THE LANCET
Haematology

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Allen E S, Srivastava K, Hsieh M M, et al. Immunohaematological complications in patients with sickle cell disease after haemopoietic progenitor cell transplantation: a prospective, single-centre, observational study.
Lancet Haematol 2017; **4:** e553–61.

Supplemental Material

Table S1. Patient characteristics among those with HPC engraftment failure or secondary graft rejection

	HLA Match		
Parameter	**Identical**	**Haploidentical**	**Total (%)**
HPC engraftment failed	0	4	4 (29)
Secondary graft rejection	5	5	10 (71)
Total	5	9	14 (100)
Alive	4	7	11 (79)
Male	2	5	7 (50)
Age, mean (years)	28.3	34.7	32.4
Sex (Donor/Recipient)			
Same (M/M)	1	0	1 (7)
Same (F/F)	0	3	3 (21)
Opposite (M/F)	3	1	4 (29)
Opposite (F/M)	1	5	6 (43)
ABO groups (Donor/Recipient)			
Identical (A/A)	1	1	2 (14)
Identical (B/B)	1	2	3 (22)
Identical (O/O)	2	4	6 (43)
Minor incompatible (O/A)	0	1	1 (7)
Minor incompatible (O/B)	1	1	2 (14)
RBC Transfusion			
Before enrollment (patients)			
Unknown history	1	0	1 (7)
1 – 10 units	1	1	2 (14)
11 – 50 units	1	1	2 (14)
> 50 units	2	7	9 (64)
After enrollment (units)			
Mean	25.6	31.4	29.4
Median	22	27	25
Range	18 – 40	14 – 63	14 – 63
Time to secondary graft rejection* (days)			
Mean	104.4	115	109.7
Median	96	91	93.5
Range	50 – 187	64 – 215	50 – 215
RBC alloantibodies at enrollment (patients)			
0 alloantibodies	3	6	9 (64)
1 alloantibody	0	2	2 (14)
2 alloantibodies	1	1	2 (14)
3 to 7 alloantibodies	1	0	1 (7)
RBC autoantibodies at enrollment (patients)			
Present	1	1	2 (14)
Absent	4	8	12 (86)
Immunohematologic Complications			
None	5	8	13 (93)
New red cell antibodies post-transplantation	0	1	1 (7)
Alloantibody directed against recipient or donor RBC antigens	0	0	0 (0)

* Secondary graft rejection is defined as the date on which chimerism studies showed no detectable donor CD3 or myeloid cells.
F – female; M – male

Table S2. Red cell antibodies reported or observed at enrollment

Antibodies				Autoantibody type in patients (n)		
Alloantibody (n)	**Autoantibody**	**Patients**	**Donors**	**Warm**	**Cold**	**Both**
0	Absent	39	60	n.a.	n.a.	n.a.
	Present	0	0	0	0	0
1	Absent	6	1*	n.a.	n.a.	n.a.
	Present	4	0	3	1	0
2	Absent	3	0	n.a.	n.a.	n.a.
	Present	2	0	1	1†	0
3 to 7	Absent	5	0	n.a.	n.a.	n.a.
	Present	2	0	1	0	1
Total (with antibodies)		22	1	5	2	1
Total (all patients)		61	61	5	2	1

* Anti-McC[a]

† Patient 170-27 was reported to have a cold autoantibody and an "auto-anti-D like" autoantibody.

n.a. – not applicable

Table S3. Red cell alloantibody specificities among recipients at enrollment

Antibody specificity				Alloantibodies				
Antigen	**ISBT Number**	**ISBT Symbol**	**Clinically Relevant**	**Reported, not detected**	**Reported and detected**	**Detected, not reported**	**Total**	**Donors with cognate antigen**
A_1	001.004	ABO1	Yes	1	0	0	1	0
M *	002.001	MNS1	No	0	1	0	1	1
S	002.003	MNS3	Yes	4	2	0	6	0
D	004.001	RH1	Yes	0	0	1	1	0
C	004.002	RH2	Yes	7	3	0	10	1
E	004.003	RH3	Yes	5	8	0	13	0
V *	004.010	RH10	Yes	1	1	1	3	0
K	006.001	KEL1	Yes	4	4	0	8	0
Le^a	007.001	LE1	No	3	1	0	4	n.t.
Fy^a	008.001	FY1	Yes	6	1	0	7	0
Fy^b	008.002	FY2	Yes	2	0	0	2	0
Jk^a *	009.001	JK1	Yes	0	1	0	1	1
Jk^b *	009.002	JK2	Yes	1	0	0	1	1
Total				34	22	2	58	

