Key words: multiple myeloma, refractory disease, idarubicin, oral application, VID.

Funding: this study was supported by Pharmacia Ltd., Erlangen, Germany and the Leukämie-Initiative Bonn e.V. The sponsors had no influence on the evaluation of data, the preparation of the manuscript or the decision to submit it for publication. Assistance with documentation was provided by Megapharm Ltd., Sankt Augustin, Germany.

Acknowledgments. We wish to thank the members of the study group: M.R. Clemens (Trier), R. Derichs (Düsseldorf), M. Gorschlüter (Bonn), A. Heyll (Düsseldorf), R. Kleinschmidt (Bonn), Y. Ko (Bonn) and C. Maintz (Eschweiler) for the inclusion of patients from their centers. Excellent data and trial management was provided by Dr. C. Hahn (Bonn).

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Stem Cell Transplantation

Allogeneic bone marrow transplantation with non-T-cell-depleted marrow from two HLA-antigen-mismatched related donors

Allogeneic BMT from 2 antigen-mismatched donors has been difficult to perform without T-cell depletion. Seven patients with advanced-stage hematologic malignancies underwent BMT with intensified graft-versus-host disease prophylaxis consisting of tacrolimus, methotrexate, and methylprednisolone. No rejection or acute GVHD ≥ grade III was observed. This preliminary result is very promising.

haematologica 2004; 89:373-375
(*http://www.haematologica.org/journal/2004/3/373*)

In a context of unrelated bone marrow transplantation (BMT), we recently demonstrated that a combination of tacrolimus (FK506), methotrexate, and methylprednisolone almost completely suppressed acute graft-versus-host disease (GVHD).[1] A transplant of non-T-cell-depleted marrow from a 1-antigen-mismatched related donor may be feasible depending on the recipient's condition.[2] The occurrence of severe acute GVHD was reported to be associated with the degree of HLA incompatibility between the donor and recipient.[3] According to Anasetti *et al.*, the difference in the incidence of severe GVHD between 1-HLA-antigen- and 2-HLA-antigen-mismatched BMT recipients was around 10%.[4] We considered it likely that this approximately 10% increase in the incidence of severe GVHD could be overcome by intensive GVHD prophylaxis with the combination of tacrolimus, methotrexate amd methylprednisolone.

The efficacy of this combination for the prophylaxis of GVHD was examined in 7 consecutive adult patients with hematologic malignancies who were transplanted using marrow from HLA-mismatched-related donors (2-antigen-mismatches in the graft-versus-host direction) without T-cell depletion in Osaka University Hospital. All the patients lacked an available HLA-identical related or HLA-phenotypically identical unrelated donor, and urgently needed transplantation. The characteristics of the patients are shown in Table 1A. The HLA disparities between donors and recipients are shown in Table 1B. Institutional review board approval was obtained for the treatment protocol, and written informed consent was obtained from the patients and their families.

All patients underwent intensified preconditioning regimens (Table 1A), and received an unmanipulated bone marrow graft. Tacrolimus was started on the day before transplantation and given at a dose of 0.03 mg/kg/day as a continuous infusion. Methotrexate was administered at the dose of 10 mg/m^2 intravenously on day 1 and at 7 mg/m^2 on days 3, 6, and 11 after grafting. Intravenous methylprednisolone was started at a dose of 2 mg/kg/day on day 1. In the absence of acute GVHD, the doses of tacrolimus and methylprednisolone were tapered as previously described.[1] Supportive care was given as previously described.[1]

All patients achieved donor type engraftment (Table 2). Three of the 7 patients did not develop clinical acute GVHD (Table 2). Three developed grade II acute GVHD at a median of day 25 (range, day 7 to 36). The acute GVHD was manifested by skin eruption preceded by a high fever and could be controlled by increasing the dose of methylprednisolone and adding mycophenolate mofetil. Two patients developed extensive chronic GVHD. One patient died of pneumonia caused by an *Aspergillus species*. One died of respiratory failure with thrombotic microangiopathy. One died of chronic GVHD-related multi-organ failure. Cytomegalovirus (CMV) antigenemia was found in 5 out of 7 patients (71.4%), but no patient developed CMV disease. All patients except for one achieved complete remission (CR) after BMT. Only 1 of 6 patients who achieved CR after BMT had a relapse (which occurred on day 284). Two patients remain in continuous CR 700 and 672 days after transplantation with 100% performance status. They have no signs of chronic GVHD under low doses of immunosuppressive agents (tacrolimus 1-2 mg/day and prednisone <10 mg/day).

