Table 2. Influence of priming with cyclophosphamide and harvest timing (before or after 14 days from the last course of chemotherapy)] on the contents of total $CD34^+$ cells, $CD34^+CD33^-$ cells and in the percentage of $CD33^-$ cells within the overall $CD34^+$ population. Cell counts are expressed as $\times 10^6$/Kg.

	priming	*Cyclophosphamide* < 14 days	> 14 days
CD34+	5.1±7.5	4.7±5.0	8.5±14.9
CD34+CD33-	2.8±3.8	4.0±4.4	3.4±3.0
% CD33-	51.3±32.0	74.7±12.0	53.5±28.0

might stimulate earlier forms of progenitors with a lower density of receptors for G-CSF on their surface.

Early mobilization (within the first two weeks) after the last block of chemotherapy mobilizes a higher proportion of $CD34^+CD33^-$ cells. Harvests collected earlier than 14 days after the last course of chemotherapy had significantly (p<0.04) higher overall percentages of $CD34^+$ $CD33^-$ cells, while the $CD34^+$ yields were not significantly different. Priming with cyclophosphamide did not produce significant differences. Values corresponding to these analyses are detailed in Table 2. These results are consistent with early reports of mobilization of CFU-GM after chemotherapy in children.[3] Grafts containing high proportions of *early* progenitors may provide faster multi-lineage hematopoietic reconstitution.

Neither $CD34^+$ cell dose nor dose of $CD34^+$ $CD33^-$ *early* progenitors seemed to influence neutrophil or platelet recovery. However, children receiving grafts containing >75% of early progenitors had a non-significant (p<0.06) tendency towards earlier platelet engraftment (25.1±24.3 vs. 57.3±54.4 days, respectively). Moreover, children receiving 10 μg/Kg of G-CSF for mobilization had significantly (p<0.02) faster platelet recovery (18.1±15.6 days in children mobilized with 10 μg/Kg and 47.9±62.1 in children mobilized with 5 mg/Kg). These results suggest that the reinfusion of an earlier, pluripotent progenitor could allow faster multi-lineage hematopoietic reconstitution.

In conclusion, doses of G-CSF of 10 mg/Kg seem to mobilise an *earlier* type of hemopoietic progenitor than doses of 5 mg/Kg in children receiving treatment for solid tumors. These *early* progenitors could provide faster multi-lineage hematopoietic reconstitution. These results should be confirmed in prospective, randomized studies.

Miguel Ortín, Leni G. Mathew, Soonie R. Patel, Barbara Millar, Nikki Pandya, Jennifer Treleaven

Department of Pediatric Haematology and Oncology, The Royal Marsden Hospital, Sutton (Surrey), UK

Correspondence: Dr. Miguel Ortín, Senior Lecturer, St. George's Hospital Medical School, Consultant Paediatric Haematologist, Director, Paediatric BMT Program, Department of Paediatric Haematology and Oncology, The Royal Marsden Hospital, Sutton (Surrey) SM2 5PT, UK. Phone: international +44.2086 613163. Fax: international +44.2086613617. E-mail: mortin@sghms.ac.uk

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Stem Cell Transplantation

Polymorphism of the α4-subunit of VLA-4 integrin and bone marrow transplantation

Integrin α4β1 is an important homing molecule on stem cells. Two genetic variants of this integrin are known, α4-mas and α4-tex. We assessed the potential influence of this polymorphism in 37 patients undergoing allogeneic bone marrow transplantation. None of the constellations of variants influenced the outcome, as determined by the recovery of leukocytes or platelets, hospitalization time, and the development of graft-versus-host disease.

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Integrin α4β1 is expressed on hematopoietic cells,[1] and plays a substantial role in the repopulation and differentiation of transplanted stem cells.[2-4] In addition, it is involved in homing of $CD34^+$ cells,[1] presumably in acute graft-versus-host disease (GvHD),[5] and in the creation of a minor histocompatibility antigen. Two known variations of the α4 subunit have been described, α4-tex and α4-mas.[6,7] The significance and biological actions of these variants are unknown. It seems likely that the α4-polymorphism could be involved in reactions associated with bone marrow transplantation (BMT).

