Table I. Characteristics of subjects according to human leucocyte antigen-matching status.

| | Identical ($n = 250$) | One-mismatched ($n = 57$) | Two or more mismatched ($n = 34$) | $P$-value |
|---|---|---|---|---|
| Recipient age (range, median) | 16–70, 54 | 25–61, 50·5 | 21–60, 46·5 | 0·03 |
| Recipient sex, female:male (unknown) | 109:141 | 43:14 | 20:14 | 0·007 |
| Previous history of HCT, no:yes | 205:45 | 27:9 | 36:19 | 0·64 |
| Disease | | | | |
| Acute leukaemia | 73 | 17 | 16 | |
| Chronic myeloid leukaemia | 16 | 3 | 2 | |
| Myelodysplastic syndrome | 48 | 18 | 8 | |
| Malignant lymphoma | 90 | 15 | 5 | |
| Multiple myeloma | 23 | 4 | 3 | 0·163 |
| Risk status | | | | |
| Standard | 52 | 8 | 3 | |
| High | 198 | 49 | 31 | 0·154 |
| HCT type | | | | |
| PBSC | 236 | 55 | 32 | |
| BM | 14 | 2 | 2 | 0·805 |
| Donor | | | | |
| Sibling | 250 | 28 | 8 | |
| Family | 0 | 29 | 26 | |
| Year of transplant | | | | |
| <2000 | 5 | 1 | 1 | |
| 2000+ | 245 | 56 | 33 | 0·922 |
| TBI | | | | |
| No | 213 | 44 | 25 | |
| Yes | 37 | 13 | 9 | 0·117 |
| ATG | | | | |
| No | 236 | 36 | 20 | |
| Yes | 14 | 21 | 14 | <0·001 |
| GVHD prophylaxis | | | | |
| CSP alone | 99 | 8 | 10 | |
| CSP + MTX | 122 | 30 | 10 | |
| FK + MTX | 5 | 13 | 12 | |
| Other | 24 | 6 | 2 | <0·001 |

HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, antithymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.

21–86%) of those who received a graft from an HLA-C mismatched donor. Although this difference was not statistically significant ($P = 0·261$), the impact of HLA-C mismatch on the incidence of aGVHD remains to be elucidated because of limited numbers of subjects for evaluation.

### *CGVHD*

Recipients of HLA-matched RICT ($n = 156$) and 42 recipients of HLA-mismatched RICT (one-locus mismatch, 32; two- to three-loci mismatch, 10) were evaluable for cGVHD. The cumulative incidence of cGVHD was 61% (95% CI, 52–67%), 69% (95% CI, 44–77%) and 67% (95% CI, 28–88%) after HLA-matched RICT, HLA one-locus-mismatched RICT and two- to three-loci-mismatched RICT respectively (Fig 2). The cumulative incidence of extensive cGVHD was 38%, 34% and 60% after HLA-matched RICT, HLA one-locus and two- to three-loci-mismatched RICT respectively. There was no significant difference between the three groups regarding the incidence of cGVHD or extensive cGVHD. Similarly, there was no significant difference between the groups in the incidence of *de novo*, quiescent and progressive cGVHD. As shown in Table III, a tendency for cGVHD was observed with increased HLA disparity in multivariate analysis ($P = 0·05$). In addition, use of PBSC grafts, no use of ATG, and high-risk disease were identified as independent risk factors for cGVHD. In patients receiving PBSC grafts, there was no association between the numbers of $CD34^+$ cells and the development of cGVHD ($P = 0·613$; HR, 0·95; 95% CI, 0·76–118).

### *Graft failure*

The incidence of graft failure was 3·7% (95% CI, 1·7–6·9%) in recipients with an HLA-matched donor, 5·7% (95% CI,


 

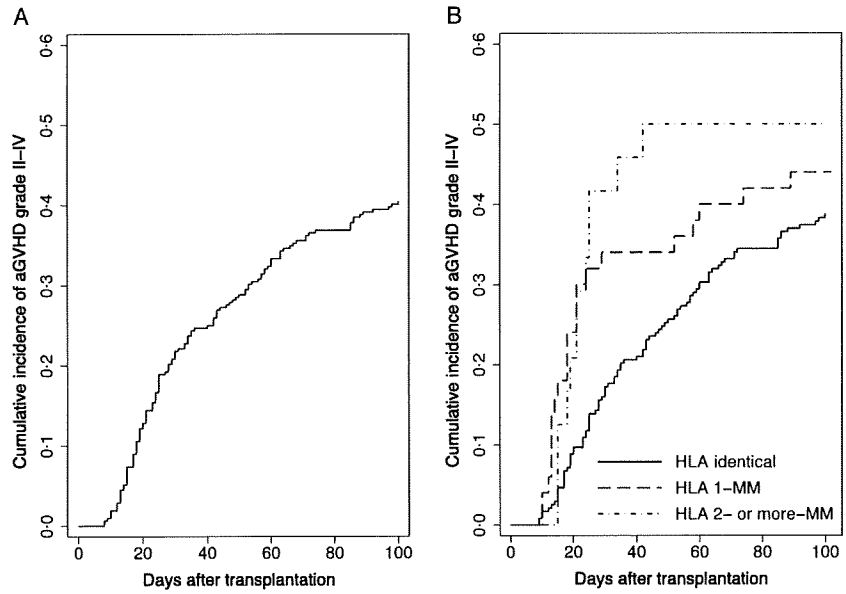


Fig 1. Incidence of grade II–IV acute graft-*versus*-host disease (aGVHD). The curves represent the cumulative incidence of grade II–IV aGVHD in patients with haematological malignancies following reduced-intensity conditioning transplant regimen from a related donor as a function of time after transplantation (A) for all available subjects ($n$ = 312) and (B) in relation to the extent of human leucocyte antigen mismatch (identical, $n$ = 238; one-locus mismatch, $n$ = 22 and two to three-loci mismatch, $n$ = 7).

1·2–15·7%) in those with a one-locus-mismatched donor, and 10·3% (95% CI, 2·2–27·4%) in those with a two- to three-loci-mismatched donor. Multivariate analysis revealed a significant increase of graft failure in patients who received a graft from a two- to three-loci-mismatched donor (HR, 8·58; 95% CI, 1·37–53·9; $P$ = 0·022, Table IV), and the extent of HLA mismatch between the donor and recipient was a continuous variable with respect to the incidence of graft failure ($P$ = 0·033). Use of ATG did not significantly influence the incidence of rejection after RICT ($P$ = 0·166).

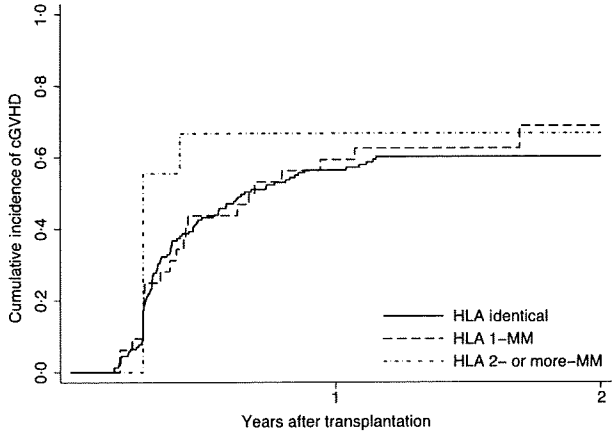


Fig 2. Incidence of chronic graft-*versus*-host disease (cGVHD). The curves represent the cumulative incidence of cGVHD in patients with haematological malignancies following reduced-intensity conditioning transplant regimen from a related donor as a function of time after transplantation in relation to the extent of human leucocyte antigen mismatch (identical, $n$ = 156; one-locus mismatch, $n$ = 32 and two-to three-loci mismatch, $n$ = 10).

### *Survival*

To elucidate the impact of HLA mismatch on transplant outcome, OS was analysed. With a median follow-up of 347 d, OS in patients who received a graft from an HLA-matched donor, a one-locus-mismatched donor and a two-to three-loci-mismatched donor was 48% (95% CI, 42–54%), 51% (95% CI, 39–61%) and 18% (95% CI, 7–32%), respectively, at 2 years after RICT. OS after HLA one-locus-mismatched RICT was comparable with that after HLA-matched RICT (Fig 3). However, OS after two- to three-loci-mismatched RICT was significantly worse post-transplant compared with that after HLA-matched RICT. Multivariate analysis identified two- to three-loci HLA mismatch (HR, 3·41; 95% CI, 2·03–5·73; $P$ < 0·001), previous history of haematopoietic cell transplantation (HR, 1·42; 95% CI, 1·00–2·02; $P$ = 0·052), and high-risk disease (HR, 2·06; 95% CI, 1·33–3·30; $P$ = 0·002) as independent risk factors for shorter survival (Table V). As only three patients with standard-risk disease received RICT from a two- to three-loci-mismatched donor, the relationship between multiple HLA incompatibility and OS was further evaluated in patients with high-risk diseases and those with standard diseases separately. In high-risk patients, the 2-year OS was 15% (95% CI, 5–30%), 52% (95% CI, 40–63%) and 43% (95% CI, 36–49%) in recipients with a two- to three-loci-mismatched donor, one-locus-mismatched donor and matched donor respectively. HLA

Table II. Uni- and multivariate analyses for possible risk factors for acute GVHD of grade II or more.

