To the editor:

Reduced-intensity versus conventional myeloablative conditioning for patients with Philadelphia chromosome–negative acute lymphoblastic leukemia in complete remission

We read with interest the results of the comparison between reduced-intensity conditioning (RIC) and conventional myeloablative conditioning (MAC) allogeneic stem cell transplantation (allo-SCT) for patients with acute lymphoblastic leukemia (ALL) in complete remission (CR), reported by Mohty et al.[1] They concluded that RIC allo-SCT was a potential therapeutic option for ALL.

Although we agree in general with their conclusion, our major concern is that the cytogenetic background between MAC and RIC might differ. Adjustment for cytogenetic risk groups was not performed in a multivariate analysis, because there was no difference in the cytogenetic distribution between MAC and RIC when analyzed among 3 risk groups: t(9;22), t(4;11), or other ($P = .10$). However, when analyzed among 4 groups, including NA/failed as one group, a significant difference was noted between MAC and RIC ($P = .02$). In fact, the number of Philadelphia chromosome–positive [Ph^+] ALL was smaller in MAC than in RIC [104/449 (23%) vs 41/127 (32%), $P = .049$]. Since the relapse incidence (RI) was higher among Ph^+ ALL patients than in the whole study population (40% ± 5% vs 31% ± 2% in MAC, and 49% ± 9% vs 47% ± 5% in RIC), lower RI in MAC might be associated with a smaller number of Ph^+ ALL. Allo-SCT has been recognized as the only curative therapy for Ph^+ ALL,[2] and there are already several reports of RIC allo-SCT for Ph^+ ALL.[3,4] It may be better to treat Ph^+ ALL and Philadelphia chromosome-negative (Ph^-) ALL as different diseases because their treatment would differ in an era of tyrosine kinase inhibitors. Therefore, it would be more practical to present data only from patients with Ph^- ALL.[5]

The results of our 121 HLA-matched allo-SCT for adult Ph^- ALL in first (81 MAC, 21 RIC) or second (14 MAC, 5 RIC) CR for patients aged ≥ 45 years (between 1998 and 2007 using the Japan Society for Hematopoietic Cell Transplantation and the Japan Marrow Donor Program database) were comparable between MAC and RIC (Figure 1A-D). In a multivariate analysis, RIC was not a significant risk factor for relapse (Hazard ratio [HR] 1.66, 95% confidence interval [CI] 0.63-4.37, $P = .30$). The variables considered in our multivariate analyses were conditioning (MAC vs RIC), age (> 50 years vs ≤50 years), sex, white blood cell counts (< 30 000/μL vs ≥30 000/μL), lineage (T vs B), disease status (first vs second CR), donor source (sibling vs unrelated), and graft-versus-host disease prophylaxis (cyclosporine-based vs tacrolimus-based). Similarly, RIC posed no significant risk factor for leukemia-free survival (HR 1.00, 95% CI 0.51-1.96, $P = .99$), nonrelapse mortality (HR 1.05, 95% CI 0.53-2.05, $P = .89$), and overall survival (HR 1.06, 95% CI 0.64-2.07, $P = .87$).

There are several ways to deal with missing data.[6] Given that the cytogenetics of 54% (244/449) for MAC and 43% (55/127) for RIC were missing in the study by Mohty et al, differences in how to handle missing data may produce different results. Because there was a difference in the cytogenetic distribution between MAC and RIC when analyzing missing data as one category, data adjusted for cytogenetic risk groups, or those of Ph^- ALL, are of considerable interest.

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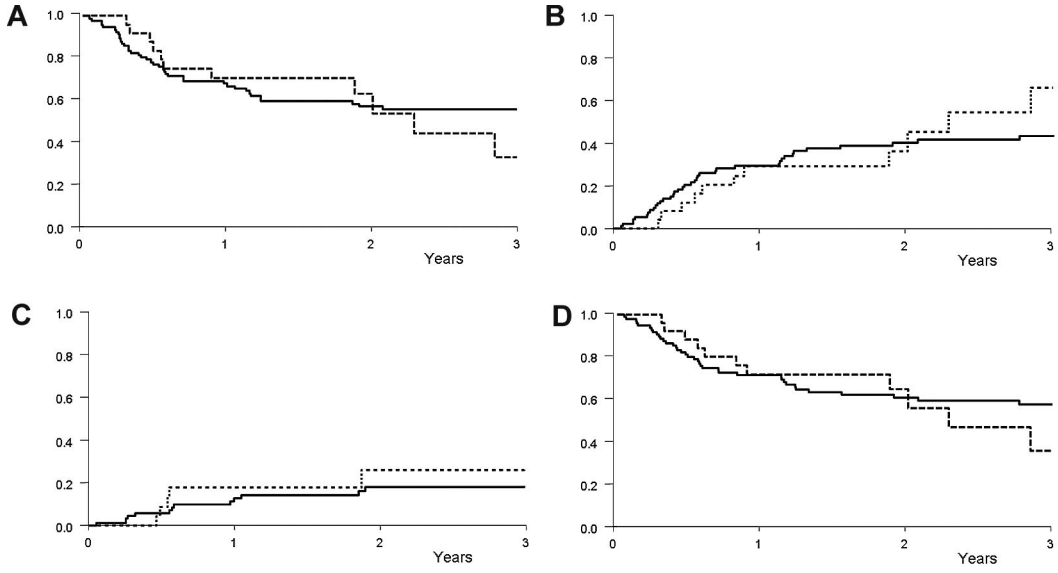