A total of 37 BMT donor-recipient pairs were genotyped for α4 variants, and for HA1 in 20 pairs who were HLA-A2-positive (Table 1). The patients received either peripheral blood stem cells (PBSC, n=27) or bone marrow (BM, n=10) from fully HLA-matched, first-degree relatives. The primers used and the typing method for α4[8] and for HA1[9] have been described elsewhere. HY was considered to be relevant in all female-to-male transplantations. An epitope prediction for α4 peptides was performed using the SYFPEITHI database (*www.uni-tuebingen.de/uni/kxi*). The scoring system of this algorithm evaluates every amino acid within a given peptide. A score over 30 means a high probability of a functionally relevant peptide. A score of 25 was achieved for the following peptides: TLKGIV(R/Q)FL (R=α4-tex, Q=α4-mas) for HLA-A*0201, IV(R/Q)FLSKTD for HLA-A3, and TLKGIV(R/Q)FL for HLA-B8. However, none of the screened peptides of the α4 subunit could be predicted to create an epitope sufficient for HLA presentation.

Regarding neutrophil engraftment, the most relevant differences occurred in mas/tex on tex/tex pairs compared to both tex/tex on tex/tex pairs (p=0.0519) and tex/tex on

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Table 1. BMT couples with relevant clinical data.

A	B	C	D	E	F	G	H	I	L	M	N	O	P	Q	R	S	T	U
1	f to m	43	37	0^- to 0^-	neg to pos	PBSC			mas/tex	mas/tex	none	11,8	7,4	12	14	3	26	M.Hodgkin
2	m to f	42	50	AB^+ to B^+	neg to pos	PBSC	hr	r	tex	mas/tex	skin	8,2	6,9	16	16	25	57	B-NHL
3	f to m	42	49	A^- to A^-	pos to pos	PBSC			tex	tex	skin	5,4	6,4	13	13	12	36	B-NHL
4	f to m	15	4	B^+ to B^-	pos to pos	BM	hr	r	mas/tex	mas/tex	none	2,1	n.a.	n.a.	14	25	20	ALL
5	f to f	44	45	A^+ to 0^+	pos to pos	PBSC	hr	r	tex	tex	none	6,5	7,3	18	19	20	36	CML
6	m to m	55	61	0^+ to 0^+	pos to pos	PBSC			mas	mas/tex	skin, gut	6,9	6	23	28	30	Fatal	Germ cell tumor
7	f to m	31	29	0^+ to 0^+	pos to pos	PBSC	hr	hr	tex	tex	none	5,2	6,9	14	15	13	43	AML
8	f to f	23	11	A^+ to 0^+	neg to neg	PBSC	hr	h	tex	tex	skin	6,9	3,9	29	29	28	51	CML
9	f to m	64	62	0^+ to 0^+	neg to neg	PBSC	r	hr	tex	tex	skin, liver	11,8	2,2	33	27	n.b.	68	MDS
10	m to m	37	34	A^+ to A^+	neg to neg	PBSC	r	r	mas	mas/tex	none	6,6	3,8	13	14	0	26	Germ cell tumor
11	m to m	2	0	0^+ to 0^+	neg to neg	BM	hr	hr	mas/tex	mas/tex	skin, liver	2,6	n.a.	28	28	49	48	MDS
12	f to m	7	5	A^+ to A^+	pos to neg	BM	r	r	mas/tex	mas/tex	skin	3,4	n.a.	18	26	23	31	ALL
13	f to m	58	57	AB^+ to A^+	neg to pos	PBSC	hr	hr	mas/tex	tex	n.a.	12,4	3,1	n.b.	n.b.	n.b.	Fatal	Aplast. anemia
14	m to f	42	31	A^+ to A^+	neg to pos	PBSC	hr	hr	tex	tex	skin, liver	3,5	5,6	16	16	12	51	CLL
15	f to m	29	27	A* to A^+	neg to neg	PBSC	hr	hr	mas/tex	mas/tex	none	2,3	3	16		15	34	Germ cell tumor
16	m to m	38	44	A^+ to 0^+	neg to neg	PBSC	h	hr	mas/tex	tex	none	7,3	3,2	13	14	12	25	AML
17	m to f	29	40	0^+ to 0^+	neg to neg	PBSC	r	r	mas/tex	tex	n.a.	4,9	7,4	n.b.	n.b.	n.b.	Fatal	Germ cell tumor
18	m to m	6	7	0^+ to 0^+	pos to pos	BM		h	tex	mas/tex	none	7,6	n.a.	12	12	20	26	Aplast. anemia
19	m to m	42	32	B^+ to B^+	neg to pos	PBSC	r	hr	tex	mas/tex	none	4,8	6,4	21	21	10	36	CML
20	m to m	59	56	0^- to A^-	neg to pos	PBSC	r	r	mas/tex	mas/tex	Skin	7,4	6,7	17	17	16	30	AML
21	f to m	28	36	A^+ to A^+	neg to neg	PBSC	h	hr	mas/tex	tex	none	n.a.	n.a.	1	1	1	13	Germ cell tumor
22	f to f	35	34	0^+ to 0^+	neg to neg	PBSC	r	r	tex	tex	none	n.i.	5,1	19	20	11	23	AML
23	m to m	38	40	A^+ to 0^+	neg to neg	PBSC	r	r	mas/tex	mas/tex	skin,gut, liver	5,1	6,6	n.b.	n.b.	n.b.	Fatal	AML
24	m to f	6	3	A^- to A^-	neg to pos	BM			mas/tex	mas/tex	skin	3,2	n.a.	n.b.	15	48	63	Thalassemia
25	m to m	61	64	A^+ to A^+	pos to pos	PBSC			tex	mas/tex	skin	4,2	4	12	13	12	55	AML
26	f to m	29	19	0^+ to 0^+	neg to pos	PBSC			tex	tex	none	9,8	15	16	19	16	Fatal	NHL
27	m to f	37	40	0^+ to 0^+	pos to pos	PBSC			mas/tex	tex	none	11,3	10	18	20	13	42	AML
28	f to m	56	59	0^+ to 0^+	neg to neg	PBSC			tex	tex	skin	8,8	6,1	23	22	18	41	MDS
29	m to m	32	38	A^+ to 0^+	neg to neg	PBSC			mas/tex	mas/tex	skin	6,1	5,8	13	12	11	36	Hodgkin's dis.
30	f to f	28	32	0^- to 0^-	neg to neg	PBSC			mas/tex	tex	skin,liver	6,4	8	16	19	13	35	CML
31	m to m	28	29	0^+ to 0^+	neg to neg	PBSC			tex	mas/tex	none	8,1	4,2	16	17	14	18	Germ cell tumor
32	f to m	6	8	0^- to 0^-	neg to pos	BM			tex	mas/tex	skin	9,2	n.a.	32	12	36	75	Fanconi anemia
33	f to m	1	7	A^+ to A^+	pos to neg	BM			tex	tex	skin	7,48	n.a.	10	19	16	48	congenital thrombopenia
34	m to m	9	2	A^+ to 0^-	pos to pos	BM			mas/tex	tex	none	2,2	n.a.	16	16	48	58	β-Thalassemia
35	m to m	15	17	A^+ to A^+	neg to neg	BM			tex	tex	skin, gut	3,37	n.a.	12	13	115	81	MDS
36	f to f	9	13	A^+ to 0^+	neg to neg	BM			tex	mas	skin	6,2	n.a.	21	20	37	45	Aplast. anemia
37	f to f	9	9	A^+ to A^+	neg to neg	BM			tex	tex	none	1,8	4,1	11	12	21	22	T-NHL

