TABLE I. Some Hematologic Data on Patient

	Age (years) and sex	Hb g/dl	HbS + HbA_2 (%)	HbF (%)	Genotype
Before transplantation					
Proposita	7, F	8.2	90	10	SS
Mother	38, F	12.3	36.8	0.5	AS
Father	40, M	14.5	37.3	0.8	AS
Sister	10, F	12.1	2.7 (Hb A_2)	0.5	AA
Posttransplantation (Proposita)					
Second month		10	10.5	1.2	AS
Third month		9	7.5	2.0	AS
Second year		10.5	8.0	0.6	AS
Third year		10.5	14.0	0.3	AS

published studies, engraftment and mixed chimerism were detected by using the hypervariable tandem repeat region of DNA [5]. This technique was not informative in some patients because the donor and recipient were usually close relatives. This study indicated that mixed chimerism and engraftment after bone marrow transplantation in patient with hemoglobinopathies associated mutations in beta globin gene region could be studied easily by DNA analysis. The ARMS technique is extremely quick and simple. This type of study would be especially useful in thalassemia, in which there is no abnormal hemoglobin to be produced.

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References

1. Blume KG, Beutler E, Bross KJ, Schmidt GM, Spruce WE, Tebliz RG: Genetic markers in human bone marrow transplantation. Am J Hum Genet 32:414–419, 1980.
2. Nesci S, Manna M, Andreani M, Fattorini P, Graziosi G, Lucarelli G: Mixed chimerism in thalassemic patients after bone marrow transplantation. Bone Marrow Transplant 10:143–146, 1992.
3. Ginsburg D, Antin JM, Smith BR, Orkin SH, Rappaport JM: Origin of cell population after bone marrow transplantation. Analysis using DNA sequence polymorphism. J Clin Invest 75:596–603, 1985.
4. Old JM, Varawalla N, Weatherall DJ: Rapid detection and prenatal diagnosis of beta thalassemia studies in Indian and Cypriot populations in the UK. Lancet 336:834–836, 1990.
5. Vermylen CH, Cornu G, Philippe M, Ninane J. Borja A, Latinne D, Ferrant A, Michaux JL, Sokal G: Bone marrow transplantation in sickle-cell anaemia. Arch Dis Child 66:1195–1198, 1991.

Itraconazole and Retinoid Resistance

To the Editor: Recent clinical studies have shown a high proportion of patients with acute promyelocytic leukemia (APL) achieve complete remission (CR) after treatment with all-*trans* retinoic acid (RA) [1,2]. Nevertheless, most patients who receive continuous RA treatment ultimately relapse and developed RA-resistant disease [3]. Clinical resistance to RA may develop and pose a serious problem for differentiation-inducing therapy. Although there are several strategies for overcoming RA resistance in APL patients, these approaches have been largely unsuccessful in overcoming RA resistance in vivo. We have treated an RA-resistant APL patient with a combination of all-*trans* RA and itraconazole, and have achieved a good clinical response.

A 30-year-old Japanese woman was diagnosed with APL in 1991 on the basis of morphology and the presence of chromosomal abnormality, t(15;17). Molecular analysis revealed a PML-RAR fusion transcript, and Southern blot analysis demonstrated the RAR-α gene rearrangement in her blast cells. She achieved CR with combination chemotherapy consisting of daunorubicin, cytarabine, 6-MP, and prednisolone. A year later, she relapsed. A second short CR was achieved after another course of chemotherapy, but she relapsed again. On second relapse, all-*trans* RA (60 mg per day orally) was commenced, and subsequently the dose of all-*trans* RA was doubled. However, RA failed to induce differentiation of APL cells. In addition, the cells were resistant to various anticancer drugs. Finally, she was treated with all-*trans* RA, 120 mg daily, plus 200 mg of itraconazole. Her peripheral blood leukocytes (WBC) and the percentage of blast cells gradually decreased, and her blast cells showed differentiation into mature granulocytes, indicating a good clinical response (Fig. 1).

Since all-*trans* RA can be catabolized by cytochrome P450 oxidative enzymes, treatment with cytochrome P450 inhibitors, such as ketoconazole and liarozole, has been used to reduce accelerated catabolism of RA and increase mean plasma all-*trans* RA concentrations in patients with APL [4]. However, to date, these approaches have failed to overcome RA resistance in vivo. Itraconazole, a triazole antifungal agent, inhibits cytochrome P450

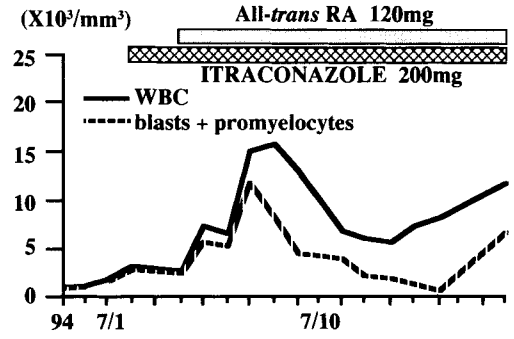

Fig. 1. Peripheral blood leukocytes (WBC) and blast cells from a patient with acute promyelocytic leukemia (APL) during treatment with all-*trans* retinoic acid (RA) and itraconazole.

enzyme activity as well as cell membrane sterol biosynthesis, thereby inhibiting the function of P-glycoprotein, an energy-dependent drug efflux pump, which decreases the intracellular accumulation of RA [5]. Thus, itraconazole would be a potentially useful drug for reversing in vivo resistance to RA, and our observations support this contention. The synergy between itraconazole and all-*trans* RA may overcome RA resistance by altering the metabolism of retinoids.