| | Evaluable ($n$ = 313) | | | | | |
|---|---|---|---|---|---|---|
| | Univariate | | | Multivariate | | |
| | HR | 95% CI | $P$-value | HR | 95% CI | $P$-value |
| HLA | | | | | | |
| Identical | 1·00 | | | 1·00 | | |
| 1-MM | 1·29 | 0·81–2·05 | 0·282 | 1·83 | 1·04–3·22 | 0·035 |
| 2 or more MM | 1·72 | 0·94–3·14 | 0·079 | 2·44 | 1·14–5·21 | 0·021 |
| | | Trend | 0·068 | | Trend | 0·010 |
| HCT type | | | | | | |
| PBSC | 1·00 | | | 1·00 | | |
| BM | 0·65 | 0·26–1·59 | 0·342 | 0·70 | 0·28–1·73 | 0·441 |
| Previous history of HCT | | | | | | |
| No | 1·00 | | | 1·00 | | |
| Yes | 1·15 | 0·75–1·77 | 0·52 | 0·90 | 0·57–1·44 | 0·663 |
| Recipient age (years) | | | | | | |
| <60 | 1·00 | | | 1·00 | | |
| ≥60 | 0·90 | 0·57–1·42 | 0·656 | 0·94 | 0·59–1·50 | 0·808 |
| Recipient sex | | | | | | |
| Female | 1·00 | | | 1·00 | | |
| Male | 1·16 | 0·82–1·66 | 0·401 | 1·28 | 0·88–1·84 | 0·196 |
| TBI | | | | | | |
| No | 1·00 | | | 1·00 | | |
| Yes | 0·96 | 0·60–1·55 | 0·881 | 0·77 | 0·43–1·38 | 0·386 |
| ATG | | | | | | |
| No | 1·00 | | | 1·00 | | |
| Yes | 0·85 | 0·50–1·44 | 0·543 | 0·55 | 0·29–1·02 | 0·057 |
| GVHD prophylaxis | | | | | | |
| CSP alone | 1·00 | | | 1·00 | | |
| CSP + MTX | 0·74 | 0·50–1·09 | 0·124 | 0·70 | 0·46–1·04 | 0·079 |
| FK + MTX | 1·07 | 0·57–2·02 | 0·826 | 0·70 | 0·32–1·50 | 0·355 |
| Other | 1·12 | 0·61–2·06 | 0·724 | 1·37 | 0·65–2·90 | 0·410 |
| Risk | | | | | | |
| Standard | 1·00 | | | 1·00 | | |
| High | 1·52 | 0·93–2·47 | 0·095 | 1·43 | 0·87–2·36 | 0·159 |

GVHD, graft-*versus*-host disease; HLA, human leucocyte antigen; MM, mismatched; HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, antithymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.

Multivariable adjusted for all variables listed.

two- to three-loci mismatch, but not one-locus mismatch, was again a risk factor for shorter survival ($P < 0·0001$, Fig 4A). In standard-risk patients, the 2-year OS was 73% (95% CI, 58–84%), 40% (95% CI, 12–67%) and 38% (95% CI, 1–81%) in recipients with a matched, one-locus-mismatched donor and two- to three-loci-mismatched donor respectively (Fig 4B). In contrast to high-risk disease, one-locus mismatch was a risk factor for shorter survival in patients with standard-risk disease ($P = 0·079$).

Of the 178 patients who died following RICT, 90 deaths were directly attributed to disease progression or relapse. Non-relapse mortality was 49%, including 60 deaths (34%) because of infection and/or GVHD, 22 deaths (12%) from other transplant-related toxicities and six deaths (3%) from other diseases. There was no difference in the cause of death between HLA-matched RICT and HLA-mismatched RICT (data not shown).

## Discussion

In this study, we found that HLA disparity was a continuous and independent risk factor for grade II–IV aGVHD and cGVHD as well as graft failure following RICT. Furthermore, two- to three-loci HLA mismatch was a risk factor for survival, and one-locus mismatch was a risk factor for survival in patients with standard-risk disease, but not in those with high-risk disease. Our finding, that HLA disparity impacts on grade II–IV aGVHD in patients receiving RICT, was quite consistent



 

Table III. Uni- and multivariate analyses for possible risk factors for chronic GVHD.

| | Evaluable ($n$ = 198) | | | | | |
|---|---|---|---|---|---|---|
| | Univariate | | | Multivariate | | |
| | HR | 95% CI | $P$-value | HR | 95% CI | $P$-value |
| HLA | | | | | | |
| Identical | 1·00 | | | 1·00 | | |
| 1-MM | 1·09 | 0·68–1·76 | 0·709 | 1·57 | 0·89–2·75 | 0·116 |
| 2 or more MM | 1·25 | 0·55–2·85 | 0·6 | 2·20 | 0·84–5·75 | 0·108 |
| | | Trend | 0·543 | | Trend | 0·050 |
| HCT type | | | | | | |
| PBSC | 1·00 | | | 1·00 | | |
| BM | 0·31 | 0·10–0·97 | 0·044 | 0·28 | 0·88–0·90 | 0·032 |
| Previous history of HCT | | | | | | |
| No | 1·00 | | | 1·00 | | |
| Yes | 0·90 | 0·56–1·42 | 0·641 | 0·75 | 0·45–1·26 | 0·274 |
| Recipient age (years) | | | | | | |
| <60 | 1·00 | | | 1·00 | | |
| ≥60 | 1·31 | 0·86–1·99 | 0·214 | 1·16 | 0·74–1·81 | 0·512 |
| Recipient sex | | | | | | |
| Female | 1·00 | | | 1·00 | | |
| Male | 1·18 | 0·82–1·69 | 0·375 | 1·27 | 0·87–1·87 | 0·221 |
| TBI | | | | | | |
| No | 1·00 | | | 1·00 | | |
| Yes | 0·76 | 0·46–1·24 | 0·265 | 0·61 | 0·33–1·11 | 0·104 |
| ATG | | | | | | |
| No | 1·00 | | | 1·00 | | |
| Yes | 0·66 | 0·36–1·23 | 0·194 | 0·42 | 0·20–0·85 | 0·016 |
| GVHD prophylaxis | | | | | | |
| CSP alone | 1·00 | | | 1·00 | | |
| CSP + MTX | 1·10 | 0·72–1·68 | 0·67 | 1·00 | 0·63–1·56 | 0·966 |
| FK + MTX | 0·76 | 0·37–1·55 | 0·444 | 0·53 | 0·23–1·24 | 0·143 |
| Other | 0·98 | 0·51–1·88 | 0·961 | 1·36 | 0·63–2·96 | 0·435 |
| Risk | | | | | | |
| Standard | 1·00 | | | 1·00 | | |
| High | 1·67 | 1·06–2·64 | 0·027 | 1·74 | 1·09–2·79 | 0·021 |

GVHD, graft-*versus*-host disease; HLA, human leucocyte antigen; MM, mismatch; HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, antithymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.
Multivariable adjusted for all variables listed.

Table IV. Uni- and multivariate analyses for host-*versus*-graft human leucocyte antigen mismatching on graft failure risk.

| | Evaluable ($n$ = 325) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Univariate | | | Multivariate | | |
| | Rejected | Not-rejected | HR | 95% CI | $P$-value | HR | 95% CI | $P$-value |
| Identical | 9 | 234 | 1·00 | Reference | | 1·00 | Reference | |
| 1-MM | 3 | 50 | 1·6 | 0·42–6·12 | 0·337 | 1·18 | 0·19–7·26 | 0·855 |
| 2 or more MM | 3 | 26 | 3·01 | 0·77–11·8 | 0·114 | 8·58 | 1·37–53·9 | 0·022 |
| | | | | Trend | 0·035 | | Trend | 0·033 |

HR, hazard ratio; MM, mismatch.
Multivariate analysis adjusted for age, sex, donor type, previous history of haematopoietic cell transplantation, total body irradiation conditioning, antithymocyte globulin conditioning, graft-*versus*-host disease prophylaxis and risk status.

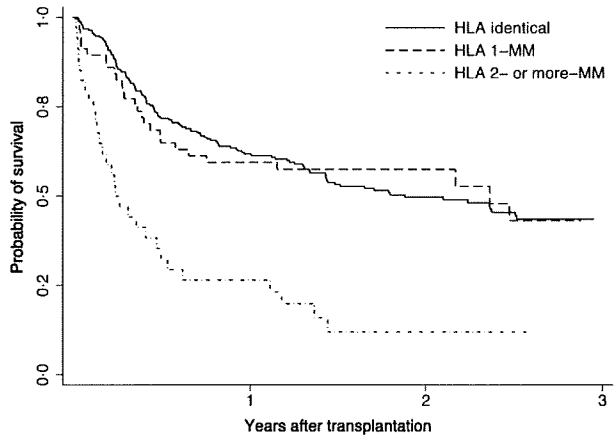


Fig 3. Overall survival (OS) based on the extent of human leucocyte antigen (HLA) mismatch. The curves represent OS in patients with haematological malignancies following reduced-intensity conditioning transplant regimen from a related donor as a function of time after transplantation in relation to the extent of HLA mismatch (identical, $n = 250$; one-locus mismatch, $n = 56$ and two to three-loci mismatch, $n = 34$).

with earlier findings in conventional myeloablative SCT (Beatty *et al*, 1985; Ringden & Nilsson, 1985; Anasetti *et al*, 1990; Anasetti & Hansen, 1994; Sasazuki *et al*, 1998; Morishima *et al*, 2002; Kanda *et al*, 2003), but has not been well described in the setting of RICT.

