Stem Cell Transplantation

Additional transplantation of *ex vivo* generated megakaryocytic cells after high-dose chemotherapy

The additional transplantation of *ex vivo* generated hematopoietic (post)-progenitor cells represents a possible approach to ameliorate high-dose chemotherapy induced cytopenia. We investigated the feasibility of the large-scale expansion and transplantation of autologous megakaryocytic cells in four patients with advanced solid tumors. Up to 1,460×10⁶ *ex vivo* generated cells were administered without adverse effects but no clear cut effect on platelet recovery was observed.

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Although hematopoietic recovery after the transplantation of mobilized peripheral blood progenitor cells (PBPC) is fast, there is still a period of neutropenia and thrombocytopenia when patients are at increased risk of developing severe infectious and bleeding complications. Theoretically, the additional transplantation of *ex vivo* generated progenitor and post-progenitor cells might lead to the production of sufficient numbers of mature functional cells within a few days after transplantation. The feasibility and efficacy of this approach with regard to neutrophil recovery has been already demonstrated,[1,2] however, a potential clinical benefit of additionally transplanted megakaryocytic cells has not yet been shown.

We have previously reported that megakaryocytic cells could be effectively generated *ex vivo* using as few as three cytokines (i.e. stem cell factor, interleukin-3 and thrombopoietin).[3] Based on these results, the present study aimed to investigate the feasibility of clinical large-scale *ex vivo* expansion of autologous CD34⁺-selected cells in cytokine-stimulated cultures, and to assess the safety of an additional transplantation of these cells.

Four consenting patients with advanced solid tumors were enrolled in this pilot study. PBPC for the standard transplants (at least 3×10⁶ CD34⁺-cells/kg) plus additional cells for the *ex vivo* cultures were collected by standard apheresis (Baxter CS3000, Baxter, Munich, Germany) following conventional-dose mobilization chemotherapy and granulocyte colony-stimulating factor (G-CSF)-mobilization.[4,5]

Ex vivo cultures were set up with CliniMACS-CD34⁺-selected cells (median purity 98.8%, range: 84.4-99.7%) at 3×10⁴ cells/mL in X-VIVO10 medium (Serva, Heidelberg, Germany) supplemented with 100 ng/mL thrombopoietin (TPO) (CellGenix, Freiburg, Germany), 10 ng/mL interleukin-3 (IL-3) (Novartis, Nürnberg, Germany), and 10 ng/mL stem cell factor (SCF) (Amgen, Mississauga, Ontario, Canada) using 300 cm² tissue culture flasks (Falcon, Becton Dickinson, Heidelberg, Germany). At indicated time points, the cultured cells were harvested, washed, and prepared for immediate infusion. *Ex vivo* generated cells were characterized by flow cytometry and colony-forming cell assays as described previously.[3,6]

It is not known whether more immature or more mature megakaryocytic cells need to be administered to improve post-transplant platelet recovery. Because of the known effect of the duration of culture on the composition of the *ex vivo* generated cell graft,[3,7] the first three patients received day 7 *ex vivo* cultured cells (more immature cells), and, when no effect on platelet recovery was observed, the transplantation of 12-day cultured cells (more mature cells) was investigated in an additional patient.

After 7 days, total nucleated cell expansion ranged between 3.1- and 9.5-fold (Table 1) with the lowest expansion rate observed for patient EXP01, whose cells had been CD34-selected after prior cryopreservation. This might have contributed to the impaired growth in culture, although, we have not yet studied this question systematically. The total nucleated cell expansion of cells from patients EXP02 and EXP03 was in the lower range of what we had observed preclinically, as was the fraction of megakaryocytic cells produced (20.3% to 25.8%).[3] Thus, CD41 production rates were rather modest (0.6 to 2.0, Table 1). The generation of more

Table 1. Large-scale *ex vivo* expansion: laboratory results and composition of additionally transplanted grafts.*

	Laboratory Results								
	Before Culture (day 0)			*After Culture*					
	Total CD34⁺ cells	*% positive*			*cells/mL*		*-fold Expansion†*		*production‡*
	(seeded at 3×10⁴/mL)	*CD34*	*CD41*	*TNC*	*CD34*	*CD41*	*TNC*	*CD34*	*CD41*
EXP01	26×10⁶	48.4	20.3	9.2×10⁴	4.5×10⁴	1.86×10⁴	3.1	1.5	0.6
EXP02	70×10⁶	33.8	25.8	18.2×10⁴	6.13×10⁴	4.7×10⁴	5.2	1.8	1.3
EXP03	75×10⁶	58.4	20.6	28.4×10⁴	16.6×10⁴	5.85×10⁴	9.5	5.5	2.0
EXP04	100×10⁶	4.7	2.6	40.6×10⁴	1.92×10⁴	1.06×10⁴	14.6	0.7	0.4