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References

1. Huang ME, Ye YC, Chen SR, Chai JR, Lu JX, Zhao L, Gu HT, Wang ZY: Use of all-*trans* retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72:567–572, 1988.
2. Castaigne S, Chomienne C, Daniel MT, Ballarini P, Berger R, Fenaux P, Degos L: All-*trans* retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. Blood 76:1704–1709, 1990.
3. Warrell RP Jr, de The H, Wang ZY, Degos L: Acute promyelocytic leukemia. N Engl J Med 329:177–189, 1993.
4. Rigas JR, Francis PA, Muindi JRF, Kris MG, Huselton C, DeGrazia F, Orazem JP, Young CW, Warrell RP Jr: Constitutive variability in the pharmacokinetics of the natural retinoid, all-*trans*-retinoic acid, and its modulation by ketoconazole. JNCI 85:1921–1926, 1993
5. Gupa S, Kim J, Gollapudi S: Reversal of daunorubicin resistance in P388/ADR cells by itraconazole. J Clin Invest 87:1467–1469, 1991.

Allogeneic Hemopoietic Progenitor Cells Transplantation for Late Graft Failure

To the Editor: Recent articles [1,2] have focused attention on peripheral blood as an alternative source of hemopoietic progenitors cells (HPCs) for allogenic transplantation. Our experience in mobilization of HPCs in normal donors, and their subsequent use in the allogeneic setting, includes a 52-year-old chronic myelogenous leukemia (CML) patient developing late graft failure 7 months after his first transplantation from his HLA-identical sister. He was conditioned with BUCY2 protocol, and GVHD prophylaxis consisted of short-course MTX and cyclosporine. No GVHD was observed. Severe pancytopenia developed by day 210. Bone marrow biopsy and aspirate were conclusive for late graft failure, as confirmed by host origin of peripheral blood lymphocytes. G-CSF and donor peripheral blood progenitor cells (PBPCs), collected after G-CSF, were administered with no benefit. A second transplant was then planned, after total doses of ATG, 90 mg/kg, and cytoxan, 200 mg/kg over 4 days, cyclosporine (3 mg/kg/day iv) was started on day −1. G-CSF, 16 μg/kg, as administered subcutaneously to the donor for 3 consecutive days. A total of 7.3×10^6/kg CD34+, 5.6×10^8/kg MNC, 2.5×10^8/kg CD3, and 4.6×10^4/kg CFU-GM was reinfused. G-CSF, 5 μg/kg, was administered after transplantation. Until neutrophil recovery, absolute neutrophil count (ANC) $> .5 \times 10^9$/l was achieved by day +9, and platelet counts $> 50 \times 10^9$/l and $> 100 \times 10^9$/l by days +28 and +36, respectively, Bone marrow examination on day +21 showed trilineage engraftment and no evidence of recurrent disease. Cytogenetic analysis showed a normal 46, XX karyotype. Acute GVHD grade III of liver and gastrointestinal tract developed by day +14 and was successfully treated with PDN, 2 mg/kg until day +19. A new flare-up of GVHD developed during the tapering off of prednisone, this time refractory to prednisone increment and to ATG and OKT3 monoclonal antibody treatment. The patient died of aGVHD and disseminated aspergillus infection on day +93 after his second transplantation.

Allogeneic transplantation using mobilized peripheral blood HPC from HLA identical donors is technically feasible [3,4]. The use of growth factor-like G-CSF induces a foreseeable increment of hematopoietic progenitor cells and therefore a simple harvest with a single leukapheresis procedure [1]. More than 100 allogeneic transplantations using peripheral blood HPC have already been performed in Europe [5]. Preliminary data show an accelerated hemopoietic recovery and no substantial increase in GVHD occurrence. A number of questions concerning the incidence of GVHD and graft failure, both related to the lymphocytic component of the graft, still remain unanswered, and these questions call for an urgent, controlled multicenter trial before a widespread, undiscriminating use of peripheral blood HPC in the allogeneic setting should take root.

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References

1. Lane TA, Law P, Maruyama M, Young D, Burgess J, Mullen M, Mealiffe M, Terstappen LWMM, Hardwick A, Moubayed M, Oldham F, Corringham RET, Ho AD: Harvesting and enrichment of hematopoietic progenitor cells mobilized into the peripheral blood of normal donors by granulocyte-macrophage colony-stimulating factor (GM-CSF or G-CSF: Potential role in allogeneic marrow transplantation. Blood 85:275, 1995.
2. Andrews RG, Briddell RA, Knitter GH, Rowley, Appelbaum FR, McNiece IK: Rapid engraftment by peripheral blood progenitor cells mobilized by recombinant human granulocyte colony-stimulating factor in non-human primates. Blood 85:15, 1995.
3. Weaver CH, Buckner CD, Longin K, Appelbaum FR, Rowley S, Lilleby K, Miser J, Storb R, Hansen JA, Bensinger W: Syngeneic transplantation with peripheral blood mononuclear cells collected after the administration of recombinant human granulocyte colony-stimulating factor. Blood 82:1981, 1993.
4. Laporte JP, Lesage S, Douay L, Lopez M, Isnard F, Norol F, Najman A, Gorin NC: Allogeneic transplantation with peripheral blood hematopoietic progenitors mobilized with G-CSF. Blood 84:347, 1995 (abstract).
5. Bacigalupo A, Majolino I, Van Lint MT, Vasta S, Valbonesi M, Lercari G, Carlier P, Occhini D, Gualandi F, Lamparelli T, Bregante S, Valeriani A, Piaggio G, Pitto A, Benvenuto F, Figari O, Marmont AM: Transplantation of rh-G-CSF mobilized allogeneic peripheral blood cells from HLA identical siblings. Bone Marrow Transplant 15 [Suppl]:44, 1995 (abstract).