Table 1A. Patients' characteristics.

N.	UPN	Disease	Age	Sex	Disease Status	Previous APBSCT	Donor	Preconditioning Regimen
1	204	ATLL	31	F	RR	yes	sibling(P)	CACyTBI
2	220	ALL	37	F	RR	no	daughter	CACyTBI
3	221	HD	26	F	RR	yes	sibling(P)	CACyTBI
4	224	AML	35	M	RR	no	sibling(M)	BuCACy
5	226	NHL	22	F	PR	no	sibling(M)	CACyTBI
6	228	AML	34	F	RR	no	mother	CACyTBI
7	234	AML	26	M	PR	no	mother	CACyTBI

ATLL: adult T-cell leukemia/lymphoma; ALL: acute lymphoblastic leukemia; HD: Hodgkin's disease; AML: acute myeloblastic leukemia; NHL: non-Hodgkin's lymphoma; RR: resistant relapse; PR: partial remission; APBSCT: autologous peripheral blood stem cell transplantation; CACyTBI: cytosine arabinoside + cyclophosphamide + total body irradiation; BuCACy: busulfan + cytosine arabinoside + cyclophosphamide. Sibling(P) and sibling(M) indicate non-inherited paternal antigen-mismatched sibling and non-inherited maternal antigen-mismatched sibling, respectively.

Table 1B. HLA disparities between donors and recipients.

N.	UPN	Recipient	Donor	N. of mismatched antigens (GVH/HVG)[a]
1	204	A26 B61 DR9/A11 B62 DR 4	A26 B59 DR4/A11 B62 DR4	2/1
2	220	A24 B52 DR15/A26 B62 DR9	A24 B60 DR4/A26 B62 DR9	2/2
3	221	A24 B61 DR14/A24 B54 DR15	A24 B39 DR9/A24 B54 DR15	2/2
4	224	A24 B67 DR12/A24 B52 DR15	A2 B35 DR8/A24 B52 DR15	2/3
5	226	A26 B54 DR4/A24 B35 DR4	A2 B46 DR 4/A24 B35 DR4	2/2
6	228	A2 B52 DR15/A11 B51 DR4	A2 B39 DR8/A11 B51 DR4	2/2
7	234	A24 B54 DR4/A26 B61 DR8	A24 B61 DR9/A26 B61 DR8	2/1

[a]GVH denotes the graft-versus-host direction and HVG the host-versus-graft (rejection) direction; [b]An HLA haplotype before or after a slash is one that is not or is shared between donors and recipients, respectively. HLA antigens that are underlined in recipients or in donors denote mismatched target antigens in the GVH or HVG reaction, respectively.

Table 2. Outcome of transplantation.

No.	UPN	Infused BM cells ($\times 10^8$/kg)	Days until ANC >0.5×10^9/L	Days until platelet >20×10^9/L	GVHD Acute	GVHD Chronic	Relapse	Status	Cause of death	Post-transplant (days)
1	204	5.15	19	30	I	Ex	no	dead	aspergillosis	329
2	220	4.22	20	108	0	–	yes	dead	relapse	583
3	221	3.17	19	101	II	–	NA	dead	tumor progression	227
4	224	2.60	27	152	0	Ex	no	dead	multi-organ failure	509
5	226	5.00	18	22	II	L	no	alive	–	+700
6	228	3.34	15	22	0	–	no	alive	–	+672
7	234	2.50	21	145	II	L	no	dead	TMA	514

ANC: absolute neutrophil count; NE: not evaluated; NA: not applicable (UPN 221 did not achieve CR after BMT); L: limited type; Ex: extensive type; TMA: thrombotic microangiopathy.

The intensified preconditioning regimen and GVHD prophylaxis with tacrolimus, methotrexate and methylprednisolone enabled stable engraftment of donor type and effectively prevented acute GVHD in non-T-cell-depleted BMT from related donors with 2-HLA-antigen mismatches. Basically, in order to achieve donor type engraftment, non-T-cell-depleted marrow has an advantage over T-cell-depleted marrow because the transplanted donor T cells contribute to the prevention of graft rejection.