The cumulative incidence of grade II–IV aGVHD after HLA-matched RICT was 39% in this study population, which is similar to that in recent reports from other groups following RICT (Levine *et al*, 2003; Martino *et al*, 2003; Wong *et al*, 2003; Bacigalupo, 2004; Diaconescu *et al*, 2004; Goggins & Rizzieri, 2004), although a recent retrospective comparison of myeloablative SCT with RICT conditioned with 2 Gy TBI and fludarabine showed less aGVHD after RICT in matched unrelated donor transplants (Sorror *et al*, 2004).

It was initially assumed that the incidence and severity of aGVHD might decrease after RICT compared with conventional SCT. Pretransplant conditioning can activate host tissues to secrete inflammatory cytokines and amplify GVHD (Xun *et al*, 1994). The relationship between conditioning intensity, inflammatory cytokine and GVHD severity was further supported by animal models (Hill *et al*, 1997) and clinical observation (Gale *et al*, 1987; Clift *et al*, 1990; Deeg *et al*, 1991). In experimental models, the development of mixed donor–host chimaerism may facilitate the establishment of anti-host tolerance (Colson *et al*, 1996; Manilay *et al*, 1998). In contrast, minimally cytotoxic conditioning may enable the persistence of host antigen-presenting cells, which would enhance presentation of host alloantigens to donor T cells (Shlomchik *et al*, 1999; Teshima *et al*, 2002; Duffner *et al*, 2004).

There are several possible explanations that can account for this unexpectedly high incidence of aGVHD after RICT. First, the median age of patients who underwent RICT in the current study was 53 years, which was much higher than that among patients who received conventional SCT. A greater age has been associated with an increased risk for GVHD after conventional SCT (Ringden & Nilsson, 1985; Gale *et al*, 1987; Anasetti *et al*, 1990; Weisdorf *et al*, 1991; Nash *et al*, 1992). Secondly, CSP alone was administered for GVHD prophylaxis in one-third of the patients in this study, whereas a combination of two agents was exclusively used in conventional SCT. Thirdly, the RIC regimens used in our study were more intensive than the non-myeloablative conditioning regimens used by the Seattle group (Sorror *et al*, 2004). These differences may counterbalance the potential beneficial aspects of reducing the intensity of conditioning. Nonetheless, the onset of GVHD was delayed in RICT; 15% of aGVHD developed between days 60 and 100 in our study, as previously reported (Mielcarek *et al*, 2003). Following conventional SCT, most of aGVHD develops within 50 d post-transplant (Snover, 1984; Beatty *et al*, 1985; Sasazuki *et al*, 1998; Morishima *et al*, 2002; Kanda *et al*, 2003).

The incidence of aGVHD rose with increasing HLA mismatch, from match through multi-loci mismatch, following RICT. In addition, the onset of aGVHD was earlier with increasing HLA mismatch. These findings are consistent with data from the myeloablative setting (Beatty *et al*, 1985; Ringden & Nilsson, 1985; Anasetti *et al*, 1990; Anasetti & Hansen, 1994; Petersdorf *et al*, 1998; Sasazuki *et al*, 1998; Morishima *et al*, 2002; Kanda *et al*, 2003). Studies from the Japan Marrow Donor Programme and others have demonstrated the importance of HLA-C mismatching in rejection, GVHD and mortality in myeloablative SCT from unrelated donors (Petersdorf *et al*, 2001, 2004; Morishima *et al*, 2002; Flomenberg *et al*, 2004; Sasazuki *et al*, 1998). We did not find a significant association between HLA-C mismatch and the development of aGVHD, but this association needs to be further investigated in a larger prospective study because only 75 donor–recipient pairs were available for analysis in this study.

We found that the use of ATG was associated with a reduction in both acute and chronic GVHD without an increased risk of graft failure, as previously shown in studies using alemtuzumab or ATG (Kottaridis *et al*, 2000; Khouri *et al*, 2001; Mohty *et al*, 2003; Nakai *et al*, 2003; Faulkner *et al*, 2004). Initial clinical trials of matched unrelated RICT or haploidentical RICT appear to be encouraging with the use of T-cell depletion (Sykes *et al*, 1999; Kottaridis *et al*, 2000; Giralt *et al*, 2001; Nagler *et al*, 2001; Chakraverty *et al*, 2002; Maris *et al*, 2003; Niederwieser *et al*, 2003; Wong *et al*, 2003; Goggins & Rizzieri, 2004). Mohty *et al* (2003) reported that a high $CD34^+$ cell dose was associated with an increased incidence of chronic, but not acute, GVHD following RICT with G-CSF-mobilised PBSC. We did not find an association between acute or chronic GVHD and the number of $CD34^+$ cells infused. Interestingly, in contrast to data from a myeloablative setting (Ringden & Nilsson, 1985; Gale *et al*, 1987; Anasetti *et al*, 1990; Weisdorf *et al*, 1991; Nash *et al*, 1992), there was no

Table V. Uni- and multivariate analyses for possible risk factors for overall survival.

| | Evaluable ($n$ = 286) | | | | | |
|---|---|---|---|---|---|---|
| | Univariate | | | Multivariate | | |
| | HR | 95% CI | $P$-value | HR | 95% CI | $P$-value |
| HLA | | | | | | |
| Identical | 1·00 | | | 1·00 | | |
| 1-MM | 1·01 | 0·67–1·53 | 0·966 | 1·03 | 0·64–1·66 | 0·899 |
| 2 or more MM | 3·21 | 2·14–4·82 | <0·001 | 3·41 | 2·03–5·73 | <0·001 |
| | | Trend | <0·001 | | Trend | <0·001 |
| HCT type | | | | | | |
| PBSCT | 1·00 | | | 1·00 | | |
| BMT | 1·19 | 0·63–2·25 | 0·598 | 1·53 | 0·79–2·94 | 0·205 |
| Previous history of HCT | | | | | | |
| No | 1·00 | | | 1·00 | | |
| Yes | 1·64 | 1·18–2·28 | 0·003 | 1·42 | 1·00–2·02 | 0·052 |
| Recipient age (years) | | | | | | |
| <60 | 1·00 | | | 1·00 | | |
| ≥60 | 1·05 | 0·73–1·51 | 0·808 | 1·09 | 0·75–1·60 | 0·65 |
| Recipient sex | | | | | | |
| Female | 1·00 | | | 1·00 | | |
| Male | 1·29 | 0·96–1·74 | 0·095 | 1·19 | 0·88–1·61 | 0·264 |
| TBI | | | | | | |
| No | 1·00 | | | 1·00 | | |
| Yes | 1·25 | 0·86–1·82 | 0·239 | 1·28 | 0·84–1·94 | 0·25 |
| ATG | | | | | | |
| No | 1·00 | | | 1·00 | | |
| Yes | 1·15 | 0·77–1·72 | 0·482 | 0·71 | 0·44–1·16 | 0·177 |
| GVHD prophylaxis | | | | | | |
| CSP alone | 1·00 | | | 1·00 | | |
| CSP + MTX | 0·92 | 0·66–1·27 | 0·614 | 0·89 | 0·64–1·24 | 0·488 |
| FK + MTX | 1·17 | 0·70–1·98 | 0·546 | 0·87 | 0·48–1·57 | 0·64 |
| Other | 0·89 | 0·52–1·51 | 0·66 | 0·69 | 0·38–1·25 | 0·223 |
| Risk | | | | | | |
| Standard | 1·00 | | | 1·00 | | |
| High | 2·21 | 1·42–3·43 | <0·001 | 2·06 | 1·31–3·25 | 0·002 |

HLA, human leucocyte antigen; MM, mis-match; HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, anti-thymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.
Multivariable adjusted for all variables listed.

increase in the incidence of aGVHD in elderly patients, as previously reported (Mohty *et al*, 2002; Wong *et al*, 2003). It has been shown in experimental models that donor T-cell responses are enhanced under stimulation with antigen-presenting cells from older mice in the context of proinflammatory milieu (Ordemann *et al*, 2002). The absence of excessive inflammation in RICT may be associated with a similar incidence of GVHD in aged recipients following RICT.

The incidence of cGVHD was similar to that following myeloablative HSCT in Japan (Kanda *et al*, 2003). A greater incidence of cGVHD was observed with increased HLA disparity, although the difference was marginal. Chronic GVHD was influenced by the use of ATG, disease status and the stem cell source, with PBSC grafts having a fourfold risk of cGVHD. It has been shown that the use of PBSC grafts instead of marrow increases the frequency of cGVHD following myeloablative HSCT (Blaise *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001). Our study suggest that the use of PBSC grafts is also a risk factor for cGVHD following RICT, although there was a large difference between the number of recipients of a PBSC graft and those who received a bone marrow (BM) graft.

