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Response

CEBPA promoter hypermethylation in a subset of myeloid/T-lymphoid leukemias with a distinct gene expression profile

With great interest we have read the letter by Terriou and coworkers, who have performed an extensive survey of *CEBPA* CpG promoter hypermethylation in T-ALL as well as in a selection of immature acute myeloid leukemia (AML) cases. This study relates to our previous work in which we identified hypermethylation of the proximal *CEBPA* promoter in a small subset of AMLs with low *CEBPA* mRNA.[1] It should be noted that the patient group we described was not primarily defined by *CEBPA* promoter hypermethylation. Instead, those cases were studied because they exhibited a unique gene expression profile. In addition to this most discriminating feature, the leukemic blasts coexpressed CD34 and T-cell antigens with myeloid markers, frequently carried *NOTCH1* mutations, and expressed the transforming gene *TRIB2*.[1,2] Using a predictive gene expression signature in an independent cohort of AML, we identified genetically and immunophenotypically similar cases with silenced *CEBPA*.

In the present study, Terriou et al found *CEBPA* promoter hypermethylation in 4/54 AML cases. These 4 cases all revealed one or more T-cell characteristics (cCD3, CD7, and/or *TCRG* rearrangement) and may therefore resemble the leukemias we described. In T-ALL, the investigators found *CEBPA* promoter hypermethylation more frequently, in 37/99 cases. Importantly, however, only a minority of those T-ALLs with methylated *CEBPA* coexpressed myeloid surface markers (CD13 and/or CD33) and CD34. The findings of Terriou et al therefore indicate that *CEBPA* proximal promoter hypermethylation in combination with an immature myeloid/T-lymphoid immunophenotype is generally rare in acute leukemia, and can be found in a small percentage of AML as well as in a small fraction of T-ALL. It will be important to apply gene expression profiling to these cases to fully assess their relationship with the leukemias that we described previously.

Together, these observations highlight the challenges in classifying particular acute leukemias as AML, ALL, or a unique entity. We believe that studies as the one performed by Terriou et al will be instrumental in obtaining a better understanding of this topic. Gene expression profiling applied to these AML and T-ALL cohorts will clearly be of added value. Combining data from multiple research groups will be a requirement to address important questions regarding prognosis, and should help to evaluate according to which protocols these relatively small patient groups are best treated.

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To the editor:

JAK2-V617F–triggered preemptive and salvage adoptive immunotherapy with donor-lymphocyte infusion in patients with myelofibrosis after allogeneic stem cell transplantation

Primary myelofibrosis is a myeloproliferative disease, and results of conventional treatment remain unsatisfactory.[1,2] Allogeneic stem cell transplantation after dose-reduced conditioning has become a reasonable, curative treatment option.[3,4] Single case reports about successful donor lymphocyte infusion (DLI) for relapsed patients provided evidence of a graft-versus-myelofibrosis effect.[5-7] Here, we report on 17 patients with either myelofibrosis (n = 16) or secondary AML post myelofibrosis (n = 1) and a median age of 52 years (range, 32-63 years) who received DLI from related (n = 5) or unrelated (n = 12) donor, either for clinical relapse (salvage DLI; n = 9) or residual disease monitored by JAK2 mutation level in peripheral blood (preemptive DLI; n = 8). Details are summarized in Table 1. One patient (no. 9) received DLI twice: once for molecular residual disease and once for reappearance of molecular disease. Sixteen patients were JAK2-V617F–positive. The median time from transplantation to first DLI was 269 days (range, 127-1570 days). The median percentage of JAK2V617 mutation level in peripheral blood before first DLI was 6.2% (range, 0.2%-72.8%) and significantly higher in patients with clinical relapse than with molecular relapse (24.7% vs 0.37%; $P = .03$). The median cell dose of the first DLI was 10^6 $CD3^+$ cells per kilogram of body weight (BW; range, 0.5-9 $\times$ 10^6 cells/kg BW). Two patients received as first DLI CD4-selected T cells (5×10^6 $CD4^+$ cells/kg BW). Second and subsequent half-log–increased DLI were given if no graft-versus-host disease (GVHD) and no significant response were observed. The median interval between the first and the second DLI was 103 days (range, 43-661 days). The response to DLI for relapsed patients was determined by the response criteria of the International Working Group,[8] and for residual disease by quantitative real time JAK2-V617F polymerase chain reaction (PCR) performed from genomic DNA from peripheral blood as recently described.[9] The sensitivity