* The 6 recipient and donor pairs were tested by red cell genotyping.
n.t. – not tested

Table S4. Patients with new alloantibodies after enrollment

	Development of new alloantibody during the clinical course			
Parameter	**Yes**	**No**	**Patients (n)**	***p****
Alloantibody reported or detected at enrollment				
Present	3	19	22	
Absent	3	36	39	
Total	6	55	61	0.66
DAT before transplantation†				
Positive	1	14	15	
Negative	5	39	44	
Total	6	53	59	1.0
Autoantibody at enrollment				
Present	1	7	8	
Absent	5	48	53	
Total	6	55	61	1.0
Alloantibody detected at enrollment‡				
Yes	3	9	12	
No	0	10	10	
Total	3	19	22	0.22
Transfusion history at enrollment§				
>50 RBCs	2	26	28	
<50 RBCs	4	24	28	
Total	6	50	56	0.67
Recipient sex				
Male	3	31	34	
Female	3	24	27	
Total	6	55	61	1.0
Donor sex				
Male	1	26	27	
Female	5	29	34	
Total	6	55	61	0.21
Diagnosis				
Hgb SS	6	48	54	
Hgb SC	0	3	3	
Hgb S-β Thalassemia	0	4	4	
Total	6	55	61	1.0
Blood group				
A	2	13	15	
B	2	10	12	
O	2	30	32	
AB	0	2	2	
Total	6	55	61	0.61
Development of new autoantibody				
Yes	2	0	2	
No	4	55	59	
Total	6	55	61	0.0082

* Fisher's exact test, 2-sided.
† Performed on 59 of 61 patients.
‡ Evaluating the 22 patients with alloantibodies reported or detected at enrollment.
§ Excluding 5 patients whose transfusion history was unknown.

DAT – direct antiglobulin test

Table S5. Transfusion support and clinical outcomes in 47 patients with sustained engraftment by immunohematologic complication

	Without immunohematologic complications			With immunohematologic complications				
					New alloantibodies detected after enrollment			
Parameter	**No alloantibody**	**Compatible alloantibody at enrollment**	**Subtotal**	**Incompatible alloantibody at enrollment only**	**Compatible***	**Incompatible**	**Subtotal**	**Total**
Patients with sustained engraftment (n)	28	11	39	3	2	3	8	47
RBCs transfused after enrollment (units)								
Mean ± SD	13.18 ± 6.58	21.27 ± 19.31	15.46 ± 11.94	51.00 ± 43	13.50 ± 0.71	16.33 ± 5.51	28.63 ± 29.69	17.70 ± 16.64
Median	11	16	12	38	13.5	19	17.5	12
Range	7 – 38	9 – 77	7 – 77	16 – 99	13 – 14	10 – 20	10 – 99	7 – 99
$p^{\dagger}$	control	0.044		0.015	0.32	0.23		
Time to transfusion independence (days)								
Mean ± SD	38.36 ± 73.08	34.82 ± 43.03	37.36 ± 65.46	418.67 ± 299.73	34.00 ± 15.56	71.00 ± 90.22	192.13 ± 251.90	63.70 ± 129.04
Median	14.5	29	16	329	34	33	109.5	19
Range	0 – 341	7 – 161	0 – 341	174 – 753	23 – 45	6 – 174	6 – 753	0 – 753
$p^{\dagger}$	control	0.29		0.009	0.21	0.59		
Time to engraftment (days)‡								
Mean ± SD	18.46 ± 7.35	21.09 ± 8.53	19.21 ± 7.67	14.67 ± 2.08	14.00 ± 0	15.33 ± 2.31	14.75 ± 1.75	18.45 ± 7.21
Median	14	18	15	14	14	14	14	14
Range	14 – 43	14 – 39	14 – 43	13 – 17	14 – 14	14 – 18	13 – 18	13 – 43
$p^{\dagger}$	control	0.35		0.21	0.23	0.46		

* The 2 patients listed are 170-29 and 170-39. A third patient, 225-01, who developed new alloantibodies after enrollment, is excluded from tabulation because she experienced graft rejection. Notably, she lost her graft on day 214 while her antibody screen was confirmed negative on day 266, and her alloantibodies were detected only 1 year after transplantation.

† Comparing the indicated group to the "no alloantibody" control group (Mann Whitney U test, 2-sided).