Although all 7 of our patients were in an advanced stage at the time of transplantation, 6 survived more than 1 year, and only 2 died of tumor progression. Thus, in the HLA-mismatched, T-cell-repleted transplant setting, although severe GVHD is suppressed, a potent graft-versus-leukemia effect may remain. Indeed, several recent studies have shown that natural killer cell alloreactivity exerted by HLA-haploidentical grafts is associated with a significant graft-versus-leukemia effect, particularly in patients with acute myeloid leukemia.[5,6] In our study, only one donor-recipient pair showed mismatches of the killer inhibitory receptor epitopes.

In conclusion, allogeneic BMT using non-T-cell-depleted marrow from 2-HLA-antigen-mismatched related donors, and GVHD prophylaxis consisting of tacrolimus, methotrexate and methylprednisolone resulted in stable engraftment of donor type and effectively prevented the development of severe acute GVHD. This 3-drug GVHD prophylaxis regimen is likely to be more effective if used in patients in an early stage of disease.

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Key words: HLA-mismatched BMT, tacrolimus, methylprednisolone, graft-versus-host disease

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Stem Cell Transplantation

Reduced-intensity allogeneic stem cell transplantation for renal cell carcinoma: *in vivo* evidence of a graft-versus-tumor effect

We report the cases of 3 patients with advanced renal cell carcinoma who underwent reduced-intensity allogeneic stem cell transplantation. In 2 partial responders, histologic analyses of metastases revealed prominent accumulation of CD8⁺ T cells and degenerative changes of clear cell carcinoma, suggestive of induction of tumor-specific cytotoxic T lymphocytes.

haematologica 2004; 89:375-376
(*http://www.haematologica.org/2004/3/375*)

Recently, reduced-intensity allogeneic stem cell transplantation (RIST) has been introduced into the treatment of renal cell carcinoma (RCC).[1-5] We report the preliminary results of RIST in 3 patients (Table 1) with advanced RCC refractory to cytokine-based therapy, and show the histologic analyses before and after transplantation. The patients and donors gave written informed consent to participate in this institutionally approved investigational protocol. The preparative regimen, consisting of cyclophosphamide and fludarabine, was entirely based on a previously reported study and included cyclosporine (CSP).[1] Patients received granulocyte colony-stimulating factor-mobilized peripheral blood stem cells from their HLA-identical siblings on day 0. Following transplantation, the degree of donor-recipient chimerism in both myeloid and T-cell lineages was assessed by polymerase-chain reaction assay according to a published method.[1]

All three patients achieved sustained myeloid and platelet engraftment with the proportion of donor cells in the peripheral blood exceeding 80% for both T cells and granulocytes within 2 months. We observed 2 partial responses in patients #1 and #3 six months and eight months after transplantation respectively: one response occurred after the development of chronic graft-versus-host disease (GVHD) and the other after acute GVHD: both coincided with full donor T-cell chimerism. Thereafter, the disease in patient #1 remained stable while GVHD responded to treatment with low-dose CSP plus steroids. Unfortunately, this patient died of bacterial pneumonia on day 554. In patient #3, chronic GVHD of the skin, salivary glands, and lung required treatment with CSP and steroids. Twenty months after transplantation, metastases started to grow despite a lack of change in GVHD. Reducing CSP and steroids caused acute respiratory failure due to chronic lung GVHD, though some regression of RCC metastases was observed. This

Table 1. Characteristics of the patients and outcome of transplantation.

Patient no.	*Age (yr)/sex*	*Histology*	*Sites of metastases*	*No. of previous systemic treatments*	*Nephrectomy*	*Age (yr)/sex of donor*	*CD34⁺ cells kg infused (×10⁶)*	*No. of CD3⁺ cells/kg infused (×10⁸)*	*GVHD*	*Response*	*Outcome*
1	64/M	Clear cell	Lung, pleura, bone, nodes	3	Yes	69/M	5.6	3.0	Extensive chronic skin, oral, salivary	PR	SD; died of pneumonia on day 554
2	58/F	Papillary	Pleura, liver, adrenal, nodes	2	No	59/M	9.7	4.3	Acute grade 3 skin, liver, GI	PD	Died of disease progression on day 68
3	56/M	Clear cell	Bone, lung, pleura, adrenal, nodes disease	3	Yes	51/F	4.9	2.4	Acute grade 2 skin, GI, extensive chronic skin, salivary, lung	PR	Died of progressive and GVHD on day 709

M: male; F: female; GVHD: graft-versus-host disease; GI: gastrointestinal; PR: partial response; SD: stable disease; PD: progressive disease.