Table 1. Results of preemptive and salvage DLI, respectively, for patients with myelofibrosis

Patient no.	Sex	Age, y	Donor	Reason for DLI	No. of DLIs	GVHD	Other complications	Best response	Further treatment	Actual status	DLI dose, per kg BW
1	m	51	MUD	Relapse	2	none	none	mCR	none	mCRat 18 mo+	1-6 × 10^6CD3$^+$
2	m	32	MUD	Relapse	3	none	none	mCR	none	mCR 3 mo	5 × 10^5-1 × 10^7 CD3$^+$
3	f	51	MUD	Relapse	4	acute (grade III), chronic (extensive)	aplasia	CR*	none	CR 29 mo+	1 × 10^6-1.3 × 10^8 CD3$^+$
4	m	55	MUD	MRD	1	none	none	mCR	none	mCR 31 mo+	1 × 10^6 CD3$^+$
5	m	49	MUD	Relapse	4	none	none	SD	2nd SCT	mCR 34 mo+	1 × 10^6-4 × 10^7 CD3$^+$
6	m	59	MUD	Relapse	2	none	none	SD	2nd SCT	died (relapse) 18 mo after 2nd SCT	1 × 10^6 CD3$^+$
7	f	34	MUD	MRD	1	none	none	mCR	none	mCR 6 mo+	1 × 10^6 CD4$^+$
8	f	63	MUD	Relapse	1	none	none	SD	2nd SCT	CR† 19 mo after 2nd SCT	1 × 10^6 CD3$^+$
9a (9b)	m	54	REL	MRD	1 (3)	chronic (limited)	none	mCR	none	mCR‡ 8 mo+	1 × 10^6 CD3$^+$; 1 × 10^6-5 × 10^6 CD3$^+$
10	m	54	MUD	Relapse	3	none	none	PD	2nd SCT	mCR 4 mo+ after 2nd SCT	1 × 10^6-9 × 10^6 CD3$^+$
11	f	44	REL	MRD	1	none	none	mCR	none	mCR 10 mo+	5 × 10^6 CD3$^+$
12	m	47	MUD	MRD	1	none	none	mCR	none	mCR 5 mo+	5 × 10^5 CD3$^+$
13	f	48	REL	Relapse	3	none	none	Ci	2nd SCT	mCR 3 mo+	1 × 10^6-1 × 10^8 CD3$^+$
14	f	53	MUD	MRD	2	none	none	mCR	none	mCR 18 mo+	1 × 10^6 CD3$^+$
15	f	60	REL	Relapse	1	acute (III)	aplasia	mCR	none	mCR 6 mo+	5 × 10^6 CD4$^+$
16	m	50	REL	MRD	1	none	none	mCR	none	mCR 2 mo+	3 × 10^6 CD3$^+$
17	m	63	MUD	MRD	1	acute (grade I), chronic	none	mCR	none	mCR 7 mo+	5 × 10^5 CD3$^+$

CR indicates complete remission; mCR, molecular complete remission; Ci, clinical improvement; SD, stable disease; PD, progressive disease; REL, relative donor; MUD, matched unrelated donor; DLI, donor-lymphocyte infusion; GVHD, graft-versus-host disease; SCT, stem cell transplantation; and MRD, molecular residual disease.

*No molecular marker; complete remission according to IWG consensus creiteria and 100% donor chimerism with quantitative PCR.

†Clinical CR on molecular basis, residual disease.