A: couples; B: gender; C: recipient's age; D: donor's age; E: blood group; F: CMV; G: preparation: H: HA1 recipient; I: HA1 donor; L: VLA4 recipient; M: VLA4 donor; N: GvHD; O: cell dose/kg10^8; P: CD34 (10^6); Q: recovery leukocytes; R: recovery granulocytes; S:recovery thrombocytes; T: dismissal; U: primary diagnosis; PBSC: peripheral bone marrow stem cells; BM: bone marrow; n.a.: no data; n.b.: criteria not reached.

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mas/tex (p=0.0571) (Table 2A). Platelet engraftment in tex/tex on tex/tex pairs was not significantly different from that of mas/tex on tex/tex pairs (p=0.2957). Regarding duration of inpatient hospitalization, a difference was observed between the tex/tex on tex/tex pairs and mas/tex on mas/tex pairs (p=0.0501). GvHD was documented in 19 of the BMT patients, and involved the skin to a variable degree. The liver was affected in five cases, the intestines in three. HY was found to be incompatible in 12 patients, 8 of whom developed GvHD (67%, p=0.4764). GvHD development was observed in 2 of 5 patients with incompatible HA1 (40%, $p\sim1$), in 4 of 7 with incompatible α4-mas (57%, $p\sim1$) and in 1 of 2 with incompatible α4-tex (Table 2B). Thus, the frequencies of GvHD in these patients did not differ significantly from those observed in patients with compatible antigens.

