Patient	Transfusion number	Days after transfusion											
		0	1	2	3	4	5	6	7	8	9	10	11
(a) Transfusions of HPA-1a/5b negative platelets													
1	1	155	94	50						55			
2	1	136	105	106		111							
3	1	78			61	45							
4	1		118	122			152						
5	1		78		95								
6	1		216	157	134			167					
7	1	143		156									
8	1	154	62	105	142	181	200						
9	1	71	76		21								
9	2		215		73								
9	3			179									
10	1		69		82							3	
10	2	44			11								
10	3			25									
10	4					98		52		64		95	
11	1	7											
11	2	40	111	32	72		161						166
12	1	113											
12	2	207			235								
13	1	171			141								
14	1		164	133	105	159							381
15	1	170	206	109	161	340							
16	1		149	98	101	78		59		90			
17	1	142	120	101			163			187			
18	1		214	139	95		74		13				
18	2						58			78		185	
19	1	115	139	137		224							
20	1		0										
20	2		492					304		350			
Mean increment		116	146	111	84	62	66	56	13			3	
SD		57	105	49	41	23	11	5					
(b) Transfusions of random platelets													
21	1		217	100	206	282							
22	1	45	10										
23	1		159	186		287							
24	1		189	168	190		228	268					
25	1	74	46	15	8	8	3	34	81	101			
28	1		12	32	63		97						
18	1		21	4	9								
19	1	43											
20	1	108	42										
Mean increment		68	87	72	8	8	3						
SD		30	86	77									

Figure 2. Platelet increments in response to individual platelet transfusions. The platelet increments ($\times 10^9$/L; ie, platelet counts after transfusion minus the pretransfusion count) are given for each transfusion. In the absence of further transfusions, increments that are rising are presumed to have a contribution from fetal platelet regeneration and are not included in the calculations of mean increments; these cells are shaded gray.

The transfusion of random platelets did result in a rise in platelet count (mean increments: 68×10^9/L and 87×10^9/L at day 0 and day 1, respectively), but this was significantly and consistently less over the lifespan of the transfused platelets than the mean increment seen with AC-Tx (116×10^9/L and 146×10^9/L on days 0 and 1, respectively). More importantly, the survival of the random platelets was only approximately half that of antigen-compatible platelets ($T_{1/2}$ of random platelets, 1.0 days; antigen-compatible platelets, 1.9 days) (Figure 1).

The range of day-1 increments varied widely, both with R-Tx (range: 10-217 $\times 10^9$/L) and AC-Tx (range: 62-216 $\times 10^9$/L). Unexpectedly large increments may occur with R-Tx when a proportion of the platelets is coincidentally HPA-1a/5b negative (estimated likelihood, 1 in 13 where the platelet pool is from 4 "random" donors)[2]; unexpectedly low increments may be seen with AC-Tx due to the presence in the fetal circulation of maternal HLA class I antibodies[3] or when platelet transfusions are inappropriately administered. Babies with NAIT should therefore have their platelet counts monitored regularly whatever treatment is used and appropriate changes to treatment instigated when necessary.

In conclusion, we agree with Kiefel et al that R-Tx is an acceptable initial treatment for NAIT where HPA-1a/5b–negative platelets are not immediately available. However, our data show that HPA-1a/5b–negative platelets give larger increments, have a longer half-life, and only occasionally fail to provide therapeutically significant platelet increments. We therefore encourage blood services in other countries to establish panels of HPA-1a/5b–negative donors to provide HPA-1a/5b–negative platelets for immediate use in cases of suspected NAIT and for intrauterine or neonatal therapy of cases of known NAIT.[4]

David Allen, Salim Verjee, Sarah Rees, Michael F. Murphy, and David J. Roberts

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Correspondence: David J. Roberts, National Blood Service—Oxford, Level 2 John Radcliffe Hospital, Headley Way, Headington, Oxford OX3 9BQ, United Kingdom; david.roberts@ndcls.ox.ac.uk.