In a conventional allogeneic transplant setting, the incidence of graft failure has been shown to be correlated with the degree of HLA mismatch between donors and recipients (Beatty *et al*, 1985; Anasetti *et al*, 1989; Petersdorf *et al*, 1998, 2001; Petersdorf *et al*, 1997). We found that HLA mismatch was also a risk factor for graft failure following RICT. The incidence of graft failure after HLA-matched RICT was comparable with that after conventional SCT in Japan

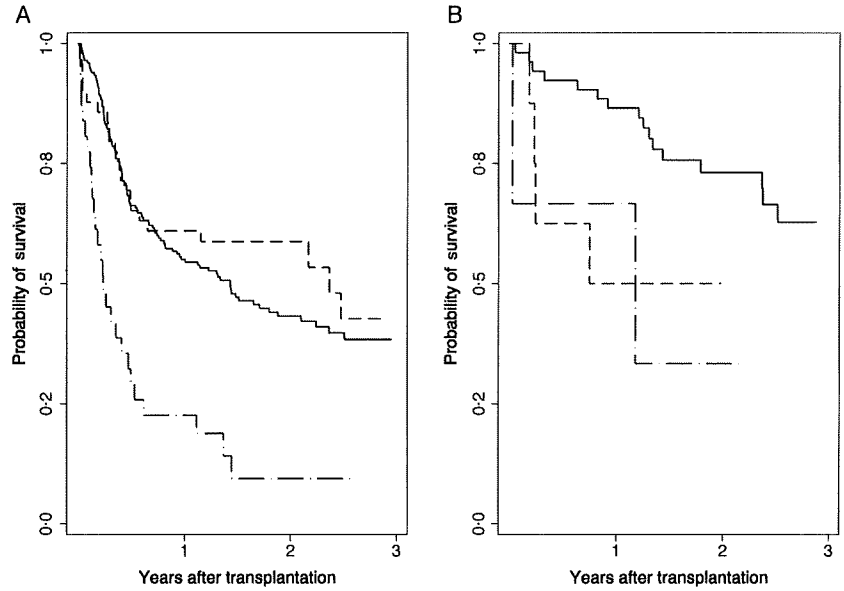


Fig 4. Overall survival (OS) according to the extent of human leucocyte antigen (HLA) mismatch and disease status. The curves represent OS as a function of time after transplantation in relation to the extent of HLA mismatch in (A) patients with a high-risk disease (total, $n = 277$; identical, $n = 198$; one-locus mismatch, $n = 48$ and two to three-loci mismatch, $n = 31$); (B) those with standard-risk disease (total, $n = 63$; identical, $n = 52$; one-locus mismatch, $n = 3$ and two- to three-loci mismatch, $n = 8$).

(Morishima *et al*, 2002; Kanda *et al*, 2003). Thus, the RIC regimens used in this study appear to have been sufficient for achieving donor cell engraftment in these patients. However, we found that the risk of rejection was extremely high (10·8%) in patients who received a graft from a two- to three-loci-mismatched donor in the HVG vector and myeloablative conditioning should be considered in this setting. Previous reports have demonstrated that the incorporation of low-dose TBI in the conditioning or the use of PBSC could reduce the incidence of graft failure (Deeg *et al*, 2001; Maris *et al*, 2003). These associations were not observed in our study probably because rate of graft failure was too low to detect a significant decrease.

The most important factor that affected OS after RICT was multiple HLA mismatch in patients with haematological malignancies. RICT from a two- to three-loci-mismatched donor resulted in a poor outcome, as has been shown in conventional SCT (Beatty *et al*, 1985; Hows *et al*, 1993; Szydlo *et al*, 1997). However, the 2-year OS after one-locus-mismatched RICT was comparable with that after HLA-matched RICT. When stratified according to disease status, one-locus mismatch was a risk factor for survival in patients with standard-risk disease, but not in those with high-risk disease. These results suggest that RICT from a one-locus-mismatched related donor may be warranted in patients with high-risk haematological malignancies when an HLA-matched sibling donor is not available. In previous studies, a younger age of patients (Faulkner *et al*, 2004) and the use of PBSC (Maris *et al*, 2003) were associated with a superior outcome after RICT, but these factors did not influence OS in the present study. However, the present study has several limitations. First, there was a large difference between the number of recipients with an HLA-matched donor and those with an HLA-mismatched donor. Secondly, since the follow-up period was short following RICT, it is too early to determine long-term outcome to these treatment regimens. Thirdly, HLA-C typing and high resolution DNA typing was not routinely performed in HSCT from a related donor.

Nonetheless, the large cohort of patients in the current study allowed us to make several important observations. First, HLA mismatch between the donor and recipient is an important risk factor for graft failure, aGVHD, cGVHD and OS after RICT. Secondly, RICT from a one-locus-mismatched related donor may represent an alternative approach in patients with high-risk haematological malignancies who lacked an HLA-matched sibling donor. These findings should be carefully confirmed in a prospective study.

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Stem Cell Transplantation

## Graft-versus-leukemia effect of allogeneic stem cell transplantation; a Japanese single center study

To clarify graft-versus-leukaemia effect of graft-versus-host disease, we studied 166 patients treated with allogeneic stem cell transplantation for haematologic malignancies. The cumulative incidence of relapse in patients with acute GVHD was significantly lower than that in patients without acute GVHD, but there was no similar GVL effect for chronic GVHD.

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The therapeutic effect of allogeneic haematopoietic stem cell transplantation (SCT) was previously assumed to be produced by high-dose chemoradiotherapy. Weiden *et al.* first demonstrated the favorable effect of graft-versus-host disease (GVHD) on leukemic relapse, and subsequent reports have confirmed such a graft-versus-leukemia (GVL) effect.[1-5] Although several reports documented a GVL effect associated with chronic GVHD (cGVHD), some patients relapse with leukemia despite developing extensive cGVHD. To clarify these issues, we studied 209 patients who underwent allogeneic SCT at our hospital for treatment of haematologic malignancies.

Table 1A summarizes patient characteristics. Of 166 patients surviving more than 30 days after SCT, 71 patients (43%) developed acute GVHD (aGVHD) on day 9~67 (median; 25). Ninety-five percent of aGVHD occurred within 40 days after SCT. The number of aGVHD+ patients was 39 for grade I, 17 for grade II, 11 for grade III and 4 for grade IV. The incidence of aGVHD+ patients from HLA-matched sibling donors (38%) was comparable to those from HLA-matched unrelated donors (59%) and HLA-mismatched sibling donors (44%). cGVHD appeared in 87 of 158 (55%) patients. The onset type of cGVHD was progressive in 9, quiescent in 16 and *de novo* in 62 patients. Relapse occurred in 47 of 166 (28%) patients, 3~102 months after SCT. Cox regression multivariate analysis showed that none of the factors affected the relapse rate in standard-risk patients while aGVHD significantly lowered the relapse rate in high-risk patients (Table 1B). Table 1C shows the relationship between aGVHD and relapse. The relapse rate was significantly lower in aGVHD ≥ grade II patients (6%), but not in cGVHD+ patients analyzed by the Fisher's exact probability test ($p<0.001$). *De novo* onset cGVHD did not reduce the rate of relapse either; 19 of 62 (31%) patients with *de novo* cGVHD relapsed and 23 of 68 (34%) without *de novo* cGVHD did. The cumulative incidence of relapse that was evaluated in patients who survived more than 100 days after SCT was significantly lower in aGVHD ≥ grade II patients than the other (Figure 1A, $p<0.05$). Estimated 5-year survival was comparable between aGVHD ≥ grade II (69%) and aGVHD-patients (75%) as well as between in cGVHD+ patients (64.0%), and in cGVHD-patients (63.9%). Figure 1b shows the overall survival in high-risk patients according to different grades of aGVHD; the 5-year probability of survival was 71% in aGVHD ≥ grade II patients, while it was 52% in the other ($p=0.17$). The probability of survival of patients who developed aGVHD ≥ grade I (66%) was significantly higher than patients without aGVHD, $p<0.05$).

This study revealed the low relapse rates amongst

**Table 1A. Patients' characteristics.**

| | |
|---|---|
| *No. of patients* | *166* |
| Gender (male/female) | 102/64 |
| Median age (range) | 24 (1-59) |
| Donor | |
| Sibling, HLA-matched | 123 |
| Sibling, HLA-mismatched | 9 (1 locus 8, 2 loci 1) |
| Unrelated, matched | 34 |
| Diagnosis | |
| AML | 57 |
| ALL | 42 |
| CML | 41 |
| NHL | 17 |
| MDS | 9 |
| Disease status at transplantation | |
| CR | 117 (incl. CML-CP 33) |
| non-CR | 49 (incl. CML-AP 5, BC 3) |
| Source of graft | |
| Bone marrow | 140 |
| PBSC | 25 |
| Cord blood | 1 |
| Conditioning regimen | |
| Chemotherapy + TBI | 115 |
| BU + CY (+ Ara-C) | 49 |
| Flu + CY | 2 |
| GVHD prophylaxis | |
| None | 20 |
| Short MTX + CyA | 127 |
| MTX | 9 |
| CyA | 3 |
| FK506 | 7 |

*AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia, NHL, Non-Hodgkin's lymphoma; MDS, myelodysplastic syndrome; CR, complete remission; CP, chronic phase; AP, accelerated phase; BC, blastic crisis; PBSC, peripheral blood stem cell; TBI, total body irradiation; BU, busulfan; CY, cyclophosphamide; Ara-C, cytarabine; Flu, fludarabine; MTX, methotrexate; CyA, cyclosporine A; FK506, tacrolimus.*