Although the total number (n=37) of patients studied here does not allow us to draw any definite conclusions, we found no evidence suggesting that mas/tex polymorphism of the α4 integrin subunit might influence the process of allo-

Table 2A. α4-genotype of the recipients and outcome of the transplantation; days in mean ± SEM. The day of transplantation was designated as day 0. The time to hematopoietic recovery was defined as the period up to the day after transplantation with an absolute leukocyte count of > 1000 per μL, an absolute neutrophil count of > 500 per μL, and an unsupported platelet count of > 20,000 per μL for more than one day.

Donor/recipient constellation (n)	*tex/tex on tex/tex (n=12)*	*tex/tex on mas/tex (n=7)*	*Time of hematopoietic recovery (day) mas/tex on tex/tex (n=6)*	*mas/tex on mas/tex (n=9)*	*mas/tex on mas/mas (n=2)*	*mas/mas on tex/tex (n=1)*
leukocytes	17.8±2.1	15.8±1	14±1.2	14.5±1.2	18±5	21
neutrophiles	18.7±1.6	17.3±1.4	13.3±0.9	14.4±0.8	21±7	20
platelets	25.6±9.1	21.5±8.8	14±2.2	15.5±3.3	15±15	37
day of discharge	45.5±5.3[a]	34.6±7.6[b]	33.8±8	29.5±2.4[a]	26[a]	45

a: in one case fatal; b; in two cases fatal.

Table 2B. Occurence of graft versus host disease (GvHD).

mHAG	*total(n)*	*GvHD(n)*	*p value*	*RR*
HY incompatible	12	8 (67%)	0.4764	1.3
HY compatible	22	11 (50%)		
HA1 incompatible	5	2 (40%)	~1	0.8
HA1 compatible	16	8 (50%)		
α4mas incompatible	7	4 (57%)	~1	1.03
α4mas compatible	27	15 (55%)		
α4tex incompatible	2	1 (50%)	~1	0.9
α4tex compatible	34	18 (53%)		

RR: relative risk, p-value by χ^2 test.

geneic BMT. We did not observe any influence of α4-mas or α4-tex on the recovery of leukocytes, neutrophils or platelets or the development of GvHD. The use of BM as a stem cell source is a more likely explanation for the delayed platelet recovery in the patient who received stem cells from the sole mas-homozygous donor. However, the number of mas-homozygous donors and recipients was too small for a conclusive assessment. To our surprise, HA1 and HY had no effect on the development of GvHD. The relevance of minor histocompatibility antigens, especially HA1 and HY, is still a controversial subject.[10] Thus, the possibility that variants of the α4 subunit may, indeed, be involved in the reaction complex related to allogeneic BMT cannot be completely excluded. As for HA1 and HY, the effect of α4 polymorphism could be dependent on other yet unidentified factors. Some co-expressed adhesion molecules on stem cells, like VLA-5 or the β2-integrins, may compensate for a functionally altered VLA-4.[2] Furthermore, the underlying diseases and the conditioning regimen must be considered. Other important factors for the success of a BMT, such as the source of the transplanted stem cells and the dose and quality of the transplanted cells, were excluded in our cases. The dose of transplanted cells ($CD34^+$ cells: range 2.2 to 15.0 $\times 10^6$) did not seem to influence cell recovery. Furthermore, no correlation was seen between the GvHD rate or the GvHD grade and the source of the transplanted stem cells. In conclusion, the fact that GvHD could not be demonstrated to be associated with an α4-variant mismatch may be interpreted as a confirmation of the non-immunogenicity of α4-variants. Whether or not further studies may reveal additional information on the relevance of α4 polymorphism for BMT remains an open question.

Guido A. Heymann, Dirk Carstanjen,° Holger Kiesewetter,* Abdulgabar Salama**

**Institute of Transfusion Medicine, Charité, Humboldt Universitätzu Berlin: °Dept. of Molecular Genetics, Research Institute of Molecular Pharmacology, Freie Universität Berlin, Germany*

Key words: VLA-4 integrin, bone marrow transplantation, polymorphism.

Correspondence: Prof. Dr. Abdulgabar Salama, Institut für Transfusionsmedizin, Charité, Humboldt-Universität zu Berlin, Campus Virchow Klinikum, Augustenburger Platz 1 13353 Berlin, Germany. Phone: international +49.30.450553011. Fax: international +49.30.450553988. E-mail: abdulgabar.salama@charite.de

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