typing of leukemias and non-Hodgkin's lymphomas. Immunological markers and their CD codes. *Neth J Med* 1998; **33**: 298–314.
3 Pizzolo G, Vincenzi C, Nadali G, Veneri D, Vinante F, Chilosi M, Basso G, Connelly MC, Janossy G. Detection of membrane and intracellular antigens by flow cytometry following ORTHO PermeaFix™ fixation. *Leukemia* 1994; **8**: 672–676.
4 Syrjala MT, Tiirkainen M, Jansson S-E, Krusius T. Flow cytometric analysis of terminal deoxynucleotidyl transferase. A simplified method. *Am J Clin Pathol* 1993; **99**: 298–303.
5 Horvatinovich JM, Sparks SD, Borowitz MJ. Detection of terminal deoxynucleotidyl transferase by flow cytometry: a three color method. *Cytometry* 1994; **18**: 228–230.
6 Knapp W, Majdic O, Strobl H. Flow cytometric analysis of intracellular myeloperoxidase and lactoferrin in leukemia diagnosis. *Rec Res Cancer Res* 1993; **131**: 31–40.
7 Knapp W, Strobl H, Majdic O. Flow cytometric analysis of cell surface and intracellular antigens in leukemia diagnosis. *Cytometry* 1994; **18**: 187–198.

G-CSF treatment in acute leukemia

TO THE EDITOR

The articles by Terpstra and Lowenberg and Rowe and Liesveld briefly touched on the use of granulocyte colony-stimulating factor (G-CSF) alone in the induction of remission in relapsed patients with myeloid leukemia and myelodysplasia following allogeneic bone marrow transplantation (BMT).[1–2] Encouraging results obtained in a limited number of patients and the changes observed at the cellular level during response might not only make this approach a therapeutic option for patients with leukemic relapse following BMT but also contribute to a better understanding of the biology of this heterogeneous disorder.

A 3-year-old Caucasian female patient was diagnosed with CALLA-positive, B precursor acute lymphoblastic leukemia (ALL) with normal cytogenetics and was treated with chemotherapy between 1985 and 1989. In 1993 she had a systemic relapse with a clonal cytogenetic abnormality [46,XX, der(3) t(1;3;11) (p36;p11;p11), add(2)(q29), del(6)(q15), del(12) (p12)]. At that time blast cells were CD19 and HLA-DR positive, CD10 negative and coexpressed myeloid markers (CD13 and CD33). She achieved and remained in remission with etoposide-containing chemotherapy until she was diagnosed with acute myeloid leukemia (AML) in April 1996. Leukemic cells carried the same cytogenetic abnormality, were Sudan black positive, CD19 positive, CD10 negative and strongly expressed myeloid markers. A fluorescence *in situ* hybridization (FISH) analysis was negative for MLL gene rearrangement.

Following remission induction and consolidation she had a matched unrelated BMT from a male donor in September 1996, conditioned with busulphan, cytarabine and cyclophosphamide. Bone marrow recovery was of donor origin as confirmed by FISH using X and Y chromosome-specific probes. She developed mild skin and gut graft-versus-host disease (GVHD). However, the response was short-lived as she experienced an early post-BMT relapse of recipient origin with the same clonal cytogenetic abnormality and 46% blasts in the bone marrow on day +100 in December 1996.

A second transplant and systemic chemotherapy were refused and after discontinuing cyclosporine she was started on G-CSF, 5 μg/kg/day subcutaneously, according to the report by Giralt *et al*.[3] A dramatic response was observed with remission induction at the end of 3 weeks of treatment with the disappearance of the abnormal clone. FISH analysis of bone marrow cells revealed 100% male cells and she continued to have mild gut GVHD.

She remained in complete clinical remission until May 1997 when she had an isolated left breast recurrence with blast cells carrying the same, biphenotypic surface markers, this time with additional CD10 positivity. Bone marrow cells were shown to be of donor origin by FISH analysis. Breast mass dramatically responded to dexamethasone treatment in a week. The patient had a Karnofsky score of 100%, was induced with chemotherapy and is being prepared for a second transplant.

Several possible explanations have been proposed for G-CSF-induced remission induction and durable remissions in relapsed AML patients following allogeneic BMT.[3] Both decreased and increased levels of endogenous G-CSF have been reported in AML.[4] The decreased levels may represent a negative feedback control mechanism or an inhibition of the hemopoietic inductive microenvironment by the mediators released from leukemic cells and may play a major role in the suppression of the normal hemopoiesis. Given the heterogeneous nature of AML, it may be reasonable to think that some patients with low G-CSF levels may benefit from pharmacological doses of G-CSF. Once normal hemopoiesis is established, suppression of malignant hemopoiesis by physical and biological means, by graft-versus-leukemia effect in post-BMT cases, may ensue. In some patients with myelodysplastic syndromes, G-CSF treatment has been shown to produce a superior stimulatory effect for normal hemopoietic cells resulting in the increase of normal neutrophils.[5] Thus, the selective or superior stimulation of donor hemopoiesis and subsequent inhibition of malignant growth with or without apoptosis-inducing effects of growth factor on the malignant clone seems to be a plausible explanation in our case. A similar response has also been reported in a BCR-ABL-positive ALL patient.[6]

An interesting aspect of our case is the isolated breast relapse while the bone marrow is still of donor origin. This can hypothetically be explained by the scarcity of donor cells in the involved extramedullary tissue, in this case breast, resulting in a lack of local anti-leukemia influence of the donor cells as suggested previously by us.[7] We think that growth factor treatment alone or in combination with immunostimulatory compounds may be an option for selected relapsed leukemia patients following allogeneic BMT.