**Table 1B. Multivariate analysis on factors affecting relapse after transplantation.**

| *Characteristics* | *Relative risk of relapse* | *95% CI* | *P value* |
|---|---|---|---|
| Standard-risk (n=79) | | | |
| Sex, female | 1,27 | 0.95-1.70 | 0.4-0.3 |
| Conditioning, including TBI | 1,19 | 0.71-2.01 | 0.4-0.3 |
| aGVHD, I-IV | 1,43 | 0.81-2.54 | 0.2-0.1 |
| cGVHD | 1,32 | 0.73-2.39 | 0.4-0.3 |
| PBSCT | 1,20 | 0.54-2.65 | 0.4-0.3 |
| High-risk (n=79) | | | |
| Sex, female | 1,25 | 0.87-1.79 | 0.2-0.1 |
| Conditioning, including TBI | 1,15 | 0.76-1.76 | 0.4-0.3 |
| aGVHD, I-IV | 0,57 | 0.38-0.85 | <0.005 |
| cGVHD | 0,90 | 0.57-1.40 | 0.4-0.3 |
| PBSCT | 0,79 | 0.44-1.42 | 0.3-0.2 |

*TBI: total body irraditation.*

**Table 1C. Effect of GVHD on the rate of relapse after SCT.**

| | | | | | |
|---|---|---|---|---|---|
| | | *Acute GVHD* *aGVHD* | | | |
| Status at SCT | Yes | | No | | |
| Standard-risk | 0% | (0/13) | 28% | (19/69) | p<0.03 |
| High-risk | 11% | (2/19) | 40% | (26/65) | p<0.02 |
| | | *Chronic GVHD* *cGVHD* | | | |
| Status at SCT | Yes | | No | | |
| Standard-risk | 19% | (8/42) | 30% | (11/37) | n.s. |
| High-risk | 29% | (13/45) | 35% | (12/34) | n.s. |

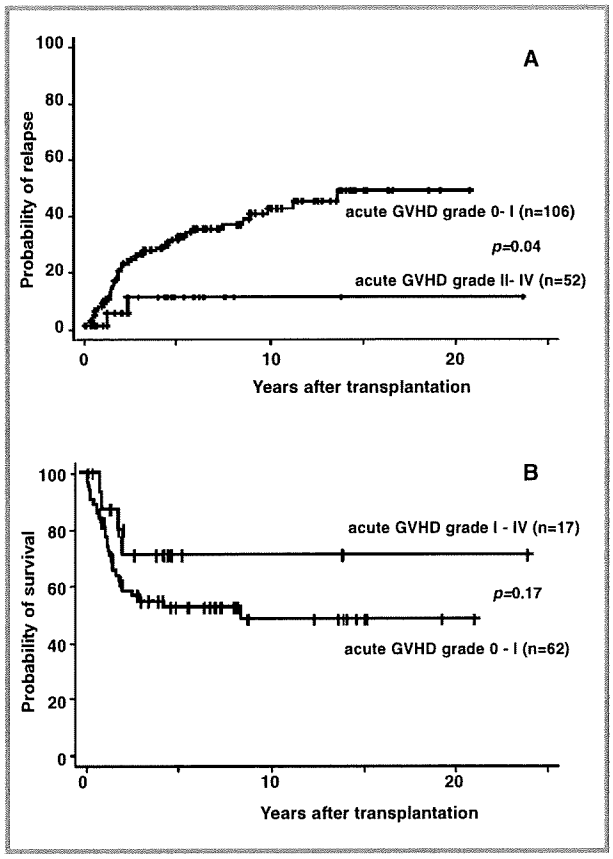


**Figure 1 A.Probability of relapse in 158 patients receiving allogeneic SCT patients grouped by the existence of acute GVHD, using Kaplan-Meier method and Wilcoxon test. B. Probability of overall survival in 79 high-risk patients grouped by the existence of acute GVHD, using Kaplan-Meier method.**

Japanese patients developing aGVHD ≥ grade II regardless of risk at SCT, supporting previous findings.[6] Development of aGVHD also contributed to improving survival in high-risk patients; when grade I aGVHD patients were included in GVHD$^+$ patients, the difference in survival rates between GVHD$^+$ and GVHD$^-$ patients became significant ($p$<0.05). To our knowledge, this is the first report documenting the significant beneficial effect of aGVHD on survival of high-risk patients after SCT. Thus, aGVHD appears to be associated with potent GVL effect. In contrast, the development of cGVHD was not associated with a decrease in the relapse rate. Weiden *et al.* reported an improvement of overall survival and a decrease of relapse rate in GVHD$^+$ patients compared with GVHD$^-$ patients.[1]

This effect was especially evident in cGVHD$^+$, and several studies supported these results.[2-5] However, cGVHD in most patients is preceded by aGVHD in Caucasians; the proportion of *de novo* cGVHD among all cGVHD patients is only 12~36%.[7,8] Thus, low relapse rates in cGVHD$^+$ patients may be affected by preceding aGVHD. Remberger M *et al.* reported that grade II aGVHD possessed GVL effect in unrelated SCT.[9]

However, aGVHD ≥ grade II did not improve the survival compared to grade I. In this study, aGVHD ≥_grade I significantly lowered relapse rate and improved the survival of high-risk patients. Thus, it may be reasonable to prevent aGVHD in standard-risk patients as much as possible and give weak immunosuppression to high-risk patients to induce grade I aGVHD. It is believed that cGVHD is useful in preventing relapses of leukemia. Since there is no other means to prevent relapses, physicians tend to intentionally induce cGVHD for aGVHD-patients with a high-risk of relapse. Such patients may be untreated even if they have developed extensive cGVHD. Our results suggest that this approach may be incorrect. cGVHD can be a fatal complication by itself and many patients succumb to infection associated with cGVHD. The best strategy to prevent relapses in high-risk patients may be to induce aGVHD ≥ grade I and treating it intensively to prevent subsequent cGVHD.

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Transfusion Medicine

## Infectious disease markers in autologous blood donors and first-time volunteer blood donors: 14 years' experience in a blood center

The proportion of blood donors with positive infectious disease markers was statistically higher in our population of 3,614 autologous donors than in our population of 276,106 first-time volunteer donors ($p<0.005$). Our data suggest that our autologous donor population is not as safe as our first-time volunteer donor population.

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In Spain, it is a current legal requirement[1] that both autologous and homologous blood donors pass the same ordinary predonation blood donor interview. Moreover, all samples from both autologous and homologous donors are tested for the presence of antibodies to HCV (anti-HCV) and HIV viruses (anti-HIV), HBsAg and RPR. If one of these tests is positive, then the donor is deferred. Moreover, it has also been suggested that the infectious markers frequency in autologous and volunteer donors may vary geographically and should be determined locally.[2]

With these controversies in mind, we decided to collect data to calculate the frequency of positive infectious disease markers in our population of autologous and first-time volunteer donors.

We evaluated the presence of the infectious disease markers from January 1989 to December 2002 both in 3,614 autologous and in 276,106 first-time volunteer blood donors who were eligible for blood donation. Both groups of donors followed the same medical history screening procedure. Minimal hemoglobin level, however, was 105 g/L for autologous donors, 125 g/L for first-time female and 135 g/L for first-time male volunteer donors.

Screening (ELISA kit) and confirmatory (RIBA kit) tests to analyze for the presence of anti-HIV 1/2 and anti-HCV were implemented in 1986 and 1989, respectively. Screening (enzyme immunoassay kit) and confirmatory (neutralization kit) tests to analyze for the presence of HBsAg were implemented in 1971. RPR was performed with RPR-Carbon (BioSystems, Barcelona, Spain) and was

**Table 1. Infectious markers in autologous and first-time volunteer blood donors per year.**

| | | *Autologous donors* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Positive HBsAg*[1] | | *Positive HIV*[2] | | *Positive HCV*[2] | | *Ind HCV*[3] | | *Positive RPR*[1] | | *ALT>88 IU/L* | |
| Year | N | n | % | n | % | n | % | n | % | n | % | n | % |
| 1989 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1990 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1991 | 24 | 1 | 4.17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1992 | 82 | 0 | 0 | 0 | 0 | 2 | 2.44 | 2 | 2.44 | 0 | 0 | 1 | 1.22 |
| 1993 | 104 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1994 | 276 | 2 | 0.72 | 0 | 0 | 17 | 6.16 | 4 | 1.45 | 0 | 0 | 1 | 0.36 |
| 1995 | 305 | 3 | 0.98 | 1 | 0.33 | 13 | 4.26 | 1 | 0.33 | 1 | 0.33 | 1 | 0.33 |
| 1996 | 248 | 0 | 0 | 0 | 0 | 7 | 2.82 | 3 | 1.21 | 1 | 0.4 | 0 | 0 |
| 1997 | 402 | 1 | 0.25 | 0 | 0 | 7 | 1.74 | 2 | 0.5 | 1 | 0.25 | 0 | 0 |
| 1998 | 232 | 1 | 0.43 | 1 | 0.43 | 6 | 2.59 | 2 | 0.86 | 0 | 0 | 1 | 0.43 |
| 1999 | 350 | 1 | 0.29 | 0 | 0 | 5 | 1.43 | 4 | 1.14 | 1 | 0.29 | 1 | 0.29 |
| 2000 | 461 | 2 | 0.43 | 0 | 0 | 10 | 2.17 | 0 | 0 | 1 | 0.22 | 0 | 0 |
| 2001 | 566 | 2 | 0.35 | 0 | 0 | 8 | 1.41 | 3 | 0.53 | 0 | 0 | 0 | 0 |
| 2002 | 544 | 5 | 0.92 | 0 | 0 | 8 | 1.47 | 2 | 0.37 | 1 | 0.18 | 0 | 0 |
| All | 3,614 | 18 | 0.5 | 2 | 0.06 | 83 | 2.3 | 23 | 0.64 | 6 | 0.17 | 5 | 0.14 |

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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.