‡ Engraftment is defined as the date on which chimerism studies showed detectable donor CD3 or myeloid cells.

n.a. – not applicable; SD – standard deviation

Table S6. Clinical outcomes in the 9 patients with immunohematologic complications, defined as new alloantibodies or red cell incompatibility or both

Patient	Age, sex	Transfusions before enrollment	Antibodies at enrollment		New antibodies after enrollment		Incompatible Antigen*	Transfusions after enrollment (n)	Time to transfusion independence (days)	Engraftment	Survival	Follow-up (years)
			Specificity	Number	Specificity	Number						
170-09	45, F	>50	M,† S, E, K, Fy^a, Fy^b, Jk^b	7	None	0	Jk^b	16	174	Yes	Alive	8
170-13	65, M	11-50	C, E, K, Fy^a, Fy^b, Jk^a, warm auto, cold auto	8	None	0	Jk^a	99	753	Yes	Died 4.5 years after transplant	4.5
170-17	34, F	>50	S, C, E, K, $Le^{a\dagger}$, Fy^a	6	None	0	C	38	329	Yes	Alive	6
170-20	35, F	11-50	E, K	2	V	1	V	20	174	Yes	Alive	5
170-28	47, F	>50	S, C, E, V, K, $Le^{a\dagger}$, Fy^a, warm auto	8	Js^a, D, probable Knops†	3	D	19	33	Yes	Alive	3.5
170-29	41, M	11 - 50	None	0	$McC^{a\dagger}$	1	None	14	45	Yes	Alive	3.5
170-31	27, M	1-10	None	0	E	1	E	10	6	Yes	Alive	3
170-39	19, M	1-10	Fy^a	1	Rh, $Le^{b\dagger}$, warm auto	3	None	13	23	Yes	Alive	1.5
225-01‡	44, F	>50	None	0	C, K, Js^a, warm auto	4	None	42	608	Engrafted, lost graft on day 214	Died 5 years after transplant	5

* Only antigens that are considered clinically significant are listed.
† Not considered clinically significant.
‡ The donor/recipient pair for patient 225-01 demonstrated minor incompatibility. All other donor/recipient pairs had identical ABO blood groups.

Significant immunohematologic events. Significant clinical events related to the immunohematologic complications occurred in only 5 of 9 patients and included prolonged reticulocytopenia in 3 patients (Figure 3) and acute hemolysis with severe anemia in 2 other patients.

(i) Prolonged reticulocytopenia. Patient 170-13 had alloantibodies including an anti-Jk^a present at enrollment. His HLA-matched donor was Jk^a antigen positive. He developed chronic immune hemolysis and reticulocytopenia (Figure 3, red line). During the next 2 years, he required 87 RBC transfusions while being treated with dexamethasone, rituximab, and bortezomib. He later remained transfusion independent for the next 2.5 years before he died from complications unrelated to the anti-Jk^a.

Patient 170-17 also had alloantibodies including an anti-C present at enrollment. Her HLA-matched donor was C antigen positive. She developed anemia and reticulocytopenia (Figure 3, blue line). Between days 74 and 329 after transplantation, she required 25 RBC transfusions. Her red cell phenotype appeared to be C negative at day 301 while being transfused C negative RBCs, and had converted to C positive at day 791. She has since remained transfusion independent for four years.

Patient 170-20 formed a new anti-V (Table 3). Her HLA-matched donor was V antigen positive. She developed reticulocytopenia (Figure 3, green line). During the next 3 months she required 8 RBC transfusions, and has since remained transfusion independent for 5 years.

(ii) Acute hemolysis with severe anemia. Patient 170-28 had multiple alloantibodies and was D antigen positive. Her HLA-matched donor was D antigen negative. On day 25, she developed hyperhemolysis, her hemoglobin reached a nadir of 4.8 g/dL, and anti-D was eluted from her RBCs (Table 3). After receiving erythropoietin, intravenous immunoglobulin (IVIG), and 3 RBC transfusions, she recovered within 2 weeks and has remained transfusion independent for 3 years.

