central nervous system involvement in a female patient who relapsed after allogeneic transplantation for erythroleukemia.

Sir,

A 40-year old female was admitted to our Division complaining of weakness, fever and purpura. Hematologic examination showed severe pancytopenia, with Hb 68 g/L, white blood cells 3.4 x 10^9/L (blasts 11%) and platelets 23 x 10^9/L. Bone marrow aspirate showed 32% blasts and 60% dyserythropoietic erythroblasts. Blasts were of large-medium size with loose chromatin and basophilic, peroxidase-negative cytoplasm. Flow cytometric immunophenotyping demonstrated expression of HLA-DR, CD13, CD33 and GlyA (50% of blasts). Cytogenetic analysis showed a normal female karyotype. A diagnosis of erythroleukemia was made. A complete remission was induced by standard chemotherapy. Allogeneic BMT had to be delayed because of severe Candida tropicalis sepsis. In the first relapse occurring 2 years after diagnosis, allogeneic bone marrow transplantation (BMT) from an HLA identical brother induced a second CR. A second relapse appeared 11 months after the BMT. The bone marrow aspirate showed 34% blasts, most of which were large, sometimes giant, bizarre and binucleated; nuclei were uniformly large, indented or lobulated in some cells, with one or two nucleoli in a rather coarse granular chromatin; cytoplasm was moderately abundant and basophilic with many azurophilic, peroxidase-positive granules, frequently clustered in a paranuclear clear Golgi area (Figure 1, A and B). Immunophenotyping showed positivity for CD33, HLA-DR, GlyA (64% of blasts) and CD71. Cytogenetic analysis showed 20% of cells with a normal male karyotype, while in the remaining 80% hyperdiploid changes were detected, with the chromosome number ranging between 93 and 97 with no preferential losses or gains of chromosomes. Structural abnormalities and Y chromosome were not found in the abnormal metaphases. More than 30 cases of near-tetraploid AML have been reported since 1971. Frequently near-tetraploidy develops as a secondary clonal event resulting from clonal evolution in more differentiated hematopoietic precursors and is commonly associated with M2 or M6 morphology and sometimes with specific karyotypic abnormalities.

Not rarely near-tetraploid AML phenotype after ABMT. Only one case showed the development of tetraploid karyotype after ABMT. Bizarre blast cell morphology can lead to an erroneous diagnosis of lymphoma or metastatic carcinoma. The occurrence of near-tetraploid AML phenotype after allogeneic BMT in our patient probably represents an unusual pattern of secondary clonal evolution, as indicated by the appearance of the abnormal clone almost three years after clinical onset of the disease and one year after allogeneic transplantation, as well as by the karyotype normalization at the third CR. The mutagenic role of chemotherapy in near-tetraploid induction in AML should be more extensively investigated.

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Key words
Near-tetraploidy, acute myeloid leukemia, allogeneic bone marrow transplantation, central nervous system involvement, erythroleukemia.

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References
A case of atypical myelodysplastic syndrome with a novel reciprocal translocation t(1;12)(q21;p13)

Reciprocal translocations as unique primary karyotypic anomalies are not frequently found in myelodysplastic syndromes. We report a case of refractory anemia with excess blasts in transformation (RAEB-t) with total absence of erythroid precursors in bone marrow. Cytogenetic study revealed that all 25 spontaneous metaphases analyzed carried a novel reciprocal translocation t(1;12) (q21; p13) as sole abnormality without detectable TEL (ETV6) gene involvement.

Sir,

A 64-year old man was admitted to hospital because of asthena and fatigue with minor exercise of sudden onset. There was no evidence of exposure to toxic agents and no fever or bleeding were noted. Physical examination showed pallor, without hepatosplenomegaly or lymph node enlargement. The complete blood count was: leukocytes 53\times 10^9/L (differential: 30% neutrophils, 60% myelocytes and metamyelocytes cells and 10% myeloid blasts); hemoglobin: 5.9 g/dL and platelets 57\times 10^9/L. Dysplastic signs were evident in myelocytes and metamyelocytes with intense hypogranular cytoplasm. Bone marrow aspiration showed hypercellularity, scarce micromegakaryocytes, 75% of markedly hypogranular myeloid cells, 22% of blast cells of myeloid appearance with out Auer rods, and 3% of lymphoid cells. Strikingly there was a total absence of erythroid cells. Cytochemical staining of blast cells was positive only for peroxidase and naphthol-AS-D-acetate esterase. Immunophenotypic markers of blast cells were positive for CD13, CD33 and HLA-DR. Conventional cytogenetic analysis of bone marrow showed a unique clonal abnormality with a reciprocal translocation t(1;12) (q21;p13) in all 25 metaphases (Figure 1). DNA and RNA were extracted from bone marrow cells at diagnosis. Southern blot with a specific probe for TEL gene after enzyme digestion with BamHI, HindIII and EcoRI, did not demonstrate that this gene was involved in the translocation. No bcr-abl fusion products were detected using a nested RT-PCR approach. A diagnosis of refractory anemia with blast excess in transformation was established on the basis of the morphologic picture.

Cytogenetic abnormalities can occur in the marrow cells of patients with de novo MDS in approximately 40-60% of cases. Detected by conventional methods, they involve mostly chromosomes 5, 7, 8, 11, 12 and 20, and have been proved to be an independent prognostic factor. Abnormalities of 12p13 in hematologic malignancies result in at least three different molecular changes: deletion of KIP1, amplification of CCND2 and rearrangement of the ETS-like gene TEL. The first description and cloning of TEL gene was carried out in a case of CMML bearing a t(5;12) and the gene has subsequently been identified as a hot spot frequently found in childhood ALL, especially in t(12;21). In MDS, translocations of 12p13 involving the TEL gene, with chromosomes 5, 10 and 3, have been reported previously. All cases displayed atypical signs such as eosinophilia or monocytosis, all carrying a dismal prognosis. Our case of MDS presented with symptoms of acute leukemia, marked normocytic anemia, thrombocytopenia and immature leukocytosis, without eosinophilia or monocytosis but with a striking total absence of erythroid medullar precursors. Despite the involvement of the breakpoint of the TEL gene in our case, and despite using a specific probe for the TEL gene, we were not able to demonstrate the presence of the TEL rearrangement, although we cannot rule out definitively. On the other hand, the breakpoint