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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 ($\times 10^6$)* | *No. of $CD3^+$ cells/kg infused ($\times 10^8$)* | *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.*

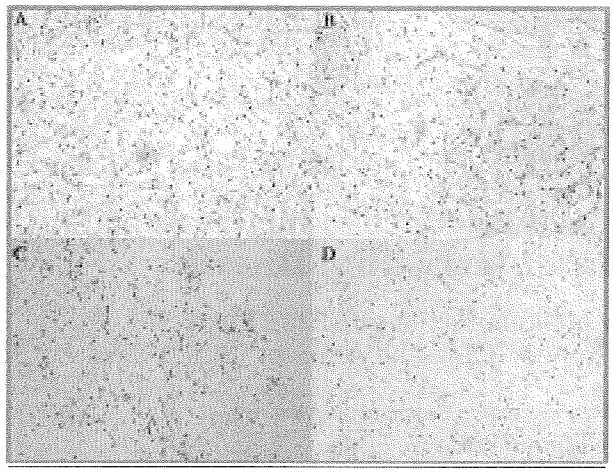

**Figure 1. Photomicrographs of pleural metastases of RCC from patient #1. (A) Before transplantation, the clear cell architecture was intact in a pleural section obtained by open-lung biopsy. (B) In the pleural section taken post-mortem, tumor cell detritus and heavy mononuclear cell infiltration were seen. Immunohistochemical staining with monoclonal antibodies against CD8 (C) and CD4 (D) antigens showed that mononuclear cells in the post-mortem pleural section were primarily CD8⁺. Original magnification, ×200.**

patient died of respiratory failure from GVHD and disease progression on day 709. Since the median survival of patients with cytokine therapy-resistant metastatic RCC is less than 6 months,[6] having stabilized disease progression for 12 months in patient #3 may be noteworthy. Patient #2 progressed soon after the transplantation and did not respond to withdrawal of CSP on day 18. To induce a graft-versus-tumor (GVT) effect, she received interleukin-2 plus donor-lymphocyte infusion with $3.0\times10^8$ CD3⁺ cells/kg on day 27. On day 45, grade III GVHD of the liver and intestine developed. Shortly thereafter lymphadenopathy in the neck and axilla regressed gradually, but there was no reduction in massive pleural effusions. Patient #2 died of disease progression on day 68.

Histologic analyses of the pleural metastasis in patient #1 revealed intact tumor cell architecture and the absence of lymphocyte infiltration before transplantation (Figure 1A). However, after transplantation we found an accumulation of mononuclear cells corresponding to a degenerative lesion of clear cell carcinoma (Figure 1B), which mainly consisted of CD3⁺CD8⁺ cells (Figure 1C), but not CD4⁺ cells (Figure 1D). The other metastases in lung, bone, and lymph nodes also showed considerable penetration by infiltrating CD8⁺ cells. These findings were also seen in patient #3. Post-mortem sections of bone, lung, pleura, adrenal, and lymph node metastases were found to contain abundant infiltrating CD8⁺ cells. In patient #2, conversely, lymphocyte infiltration and destruction of tumor were not present in metastatic sites examined post-mortem, except for a small number of lymphocytes seen in the supraclavicular lymph node and the primary lesion of the left kidney as well as minimal destruction of the primary tumor.

Our study showed that the GVT effect was closely associated with infiltration of CD8⁺ cells without infiltration of CD4⁺ cells, although there is a possibility that T cells seen after death may differ from those at the time of response.

These findings suggest that the GVT effect after RIST may be mediated by induction of tumor-specific cytotoxic T lymphocytes (CTL) rather than induction of lymphocytes which secrete cytokines locally, since cytokine secretion and cytotoxicity are mainly functions of CD4⁺ cells and CD8⁺, cells respectively. The future direction of this study will be to isolate tumor antigens exclusively or preferentially presented by tumor cells, and generation of CTL specific for these tumor antigens.

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HEMATOLOGY

# Mycophenolate Mofetil Is Effective and Well Tolerated in the Treatment of Refractory Acute and Chronic Graft-versus-Host Disease

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**Abstract**

We enrolled 11 patients with refractory graft-versus-host disease (GVHD) in a prospective trial evaluating the efficacy of mycophenolate mofetil (MMF). Four (67%) of the 6 patients with acute GVHD and all 5 patients with chronic GVHD responded to MMF. Ten (91%) of the 11 patients were able to decrease steroid use (median decrease, 86%; range, 25%-100%). After a median follow-up of 18 months (range, 1-65 months), 7 patients (64%) remained alive. The adverse events were infectious complications (36%), diarrhea (27%), and neutropenia (18%); the only patient discontinuing MMF did so because of grade 4 neutropenia. This preliminary study suggests that MMF is a well-tolerated agent and has a beneficial effect in the treatment of refractory acute and chronic GVHD.

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*Key words:* Mycophenolate mofetil; Allogeneic stem cell transplantation; Mismatched donor; Graft-versus-host disease

## 1. Introduction

Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after allogeneic stem cell transplantation (SCT) [1]. Cyclosporin A (CSA), tacrolimus (FK506), and steroids are effective in the treatment of both acute GVHD and established chronic GVHD [1-3]. However, patients who fail to respond to standard therapy have a poor prognosis [4,5]. The therapeutic options for these patients are limited, and salvage therapies have produced disappointing results to date [6-11].

Mycophenolate mofetil (MMF; CellCept; Roche Diagnostics, Indianapolis, IN, USA) is an ester prodrug of the active immunosuppressant mycophenolic acid, which is a noncompetitive reversible inhibitor of inosine monophosphate dehydrogenase [12-14]. This inhibition blocks the de novo synthesis of guanosine nucleotides, necessary substrates for DNA and RNA synthesis. Lymphocytes depend on this pathway and do not possess the salvage pathways of other cells [14]. This drug has been successfully tested in multicenter randomized trials for preventing renal transplant rejection [15] and has been used in limited trials for the treatment of acute and chronic GVHD [12,16-23]. These retrospective reports have suggested that MMF is an effective agent in these settings. The toxicity profile of MMF, such as upper and lower enteritis, cytopenia, and lack of renal toxicity, is not cross-reactive with the toxicity profiles of CSA, tacrolimus, and steroids, making MMF an attractive candidate for combination therapy.

In February 2000, we began a prospective single-center study in which we analyzed the efficacy of MMF in combination with CSA, tacrolimus, or steroids in the treatment of acute and chronic GVHD in a series of 11 allograft recipients with refractory GVHD.

## 2. Patients and Methods

### *2.1. Patients*

Eleven patients with refractory GVHD who had undergone allogeneic SCT between December 1997 and April 2004 were enrolled in this prospective trial. Eligibility criteria were the presence of refractory acute or chronic GVHD after treatment with steroids, CSA, and/or tacrolimus, and the absence of relapse at the time of study enrollment. The protocol received Institutional Review Board approval, and

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signed informed consent was obtained from every patient before study entry.

The patients' characteristics are shown in Table 1. The median age was 46 years (range, 28-66 years). The patients had undergone matched sibling (n = 7), related (n = 3), or unrelated (n = 1) allogeneic transplantation without T-cell depletion. GVHD prophylaxis included CSA and methotrexate for 6 patients, CSA alone for 2 patients, and tacrolimus and methotrexate for 2 patients. Antithymocyte globulin was administered in association with CSA and methotrexate to 1 patient who had received a related transplant mismatched at 2 loci.

### 2.2. GVHD Treatment

The assessment and grading of acute and chronic GVHD were primarily based on clinical findings and were carried out by following the commonly accepted diagnostic criteria [9,10,24,25]. Diagnosis was supported by skin, liver, or gut biopsies whenever indicated and clinically possible. The ocular involvement of chronic GVHD was diagnosed by the Schirmer test. Patient 1 developed skin and liver disease early after cord blood transplantation. The diagnosis of acute GVHD for this patient was based on skin and liver biopsy results, and the patient showed refractoriness to combination treatment with CSA and methylprednisolone (mPSE), suggesting a lower possibility of periengraftment syndrome after cord blood transplantation.

First-line treatment for acute GVHD of grade II or higher or for chronic GVHD consisted of a combination of CSA or tacrolimus with steroids. mPSE was initially administered to patients with acute GVHD of grade II to IV at a dosage of 2 mg/kg per day for 1 to 2 weeks; then the patients were switched to prednisolone (PSE). The tapering schedule for PSE was a dosage reduction of 0.1 to 0.2 mg/kg per week in the responsive cases. PSE was initially administered to patients with chronic GVHD at a dosage of 1 mg/kg and then tapered slowly. If partial or complete resolution of symptoms did not occur or if patients became dependent on steroids (defined as the need for >30 mg/day PSE for more than 6 weeks), they were considered refractory to treatment and were switched to MMF therapy. The blood levels of CSA and tacrolimus of all patients who had been given these drugs reached their target points before MMF treatment was initiated.