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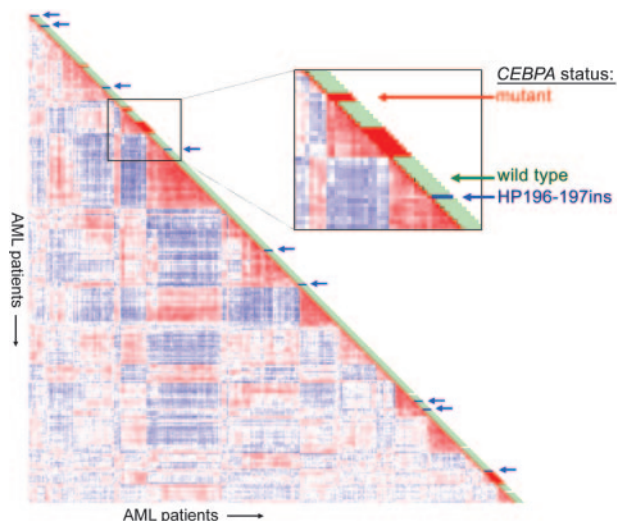
To the editor:

A recurrent in-frame insertion in a *CEBPA* transactivation domain is a polymorphism rather than a mutation that does not affect gene expression profiling–based clustering of AML

Mutations in *CEBPA,* the gene encoding the transcription factor CCAAT/enhancer binding protein alpha (C/EBPalpha), have been reported in multiple studies, and are found in approximately 8% of patients with acute myeloid leukemia (AML).[1,2] Specific regions of the gene tend to be most commonly mutated: (1) in-frame insertions in the basic/leucine zipper (bZIP) region and (2) truncating out-of-frame insertions or deletions in the N-terminus.[1,2] Although mutations are most frequently found in these 2 regions, other abnormalities have been described as well.[2] Fröhling et al reported in 6 of 236 AML cases the existence of an in-frame insertion mutation of 6 nucleotides.[3] This insertion is predicted to

result in a histidine-proline duplication (HP196-197ins) in a transactivation domain of C/EBPalpha. In vitro studies have suggested that this proline-histidine–rich region may play a role in antiproliferative control, although this notion has not been supported by in vivo experiments.[4,5] Remarkably, in none of the other initial *CEBPA* mutation studies was the insertion reported as either a mutation or a polymorphism, notwithstanding the fact that investigators frequently applied single-strand conformation polymorphism (SSCP) analysis or nucleotide-sequenced the complete *CEBPA* cDNA (see Leroy et al[2] for references). More recently, one other group described the HP duplication in 20 (20%) of 100 AML

Figure 1. Correlation view of 285 AML cases including *CEBPA* status. Pairwise correlations between samples are displayed using 2856 probe sets as described.[8] Colors of boxes visualize Pearson correlation coefficient: red indicates higher positive correlation; blue indicates higher negative correlation. Bars next to each sample represent *CEBPA* status: mutation in bZIP region and/or N-terminus (red), presence of HP196-197ins (blue), or neither (green). For 3 specimens, depicted in white, no material was available for dHPLC analysis. The figure was generated using HeatMapper software (http:www.erasmusmc.nl/hematologie/heatmapper/).[10]

samples.[6] In this study, the insertion was reported in 7 (39%) of 19 healthy volunteers as well, questioning its role in AML.[6]

In a cohort of 285 AML cases, we previously selectively screened for the 2 major mutation types and identified 17 patients with mutations.[7,8] Here, we asked whether this cohort also included cases with HP196-197ins. By means of a denaturing high-performance liquid chromatography (dHPLC) approach[9] and subsequent nucleotide sequencing, we identified the heterozygous HP196-197ins in 9 patients (3.2% of 282 available samples). We also screened an independent second cohort of 305 AML cases, and again found 12 cases (3.9%) to present with this duplication. Finally, we analyzed a series of 274 nonleukemic blood samples and found 22 individuals (8.0%) to carry the same insertion.

Cases with *CEBPA* mutations were found predominantly in 2 distinct gene expression clusters[8] (Figure 1). We asked whether cases with HP196-197ins associated with specific gene expression clusters as well. The 9 specimens carrying HP196-197ins in the first cohort of 285 AML cases did not cluster with *CEBPA* mutant

cases. Moreover, they did not belong to one single previously defined cluster of AML, but were spread out over several subgroups instead (Figure 1).

We conclude that HP196-197ins represents a common *CEBPA* polymorphism, rather than a mutation, that does not influence gene expression profiling–based clustering of AML specimens. Whether the higher percentage of HP196-197ins observed in nonleukemic samples compared with AML cases is due to chance, or represents an important difference, remains to be elucidated in larger series.

Bas J. Wouters, Irene Louwers, Peter J. M. Valk, Bob Löwenberg, and Ruud Delwel

Correspondence: Ruud Delwel, Erasmus Medical Center, Department of Hematology, Room Ee1342, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands; e-mail: h.delwel@erasmusmc.nl.

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