Correspondence: S Savaşan
Received 11 July 1997; accepted 21 August 1997

S Savaşan[1]
E Abella[1,2]
AJ Akhtar[2]
Y Ravindranath[1]

Barbara Ann Karmanos Cancer Institute and [1]Children's Hospital of Michigan, Division of Hematology/Oncology and [2]Bone Marrow Transplantation Unit, Harper Hospital, Wayne State University, Detroit, MI, USA

References

1 Terpstra W, Lowenberg B. Application of myeloid growth factors in the treatment of acute myeloid leukemia. *Leukemia* 1997; **11**: 315–327.
2 Rowe JM, Liesveld JL. Hematopoietic growth factors in acute leukemia. *Leukemia* 1997; **11**: 328–341.
3 Giralt S, Escudier S, Kantarjian H, Deisseroth A, Freireich EJ, Andersson BS, O'Brien S, Andreeff M, Fisher H, Cork A, Hirsch-Ginsberg C, Trujillo J, Stass S, Champlin RE. Preliminary results of treatment with filgrastim for relapse of leukemia and myelodysplasia after allogeneic bone marrow transplantation. *New Engl J Med* 1993; **329**: 757–761.
4 Shinohara K, Oeda E, Nomiyama J, Inoue H, Kamei S, Tajiri M, Ichikawa T, Kuwaki T, Tachibana K. The levels of granulocyte colony-stimulating factor in the plasma of the bone marrow aspirate in various hematological disorders. *Stem Cells* 1995; **13**: 421–427.
5 Gabrilove JL, Jakubowski A. Granulocyte colony-stimulating factor: preclinical and clinical studies. *Hematol Oncol Clin North Am* 1989; **3**: 427–440.
6 Keil F, Kalhs P, Haas OA, Fritsch G, Lechner K, Mannhalter C, Greinix HT. G-CSF stimulation of donor myelopoiesis prolongs survival of relapsed BCR-ABL-positive acute lymphoblastic leukemia after allogeneic marrow transplantation. *Bone Marrow Transplant* 1996; **18**: 655–657.
7 Savaşan S, Abella E, Karanes C, Ravindranath Y. Recurrent breast relapses in a patient with acute lymphoblastic leukaemia following allogeneic bone marrow transplantation. *Acta Haematol* 1997; **871**.

Acute myeloid leukemia following 3M (mitoxantrone, mitomycin and methotrexate) chemotherapy for advanced breast cancer

TO THE EDITOR

The risk of secondary acute myeloid leukemia (AML) among subjects with breast cancer receiving standard-dose cyclophosphamide and doxorubicin has been reported equivalent to that of the general population.[1] Recently an increased frequency of therapy-related leukemias has been documented, paralleling the more widespread use of dose-intensive therapies and the longer survival of cancer patients.[2]

Alkylating agents[3] or more recently, epidophyllotoxins-related[4] secondary AML have been described, but also growth factors or different drug combinations seem to affect cellular proliferation, especially in subjects with genetic susceptibility.[5]

Because of the effectiveness of chemotherapeutic agents such as topoisomerase II inhibitors (epidophyllotoxins, anthracyclines and their derivates) it is important to define their leukemogenic potential in patients with advanced diseases and mainly in adjuvant regimens for early cancer.

We describe three cases of secondary AML observed in the last year in our Institution, which occurred in women treated with 3M (methotrexate, mitoxantrone, mitomycin) regimen for advanced breast cancer.

Clinical and biologic features are described and the possible relationship between these drugs and the onset of acute leukemia is discussed.

Three women with a history of advanced breast cancer treated with mitoxantrone (8 mg/m^2 intravenously every 3 weeks), mitomycin (8 mg/m^2 intravenously every 6 weeks) and methotrexate (30 mg/m^2 intravenously every 3 weeks) (3M) were admitted in 1996 to our Department for acute leukemia. Clinical characteristics of the patients are shown in Table 1.

Patient No. 1, a 44-year-old woman, was well until 4 years earlier, when breast cancer occurred. She was treated with radical surgery plus six courses of cyclophosphamide, methotrexate and 5-fluorouracil (CMF). One year after the initial diagnosis, she had recurrence of the disease with bone metastases and was treated with six courses of 3M followed by medroxyprogesteron acetate (MAP) and radiation therapy (RT) on the bone lesions. About 6 months from the start of 3M a decrease of white blood cells and platelets count was noted. Eighteen months later she was admitted to our Department because of a hemorrhagic syndrome: the bone marrow examination revealed AML M0 according to FAB criteria. The patient died 4 days later.

Patient No. 2, a 60-year-old woman, had a history of breast cancer treated with radical surgery at 50 years. Six years later, bone metastases developed and she was treated with eight courses of CMF and RT to lesions of the skeleton. At the age of 58, the disease progressed and she was therefore treated with seven courses of 3M followed by MAP. One year after the end of the therapy, piastrinopenia and hemorrhagic syndrome occurred: bone marrow examination disclosed acute promyelocytic leukemia. All-*trans* retinoic acid therapy was started and at +10 months from diagnosis the patient is alive and well.

Patient No. 3 was a 52-year-old woman. She was well until 10 years earlier when she underwent radical surgery and breast RT for breast cancer. Thirty months before admission, the patient received five courses of 3M followed by tamoxifen for disease recurrence to skeleton and skin. After that a progressive anemia and leucopenia developed: bone marrow examination revealed refractory anemia. Transfusional therapy was prescribed. Two weeks before admission acute monocytic leukemia developed; the patient was treated with

Correspondence: LMA Melillo
Received 20 February 1997; accepted 5 September 1997