MMF was started at a dosage of 1500 mg/day except for 1 patient (no. 1), who received MMF at a dosage of 1000 mg/day because of low body weight (<50 kg) and coexisting pancytopenia. MMF was given orally, and the starting dose was maintained if it was tolerated. Patients were treated with MMF in addition to CSA and steroids (n = 2), tacrolimus and steroids (n = 6), or steroids alone (n = 3). The median time from GVHD onset to the initiation of MMF treatment was 17 days (range, 7-55 days) for acute GVHD and 82 days (range, 59-560 days) for chronic GVHD. The duration of therapy ranged from 30 days to more than 900 days (median, 133 days).

### 2.3. GVHD Monitoring

Response to MMF was assessed for each organ involved, as has been described previously [1,12,18,20]. A complete response was defined as complete resolution of clinical and/or biological signs (skin changes, digestive symptoms, bilirubin level, oral lesions, and joint, lung, and ocular clinical manifestations) that allowed a decrease in dosage or the discontinuation of steroid treatment. A partial response was defined as an improvement in but not a resolution of these clinical and/or biological signs. Stable disease was defined as stable organ involvement. An evaluation of no response referred to the progressive worsening of chronic GVHD. The patients were regularly monitored by full clinical and laboratory evaluations and by pathologic examinations in some cases. Adverse events attributed to MMF were graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0).

## 3. Results

### 3.1. Response to MMF in Refractory Acute GVHD

Response, complication, and survival data from the 6 patients who received MMF treatment for refractory acute GVHD are listed in Table 2. Four (67%) of the 6 patients responded to MMF treatment (Table 2). Although acute GVHD of the gut in patient 3 was resolved by MMF treatment, the patient was considered a nonresponder because of no response to the acute GVHD of the skin and liver. The median time for a patient to show initial signs of response to MMF was 13 days (range, 5-63 days). This interval was calculated as the time to the first objective signs of any improvement, not as the time to maximum response. The responses of these 6 patients according to the involved organs are shown in Figure 1. There was no preference for response according to involved organs.

### 3.2. Response to MMF in Refractory Chronic GVHD

All 5 patients with refractory chronic GVHD responded to MMF therapy and survived thereafter, allowing a decrease in the dosage or discontinuation of steroid treatment in 4 patients (Table 2). The median time to show initial signs of a response was 50 days (range, 27-180 days). Dosage reduction or discontinuation of steroid treatment was possible in 4 of the 5 patients.

### 3.3. Toxicity and Complications

The most common adverse event associated with MMF treatment was diarrhea, which occurred in 3 patients (27%). One patient (no. 9) had to discontinue MMF treatment because of grade 4 neutropenia that was attributed to MMF. Another patient (no. 2) also developed grade 4 neutropenia but required only a dosage adjustment. There were 6 infectious episodes during treatment (cytomegalovirus [CMV] antigenemia, n = 3; CMV pneumonia, n = 1; *Pseudomonas* septicemia, n = 2). The 2 patients with acute GVHD who did not respond to MMF therapy died of progressive acute GVHD and infection. Two other patients experienced relapse of disease while receiving MMF and died of disease progression.

**Table 1.**
Patient Characteristics*

| Patient | Age, y/ Sex | Diagnosis | Donor (Sex) | Graft | Conditioning Regimen | GVHD Prophylaxis | Indication to MMF, GVHD Duration before MMF Tx, d | aGVHD Onset Posttransplantation, d/Grade/Sites | aGVHD Tx before MMF (Response) | cGVHD Onset Posttransplantation, d/Grade/Sites | cGVHD Tx before MMF | Concomitant Tx with MMF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 66/M | ALL (CR) | 2 Loci mis, UR (F) | CB | FL/L-PAM/ TBI4 | CSA | aGVHD, 12 | 12/IV/skin, liver | CSA, mPSE (NR) | NE | — | CSA, mPSE |
| 2 | 30/F | Marginal zone B-cell lymphoma (PR) | 2 Loci mis, REL (F) | PB | CY/Ara-C/ TBI12 | FK506/ MTX | aGVHD, 18 | 11/II/skin | FK506, mPSE, PUVA (NR) | No | — | FK506, mPSE |
| 3 | 46/M | CML (CP) | Matched, REL (M) | PB | CY/TBI12 | FK506/ MTX | aGVHD, 16 | 21/III/skin, liver, gut | FK506, mPSE (NR) | No | — | FK506, mPSE |
| 4 | 28/M | CML (BC)† | 2 Loci mis, REL (M) | PB | FL/BU | CSA/MTX/ ATG | aGVHD, 7 | 8/III/skin, liver | CSA, mPSE (skin, CR; liver, NR) | NE | — | CSA, mPSE |
| 5 | 53/F | Diffuse large B-cell lymphoma (refractory)† | Id sibling (M) | PB | FL/BU | CSA | aGVHD, 44 | 13/III/skin, gut | FK506, mPSE (skin, NR; gut, CR) | No | — | FK506, mPSE |
| 6 | 33/M | Nasal NK/T lymphoma (PR) | Id sibling (F) | PB | CY/TBI12 | CSA/MTX | aGVHD, 55 | 36/III/skin, liver, gut | FK506, PSE (NR) | No | — | FK506, PSE |
| 7 | 61/M | AML (CR) | Id sibling (M) | PB | FL/BU | CSA/MTX | cGVHD, 126 | 25/III/skin, liver, gut | CSA, mPSE (CR) | 82/ext/liver, mouth, ocular | CSA, PSE | PSE |
| 8 | 32/F | AML (CR) | Id sibling (M) | PB | CY/Ara-C/ TBI12 | CSA/MTX | cGVHD, 285 | 35/II/skin, liver | CSA, PSE (CR) | 560/ext/skin, liver | PSE | PSE |
| 9 | 32/M | CML (CP) | Id sibling (M) | PB | CY/Ara-C/ TBI12 | CSA/MTX | cGVHD, 5 | 25/III/skin, liver, gut | FK506, mPSE (skin/liver, NR‡; gut, CR) | 59/ext/skin | FK506, PSE | FK506, PSE |
| 10 | 52/M | AML from RAEB | Id sibling (F) | PB | CY/Ara-C/ TBI12 | CSA/MTX | cGVHD, 1776 | No | — | 79/ext/liver, mouth, ocular | FK506, PSE | FK506, PSE |
| 11 | 59/M | ALL (CR) | Id sibling (M) | PB | FL/BU | CSA/MTX | cGVHD, 235 | No | — | 126/ext/liver | PSE | PSE |

*GVHD indicates graft-versus-host disease; MMF, mycophenolate mofetil; Tx, therapy; aGVHD, acute GVHD; cGVHD, chronic GVHD; ALL, acute lymphoblastic leukemia; CR, complete response; mis, mismatched; UR, unrelated; CB, cord blood; FL, fludarabine; L-PAM, melphalan; TBI, total body irradiation; CSA, cyclosporin A; mPSE, methylprednisolone; NR, no response; NE, nonevaluable; PR, partial response; REL, relative; PB, peripheral blood stem cell; CY, cyclophosphamide; Ara-C, cytarabine; FK506, tacrolimus; MTX, methotrexate; PUVA, psoralen and ultraviolet A irradiation; CML, chronic myeloid leukemia; CP, chronic phase; BC, blast crisis; BU, busulfan; ATG, antithymocyte globulin; Id, identical; NK/T, natural killer/T-cell; PSE, prednisolone; AML, acute myeloid leukemia; ext, extensive; RAEB, refractory anemia with excess of blasts.

†Patient 4 had a history of allogeneic stem cell transplantation, and patient 5 had a history of autologous stem cell transplantation.

‡Progressive type of chronic GVHD of the skin and liver developed subsequently.

**Table 2.**
Response and Toxicity*

| Patient | Response to MMF (Time from MMF Initiation to Response, d) | | | | | | Reduction in Steroid Dosage† | MMF Tx Duration, (Cause of Disruption) | Infections during MMF Tx | Adverse Events (Grade) | Outcome (Cause of Death), Time Posttransplantion |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Skin | Liver | Gut | Joints | Ocular | Mouth | | | | | |
| 1 | NR | NR | — | — | — | — | 63% | 30 d (death) | *Pseudomonas* septicemia, CMV-Ag | No | Dead (aGVHD, *Pseudomonas* septicemia), 54 d |
| 2 | CR (63) | — | — | — | — | — | 100% | 384 d (efficacy) | CMV-Ag | Neutropenia (4)‡ | Alive CR, 26+ mo |
| 3 | NR | NR | CR (7) | — | — | — | 80% | 111 d (death) | *Pseudomonas* septicemia, CMV pneumonia | No | Dead (aGVHD, CMV pneumonia), 147 d |
| 4 | — | CR (5) | — | — | — | — | 87% | 204 d (relapse) | CMV-Ag | No | Dead (relapse), 209 d |
| 5 | CR (10) | — | — | — | — | — | 90% | 89 d (relapse) | No | No | Dead (relapse), 145 d |
| 6 | CR (15) | CR (15) | CR (15) | — | — | — | 80% | 133 d (efficacy) | No | diarrhea (3)‡ | Alive CR, 68+ mo |
| 7 | — | CR (70) | — | — | CR (70) | CR (360) | 100% | 825 d (efficacy) | No | No | Alive CR, 25+ mo |
| 8 | PR (57) | SD | — | — | — | — | 25% | 110 d (efficacy) | No | Diarrhea (1)‡ | Alive CR, 66+ mo |
| 9 | SD | PR (27) | — | — | — | — | 85% | 56 d (neutropenia) | No | Neutropenia (4)‡ | Alive CR, 64+ mo |
| 10 | — | PR (180) | — | — | PR (180) | PR (180) | 0% | 30+ mo | No | Diarrhea (1)‡ | Alive CR, 91+ mo |
| 11 | — | CR (31) | — | — | — | — | 100% | 16+ mo | No | No | Alive CR, 27+ mo |

*CMV-Ag indicates cytomegalovirus antigenemia; SD, stable disease. Other abbreviations are expanded in the first footnote to Table 1.
†Percent reduction in steroid dosage at the end of MMF treatment or at last follow-up.
‡Neutropenia in patients 2 and 9 was resolved with a reduction in MMF dosage and MMF discontinuation, respectively. Diarrhea in patients 6, 8, and 10 was resolved with supportive medication.