Figure 1. Survival probabilities. (A) Leukemia-free survival according to conditioning regimen: 58% ± 5% in myeloablative conditioning (MAC) vs 63% ± 11% in reduced-intensity conditioning (RIC) at 2 years ($P = .90$). (B) Nonrelapse mortality according to conditioning regimen: 40% ± 5% in MAC vs 36% ± 11% in RIC at 2 years ($P = .79$). (C) Relapse incidence according to conditioning regimen: 18% ± 5% in MAC vs 26% ± 11% in RIC at 2 years ($P = .27$). (D) Overall survival according to conditioning regimen: 59% ± 5% in MAC vs 63% ± 11% in RIC at 2 years ($P = .82$). Solid curve indicates MAC; dashed curve, RIC; x-axis, years after transplantation; and y-axis, probability.

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Contribution: S.N., Y.I., and K.M designed the research; S.N., Y.I., and R.S. performed the statistical analysis and interpreted the data; M.I., H.T., and K.H. provided the data of patients; K.K., and R.S. collected the data of patients; and S.N., Y.I., and R.S. wrote the manuscript.

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To the editor:

Dysregulation of the HIF pathway due to *VHL* mutation causing severe erythrocytosis and pulmonary arterial hypertension

Hereditary erythrocytosis can be caused by mutations in genes involved in the hypoxia-inducible factor (HIF) pathway.[1-3] For example, Chuvash polycythemia is caused by an R200W substitution in the von Hippel–Lindau protein (VHL).[1] There is increasing evidence linking VHL-HIF dysregulation to altered vascular physiology, and a mouse model of Chuvash polycythemia develops pulmonary arterial hypertension (PAH).[4-6] Recently, we reported an autosomal dominant erythrocytosis associated with an activating *EPAS-1* (*HIF-2A*) mutation in which there was late-onset PAH in some family members.[7] We now report a patient with severe erythropoietic dysregulation and PAH who is a compound heterozygote for novel *VHL* mutations.

A 2-month-old boy presented with increasing dyspnea and hypoxia requiring emergency ventilation and inotropic support. Echocardiography showed right ventricular dysfunction and hypertrophy. Severe PAH was confirmed by cardiac catheterization. Pulmonary artery systolic pressure was 91 mm Hg (approximately twice systemic values). Infusions of nitric oxide, prostacyclin, and sildenafil were required to allow discontinuation of ventilation. Treatment with vasodilators, diuretics, and bosentan was continued on eventual discharge from hospital.

Consistently raised hemoglobin (Hb) concentrations (> 21 g/dL) prompted further investigation. Serum erythropoietin (EPO) concentration was grossly elevated at 4120 IU/L. Diagnostic imaging and selective venous sampling provided no evidence of an EPO-secreting lesion. We hypothesized that this unusual phenotype was explicable by congenital dysregulation of the HIF pathway. Gene sequencing revealed heterozygous mutations in exon 2 (376 G>A) and exon 3 (548 C>T) of *VHL* (Figure 1A), predicting the amino acid changes Asp126Asn (D126N) and Ser183Leu (S183L), respectively.

To examine the functional consequences of the mutations, VHL-null renal carcinoma cells were transfected to generate cell pools stably expressing wild type (WT) or mutant proteins (Figure 1C). Function was assessed by measurement of the pH of cell culture media. Impaired or absent VHL function results in more rapid acidification because of HIF-mediated enhancement of glycolysis and suppression of mitochondrial respiration.[8,9] As expected, expression of WT VHL increased media pH while an inactivating VHL mutation (N78S) had no effect. In contrast, each of the D126N and S183L mutants exhibited an intermediate effect (Figure 1B). Pools expressing mutant proteins consumed more glucose and produced more lactate compared with WT, consistent with enhanced glycolytic metabolism (Figure 1B). To confirm that D126N and S183L mutations impair the ability of VHL to regulate HIF, we examined HIF-1α protein levels (Figure 1C) and the expression of HIF target genes *PHD3* and *GLUT-1* (Figure 1D), all of which were elevated in comparison to WT.

Thus, our patient has compound heterozygosity for novel mutations in VHL, which impair the ability to regulate HIF. Strikingly, EPO levels are greatly in excess of those observed in previous patients with inherited VHL-HIF dysfunction, suggesting that this patient has a more severe defect in HIF regulation. We observed that D126N and S183L were expressed at lower levels in transfected cells compared with WT. Because stably transfected cell pools exhibit a range of expression of the introduced protein, we examined this in multiple clonal sublines, with similar results. We hypothesized that this could reflect intrinsic differences in the