‡After first DLI.

of this quantitative PCR is 0.01%. For one patient without JAKV617F mutation molecular remission was determined by 100% donor chimerism with quantitative PCR using genetic polymorphisms.

No treatment-related mortality after DLI was observed. Acute GVHD grade II through IV was seen in 3 patients (18%), which occurred only in the salvage DLI cohort. In this cohort, complete remission was seen in 4 patients (44%), which was complete on molecular level in 3 of them. In 2 of them complete remission was associated with acute GVHD (grade III). All other patients with clinical relapse (n = 5) developed no GVHD, but best response was only clinical improvement or stable disease. In contrast to salvage DLI, all patients who received preemptive DLI responded with complete molecular remission (100%), and none of these patients developed any signs of grade II through IV acute GVHD. Only in one patient was mild chronic GVHD of the liver noted. The median time to achieve complete remission was 79 days (range, 31-495 days). The overall rate of complete molecular remission rate was 68%, which was higher in the preemptive group than in the salvage group (100% vs 44%; $P = .04$).

This report provided evidence for a strong donor T cell–mediated graft-versus-myelofibrosis effect. Adoptive immunotherapy for molecular residual disease monitored with highly sensitive PCR for JAK2-V617F mutation seems to be more effective and less toxic than using donor lymphocyte infusion for clinical relapse and should be implemented in further clinical trials.

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Contribution: N.K. conceived the idea and study proposal and wrote the manuscript; H.A. and A.B. performed quantitative JAK2V617 PCR; E.K. and F.A. provided study material and analyzed data; N.K., U.B., B.F., and A.Z. analyzed and interpreted JAK2V617 PCR results; O.B. and M.K. performed bone marrow histology examination; Y.H. provided JAK2 analysis; and all authors reviewed and approved the manuscript.

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To the editor:

Older age does not influence allogeneic peripheral blood stem cell mobilization in a donor population of mostly white ethnic origin

Several factors that affect peripheral blood stem cell (PBSC) mobilization were recently identified in a large cohort of healthy donors; factors included ethnic origin, weight, and the total dose of rhG-CSF received for mobilization.[1] However, the median age of this population was 40 years, which does not necessarily reflect current practices in allogeneic stem cell transplantation since the decrease of transplant-related mortality associated with the use of reduced-intensity conditioning (RIC) regimens has led to offering this therapeutic modality to older patients, and in turn soliciting older related donors. The question whether age has a negative impact on mobilization remains controversial.[1-5]

We retrospectively evaluated 129 consecutive related adult donors who underwent PBSC mobilization and collection at the Institut Paoli-Calmettes (Marseille, France) between January 2005 and December 2007. Median age was 51 years (range, 19-70 years) among 44 donors aged 55 years or older. All donors received 4 to 5 days of G-CSF (filgrastim; Amgen, Thousand Oaks, CA) given once daily in the evening, with the first leukapheresis initiated on the morning of day 5. Although the recommended dose for rhG-CSF is 10 μg/kg per day for stem cell mobilization, most donors received a total dose of rhG-CSF that was rounded to the lowest multiple of 300 μg, thus allowing the daily use of a whole number of vials: as a consequence, the median received dose per kilogram of body weight of rhG-CSF was 8.9 μg, which may contribute to the lower number of circulating $CD34^+$ cells observed in our cohort of patients (59.5/μL), compared with the recently published cohort of North American donors (84/μL).[1]

To identify factors affecting circulating $CD34^+$ cell counts as a surrogate maker for stem cell mobilization, we first performed a univariate analysis, including age, sex, weight, height, total G-CSF dose, and G-CSF dose per kilogram of body weight. Ethnicity was not tested as an explanatory factor for stem-cell mobilization, because our cohort of donors was smaller and more homogeneous, mostly with individuals of white descent. Variables associated with higher post-G-CSF $CD34^+$ cell counts were donor weight ($P < .003$) and the total dose of G-CSF