## 4. Discussion

Even with the best immunosuppressive regimens using CSA, tacrolimus, and steroids, many patients still succumb to acute and chronic GVHD. These patients are likely to die of GVHD itself or from infectious complications secondary to prolonged immunosuppression, as well as to the depression of their immune system by GVHD [1-11]. We attempted to improve the prognosis of such patients by combining MMF with other commonly used immunosuppressive agents. Four (67%) of 6 patients with refractory acute GVHD responded with no subsequent development of chronic GVHD, and MMF therapy was eventually stopped in 2 of these patients because of successful outcomes. Additionally, all 5 patients with refractory chronic GVHD who were treated with MMF showed improvements of clinical symptoms, and MMF was discontinued in 2 patients. These results seemed comparable to the outcomes reported for previous studies on treatment of acute GVHD (response rates, 31%-71%) and chronic GVHD (response rates, 46%-77%) [12,16-23]. In addition, the administration of MMF allowed a dosage reduction of steroids in 10 of the 11 patients. The remaining patient (no. 10), who had been treated with a combination of 7.5 mg PSE daily and the maximum dose of tacrolimus before the initiation of MMF therapy, became free of tacrolimus treatment despite continuing the same PSE dosage thereafter. These findings suggest that MMF may be an effective salvage treatment for refractory GVHD.

Although all 5 patients with chronic GVHD in the current study have maintained good clinical conditions after the initiation of MMF treatment, only 2 patients (33%) with acute GVHD have survived. The difference between the 2 groups in the rate of response to MMF may partly account for this observation. Another explanation is that 4 of the 6 patients with acute GVHD had advanced disease at the time of transplantation, whereas only 1 of the 5 patients with chronic GVHD had advanced disease.

Several reports have shown that the response to MMF developed within 2 months after MMF introduction, irrespective of whether acute or chronic GVHD was targeted [20,22,23]. The median time for a patient to show initial signs of response to MMF treatment was 31 days (range, 5-180 days) in the present study. This interval was the time to the start of any improvement and not the time to maximum response. Of note is that 3 (33%) of 9 responders began to show improvements in GVHD more than 2 months after MMF initiation (at 63, 70, and 180 days). These findings suggest that MMF should be continued for at least 3 months to provide an opportunity for late responses to develop.

MMF was generally well tolerated. Of note is that treatment with MMF was not discontinued for adverse events except in a single patient who responded to MMF but experienced grade 4 neutropenia that required the discontinuation of MMF therapy. Other adverse events were resolved with supportive medication or by reducing the MMF dosage. Our findings may serve to strengthen the advantage of MMF, which causes a relatively small number of adverse events including nephrotoxicity and liver toxicity compared with other new immunosuppressive drugs [19].

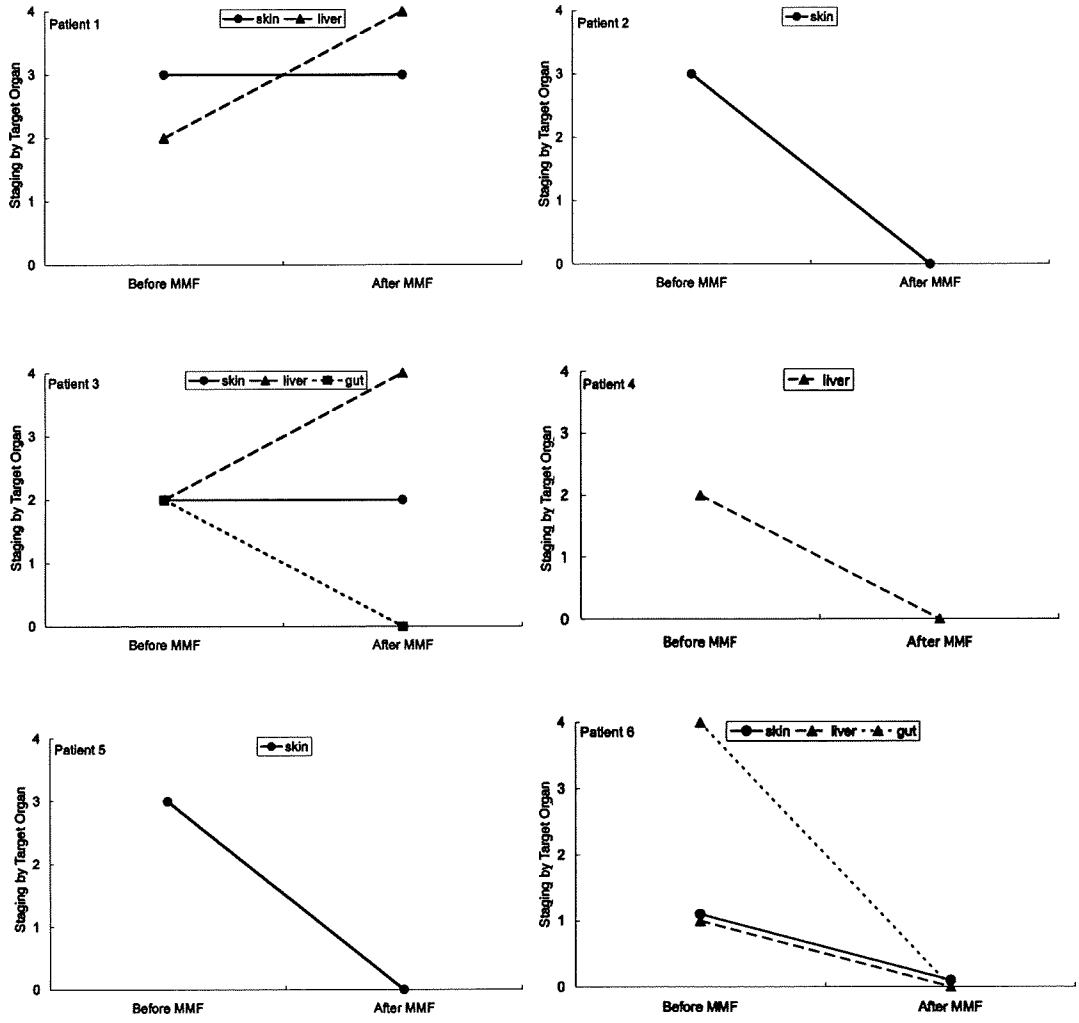


**Figure 1.** Response to mycophenolate mofetil (MMF) by target organ in 6 patients with acute graft-versus-host disease.

Six opportunistic viral or bacterial infections occurred in 4 of the patients. Two patients died from infection (*Pseudomonas* septicemia in one patient and CMV pneumonia in the other) coinciding with progressive acute GVHD, which developed while these patients received MMF. These findings may be consistent with previous reports that the use of MMF in allogeneic SCT was an independent risk factor for CMV infection [26] and was associated with a high risk of serious viral or bacterial infections [20,22]. However, it is difficult to accurately ascertain the negative impact of MMF on infectious complications in such a small retrospective study that lacks a comparison group in terms of salvage GVHD treatment.

In the current study, 2 patients relapsed during MMF therapy, although both patients were at high risk of relapse at the time of transplantation (Tables 1 and 2). Recently, Shapira et al [27] reported that MMF does not impair graft-versus-leukemia (GVL) effects or reduce lymphokine-activated killer cell activity in mice, whereas CSA had already been shown in mice [28] and in clinical practice [29] to suppress the GVL effects inducible by allogeneic donor lymphocytes. A study that compared tacrolimus with CSA for GVHD prophylaxis has shown that the relapse rate among recipients of HLA-matched transplants from siblings was significantly higher in the tacrolimus group than in the CSA group [30], indicating that tacrolimus may compromise the GVL effects more significantly than CSA. However, whether MMF treatment is irrelevant to disease relapse is still unknown.

No patients in the current study developed thrombotic microangiopathy (TMA) during treatment with MMF. TMA is a syndrome of microangiopathic hemolytic anemia, thrombocytopenia, and renal dysfunction [31]. The association of TMA with immunosuppressive agents given after SCT, such as CSA, tacrolimus, and sirolimus, is well established [31,32]. Despite the unknown etiology of TMA, the pathologic finding of endothelial injury is commonly seen in patients with TMA. Of note is that no literature review has reported that MMF induces endothelial toxicity. These findings suggest that MMF, if used instead of CSA and tacrolimus, could have a benefit in decreasing the risk of TMA after SCT.