	Composition of Additionally Transplanted Grafts					
	TNC		*CD34⁺ cells*		*CD41⁺cells*	
	total	*per kg b.w.*	*total*	*per kg b.w.*	*total*	*per kg b.w.*
EXP01	80×10⁶	1.54×10⁶	39.0×10⁶	0.75×10⁶	16.1×10⁶	0.31×10⁶
EXP02	363×10⁶	5.19×10⁶	122.5×10⁶	1.75×10⁶	93.8×10⁶	1.34×10⁶
EXP03	710×10⁶	9.47×10⁶	414.8×10⁶	5.53×10⁶	146.3×10⁶	2.0×10⁶
EXP04	1,460×10⁶	14.6×10⁶	69.0×10⁶	0.69×10⁶	38.0×10⁶	0.38×10⁶

**Positively-selected CD34⁺ cells were cultured in cytokine-supplemented serum-free medium for either 7 days (EXP01, EXP02, EXP03) or 12 days (EXP04). Expansion cultures were initiated with 3×10⁴ CD34⁺ cells/ml with a total of 0.5×10⁶ CD34⁺ cells/kg and 1.0×10⁶ CD34⁺ cells/kg for EXP01 and EXP02 – EXP04, respectively. †fold expansion = numbers of cells after a given culture period divided by the number of cells at day 0 of culture, ‡production: number of CD41⁺ cells produced per seeded CD34⁺ cell (%CD41 multiplied by TNC expansion rate/100). TNC: total nucleated cells.*

Table 2. High-dose chemotherapy and transplantation of PBPC plus *ex vivo* generated cells: hematopoietic recovery parameters.*

	Neutrophils (ANC)†				*Platelets (PLT)*		
	Days with ANC		*Days to ANC*		*Days with PLT*	*Days to PLT*	*Number of PLT transfusions*
	< 100/μL	*< 500/μL*	*> 100/μL*	*> 500 /μL†*	*< 20,000/μL*	*> 20,000/μL†*	
EXP01	6 (5)‡	8 (6)	8 (9)	9 (10)	5 (4)	11 (10)	1(3)
EXP02	5	6	9	10	7	12	1
EXP03	4	6	8	9	2	9	3
EXP04	5	6	10	10	5	12	1

**Patients with advanced solid tumors (stage IV breast cancer, relapsed non-seminomatous germ cell tumor, stage IIIC non-seminomatous germ cell tumor, relapsed metastatic sarcoma) received high-dose VIC-chemotherapy (etoposide 1,500 mg/m², ifosfamide 12,000 mg/m², carboplatin 1,500 mg/m²) followed by the transplantation of unmanipulated PBPC on day 0 plus ex vivo expanded cells on day +1. Patient EXP01 underwent tandem HD-CT consisting of HD-VIC and HD-TC (thiotepa 200 mg/m², cyclophosphamide 1,5000 mg/m²) with the additional transplantation of ex vivo expanded cells after the second HD-CT only. Unmanipulated PBPC grafts contained 4.9×10⁶ CD34⁺ cells/kg (EXP01, 1st HD-CT), 3.0×10⁶ CD34⁺ cells/kg (EXP01, 2nd HD-CT), 3.4×10⁶ CD34⁺ cells/kg (EXP02), 4.8×10⁶ CD34⁺ cells/kg (EXP03), and 4.1×10⁶ CD34⁺ cells/kg (EXP04). Post transplant, G-CSF was administered daily at 300 μg (< 75 kg b.w.) and 480 μg (> 75 kg b.w.) daily until neutrophil recovery. †Neutrophil recovery: first of two consecutive days after transplantation with ANC > 100/μL and 500/μL; platelet recovery: first of 7 consecutive days after transplantation with transfusion-independent platelet counts > 20,000/μL. ‡For comparison, hematopoietic recovery after the first course of HD-CT (HD-VIC, transplantation of unmanipulated cells only, no additional transplantation of ex vivo expanded cells) in patient EXP01 is shown in brackets.*

immature cells was reflected by an up to 5.5-fold expansion of CD34⁺-cells. An amplification of progenitor cells was only observed for patient EXP03 (1.2-fold and 2.9-fold for CFU-Meg and CFU-GM, respectively). After 12 days of culture, cells from patient EXP04 were amplified about 15-fold but hardly any megakaryocytic cells had been produced (Table 1). Instead, *ex vivo* generated cells were mainly characterized by the expression of myelo-monocytic markers (*data not shown*). A definitive reason for this insufficient expansion result could not be identified and has, therefore, remained unexplained.

The additional transplantation of *ex vivo* generated cells containing up to 1.5×10⁷/kg total nucleated cells and a maximum of 2×10⁶/kg CD41⁺-cells, which compares well with the number of additionally transplanted megakaryocytic in previous studies,[7] was safe and there were no side effects. Rapid hematopoietic recovery was observed in all patients (Table 2) and the recovery patterns were within the range normally observed after non-myeloablative high-dose chemotherapy. However, the transplantation of expanded cells had no clear cut effect on either the platelet nadir and time to platelet recovery, or on the number of platelet transfusions required (Table 2).

Taken together, an effect of additionally transplanted megakaryocytic cells on platelet recovery after high-dose chemotherapy has not yet been convincingly demonstrated. Additional studies, utilizing improved expansion methods and, possibly, exogenous TPO administration after transplantation, are certainly warranted.

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