TAFI in plasma confers a significant risk of acute CAD. Thus, functional TAFI plasma levels above the 126% cut-off increased the risk of acute CAD almost 4-fold. To our knowledge, this is the first case-control study that unequivocally establishes that high levels of functional TAFI are associated with an increased risk of acute CAD in patients under the age of 80 years. We hypothesize that high functional TAFI levels may represent a significant thrombotic biomarker for the risk of acute CAD. Knowledge of the pathophysiological role of functional TAFI should lead to a better understanding of the mechanism of thrombotic disease.

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Stem Cell Transplantation

High-dose granulocyte colony-stimulating factor mobilizes a higher proportion of *early* CD34⁺CD33⁻ hemopoietic progenitors in children receiving treatment for solid tumors

A relationship between dose of granulocyte colony-stimulating factor (G-CSF) and maturational stage of the progenitors mobilized in healthy adult donors has been suggested.[1] In this study we characterize the progenitors mobilized by 2 different dosages of G-CSF in children receiving autologous grafts after intensive treatment for solid tumors.

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From 1997 to 2001, 55 children received an autologous peripheral blood progenitor cells (PBPC) transplant as consolidation treatment for solid tumors at The Royal Marsden Hospital. Indications for transplant were: neuroblastoma (27 cases), rhabdomyosarcoma (8), Hodgkin's disease (7), Wilm's tumor (4), non-Hodgkin's lymphoma (2), Ewing's sarcoma (4), germ cell tumor (2) and synovial sarcoma (1). Data on the mobilization and harvest procedures were available in 51/55 cases (31 boys, 20 girls, median age 6.0±4.4 years). All children received G-CSF (5 μg/Kg in 35 cases and 10 μg/Kg in 16 cases) for four consecutive days. The first harvest session was performed on the 5th day. If an insufficient number of CD34⁺ cells was harvested (<2.5x10⁶ CD34⁺ cells/Kg), the patient received a 5th dose of G-CSF on that day and a second harvest session was performed on the 6th day. Overall, a second harvest was performed in 45 cases. In addition, 24 patients received *priming* with cyclophosphamide (1.5 g/m²) prior to mobilization with G-CSF. The average time from the last course of chemotherapy to the first harvest session was 28.3±23.9 days.

Conditioning regimens included melphalan (33 cases), busulphan plus melphalan (10), thiotepa plus etoposide (2), carboplatin alone (9), and carboplatin plus melphalan (1).

Endpoints for this study were: numbers of CD34⁺, CD34⁺CD33⁺ and CD34⁺CD33⁻ cells harvested, time to neutrophil and platelet engraftment and influence of harvest timing and cyclophosphamide priming on the maturation stage of these progenitors. High doses of G-CSF appear to mobilize a higher proportion of *early* CD34⁺CD33⁻ progenitors.

The most relevant data on the qualitative contents of harvests are shown in Table 1. There were no significant differences in overall number of CD34⁺ or CD34⁺CD33- cells harvested after mobilization with either 5 or 10 μg/Kg of G-CSF. However, the percentage of CD34⁺CD33⁻ cells within the CD34⁺ population was significantly (p<0.05) higher in patients receiving 10 mg/Kg of G-CSF. A similar dose-dependent effect has been reported in healthy adult donors.[1,2] A possible explanation is that high doses of G-CSF

Table 1. Most relevant results of the mobilization/harvest procedures according to the dosage of G-CSF. All values are expressed as number of cells×10⁶ per Kilogram body weight. In the last column, values are expressed as percentages. 1st: first harvest; 2nd: second harvest; total: first plus second harvests.

	CD34⁺			*CD34⁺CD33⁻*			*% CD33⁻ within overall CD34⁺*		
	1st	2nd	total	1st	2nd	total	1st	2nd	total
5 mg/Kg	4.9±8.0	4.4±8.3	8.6±14.8	1.3±1.4	1.6±1.7	2.7±2.8	47.6±30.4	57.9±26	50.9±27.2
10 mg/Kg	2.3±2.2	2.0±1.4	4.2±3.5	2.2±2.1	1.7±1.4	3.7±3.5	73.8±23.4	82.8±16	74.3±23.5

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.

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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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