Patient 170-39 had an Rh genotype of *(C)ceS* and *weak D type 4.2.2/ceAR*, which was shared by his 16 year-old HLA-matched sibling donor. An alloantibody against a high-prevalence Rh antigen was detected on day 1 (Table 3), and he developed hyperhemolysis following day 10. He tolerated 2 RBC transfusions from his HPC donor. At a hemoglobin of 3.1 g/dL, transfusion of 1 RBC unit with the homozygous Rh genotype *(C)ceS* caused acute intravascular hemolysis. Within 2 days, his hemoglobin concentration reached a nadir of 2.4 g/dL, at which time a third RBC unit from his HPC donor was transfused. After treatment with erythropoietin, IVIG, and PEGylated bovine carboxyhemoglobin (Sanguinate; Prolong Pharmaceuticals, South Plainfield, NJ), he recovered within 2 weeks. Although his hemoglobin concentration at enrollment was 6.6 g/dL, it remained at or above 11 g/dL for the follow-up of 1.5 years.

Supplemental Reading List

1. Arnold SD, Bhatia M, Horan J, Krishnamurti L. Haematopoietic stem cell transplantation for sickle cell disease - current practice and new approaches. *British journal of haematology* 2016; **174**(4): 515-25.

2. Walters MC, De Castro LM, Sullivan KM, et al. Indications and Results of HLA-Identical Sibling Hematopoietic Cell Transplantation for Sickle Cell Disease. *Biol Blood Marrow Transplant* 2016; **22**(2): 207-11.

3. Walters MC, Patience M, Leisenring W, et al. Bone marrow transplantation for sickle cell disease. *N Engl J Med* 1996; **335**(6): 369-76.

4. Krishnamurti L, Blazar BR, Wagner JE. Bone marrow transplantation without myeloablation for sickle cell disease. *N Engl J Med* 2001; **344**(1): 68.

5. Ozdogu H, Boga C. Hematopoietic Stem Cell Transplantation in Adult Sickle Cell Disease: Problems and Solutions. *Turkish journal of haematology : official journal of Turkish Society of Haematology* 2015; **32**(3): 195-205.

6. Badros A, Tricot G, Toor A, et al. ABO mismatch may affect engraftment in multiple myeloma patients receiving nonmyeloablative conditioning. *Transfusion* 2002; **42**(2): 205-9.

7. Maciej Zaucha J, Mielcarek M, Takatu A, et al. Engraftment of early erythroid progenitors is not delayed after non-myeloablative major ABO-incompatible haematopoietic stem cell transplantation. *British journal of haematology* 2002; **119**(3): 740-50.

8. Vidler JB, Gardner K, Amenyah K, Mijovic A, Thein SL. Delayed haemolytic transfusion reaction in adults with sickle cell disease: a 5-year experience. *British journal of haematology* 2015; **169**(5): 746-53.

9. Noizat-Pirenne F, Lee K, Pennec PY, et al. Rare RHCE phenotypes in black individuals of Afro-Caribbean origin: identification and transfusion safety. *Blood* 2002; **100**(12): 4223-31.

10. Avent ND, Martinez A, Flegel WA, et al. The BloodGen project: toward mass-scale comprehensive genotyping of blood donors in the European Union and beyond. *Transfusion* 2007; **47**(1 Suppl): 40s-6s.

11. Meyer S, Vollmert C, Trost N, et al. High-throughput Kell, Kidd, and Duffy matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry-based blood group genotyping of 4000 donors shows close to full concordance with serotyping and detects new alleles. *Transfusion* 2014; **54**(12): 3198-207.

12. Hashmi G, Shariff T, Zhang Y, et al. Determination of 24 minor red blood cell antigens for more than 2000 blood donors by high-throughput DNA analysis. *Transfusion* 2007; **47**(4): 736-47.

13. St-Louis M, Perreault J, Lavoie J, et al. [Genotyping of 21,000 blood donors in Quebec and RHD analysis]. *Transfus Clin Biol* 2010; **17**(4): 242-8.

14. Evers D, Middelburg RA, de Haas M, et al. Red-blood-cell alloimmunisation in relation to antigens' exposure and their immunogenicity: a cohort study. *The Lancet Haematology* 2016; **3**(6): e284-92.

15. Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, editors. Technical Manual. 18 ed. Bethesda MD: AABB; 2014.

16. Sippert EA, Visentainer JE, Alves HV, et al. Red blood cell alloimmunization in patients with sickle cell disease: correlation with HLA and cytokine gene polymorphisms. *Transfusion* 2017; **57**(2): 379-89.

17. Negre O, Eggimann AV, Beuzard Y, et al. Gene Therapy of the beta-Hemoglobinopathies by Lentiviral Transfer of the beta(A(T87Q))-Globin Gene. *Human gene therapy* 2016; **27**(2): 148-65.