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Graft-versus-host disease

Treatment of steroid-resistant acute graft-versus-host disease with daclizumab and etanercept

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Summary:

Steroid-resistant acute GVHD (aGVHD) following allogeneic hematopoietic stem cell transplantation (alloHSCT) continues to be associated with a high mortality. We report the results of a phase II study of treatment of steroid-resistant aGVHD with the IL-2 receptor antibody daclizumab combined with the TNF-receptor fusion protein etanercept. Treatment consisted of daclizumab 1 mg/kg given i.v. on days 1, 4, 8, 15, 22 and etanercept 16 mg/m^2 s.c. on days 1, 5, 9, 13, 17. A total of 21 patients (age 15–61 years) with steroid-resistant aGVHD after alloHSCT were included in the study. Donor types were HLA-matched related ($n = 6$), HLA-matched unrelated ($n = 14$), and HLA-mismatched unrelated ($n = 1$). Eight patients achieved complete, and six showed partial remission of aGVHD. Seven patients did not respond. Four of 21 patients are currently alive with a median follow-up of 586 (185–1155) days. Three patients died due to relapsed malignancy. Treatment-related mortality was due to infectious complications ($n = 11$) or organ failure due to aGVHD ($n = 3$). In total, 12 patients developed subsequent chronic GVHD. In conclusion, the data demonstrate an acceptable response rate of the combination of daclizumab and etanercept in the treatment of steroid-resistant aGVHD. Nevertheless, long-term mortality due to infectious complications and chronic GVHD remains high.

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Steroid-resistant acute GVHD (aGVHD) is associated with high morbidity and mortality. It is defined as progression of aGVHD symptoms and manifestations while patients are receiving full dose corticosteroid treatment. The high mortality in steroid-resistant aGVHD is mainly caused by a high rate of lethal infectious complications due to profound immunosuppression or is a result of organ damage by aGVHD itself.[1]

In the past, different approaches have been used in treatment of steroid-resistant aGVHD, which may be distinguished by mode of action. One treatment option is based on drugs, which are directly cytotoxic to the effector cells of aGVHD (ATG, OKT3, visilizumab, mycophenolate mofetil (MMF), pentostatin, sirolimus, anti-CD147, photopheresis).[2–13] A different approach is based on blockage of cytokines involved in the pathogenesis of aGVHD (antibodies against TNF and IL-2 or IL-1 antagonist).

Several approaches using antibodies against cytokines involved in the pathophysiology of aGVHD have been used in treatment of aGVHD. Murine antibodies against the IL-2 receptor have been studied with high response rates, but relapses of aGVHD have been frequently observed and prolonged administration of murine IL-2 receptor antibodies was associated with the development of neutralizing antibodies.[14–17] The availability of humanized IL-2 receptor antibodies daclizumab and basiliximab offered the opportunity for prolonged administration of treatment with a low toxicity profile.[18–20] Two phase II trials have evaluated the efficiency of daclizumab in the treatment of steroid-refractory aGVHD.[19,20] In both studies, activity of daclizumab has been observed. However, a significant failure rate on daclizumab as a single agent in treatment of steroid-refractory aGVHD was observed requiring additional immunosuppressive agents (ATG), and subsequent rates of infections and infectious mortality were significant.

TNF-α is a central cytokine in aGVHD with special implications in intestinal and cutaneous manifestations.[21] In the past, strategies using antibodies against TNF have been explored in prophylaxis and treatment of aGVHD. While the use of murine antibodies in treatment of aGVHD resulted in marked improvement of skin and gut manifestations, frequent relapses were observed after withdrawal of treatment due to the short half-life of these agents.[22–24] Results from phase II trials of treatment of aGVHD

with the monoclonal humanized antibody infliximab suggest a significant response rate, especially in gut GVHD, hampered by a high mortality due to infectious complications.[25–30]

Etanercept is a TNF-receptor fusion protein which, unlike infliximab, does not eliminate TNF-positive cells via antibody-dependent cytotoxicity (ADCC) and induction of monocyte apoptosis. The elimination of TNF-positive cells has been associated with an increased rate of infectious mortality. In agreement with this observation, a meta-analysis comparing infliximab with etanercept in rheumatoid arthritis found a lower risk of infections with mycobacteria in patients treated with etanercept.[26,31] Case reports using etanercept in the treatment of acute and chronic GVHD suggest activity comparable to infliximab.[25,28,32] In addition, etanercept has been successfully used in prevention of experimental GVHD-associated pulmonary complications and might be effective in the treatment of idiopathic pneumonia syndrome in patients.[33,34]

We present data of a phase II study combining daclizumab with etanercept, aiming to increase response rates in steroid-refractory aGVHD without increasing infection related mortality.

Patients and methods

Methods

In total, 21 patients, transplanted at the Division of Haematology and Oncology of the University of Rostock between March 2001 and February 2004, with aGVHD not responding to or progressing on treatment with methylprednisolone (MP) 2 mg/kg/day for at least 3 days ($n=19$), or with progression of aGVHD after dose reduction of MP at a dose >1 mg/kg MP ($n=2$) were eligible for treatment with daclizumab and etanercept. Exclusion criteria were uncontrolled viral infections and treatment with other immunosuppressive agents except for calcineurin inhibitors and MP. Due to lethal invasive aspergillosis in two study patients with pre-existing active pulmonary aspergillosis throughout the study period, an amendment made after 16 patients excluded patients with evidence of active invasive aspergillosis at the time of initiation of treatment with daclizumab and etanercept. Diagnosis of aGVHD was made clinically and confirmed histologically in 20 of 21 patients. One patient (UPN 376) refused biopsy but upper gastrointestinal endoscopy showed typical signs of aGVHD. Each organ was staged grade 1 through 4 aGVHD according to standard criteria.[35] The overall clinical grade was defined by grades of organ involvement according to the modified Keystone criteria.[35] Partial remission was defined as 50% reduction of all symptoms of aGVHD (skin involvement, diarrhea, hyperbilirubinemia) and reduction of the clinical grade of aGVHD. Complete remission was defined as resolution of all symptoms of aGVHD for at least 1 month. The protocol was reviewed and approved by the Institutional Review Board and written informed consent was obtained from each patient.

Treatment consisted of daclizumab 1 mg/kg given i.v. on days 1, 4, 8, 15, 22 and etanercept 16 mg/m^2 given s.c. on days 1, 5, 9, 13, 17. Calcineurin inhibitors (cyclosporin (CsA) or tacrolimus) were continued, and MP was continued with 2 mg/kg/day with dose reduction starting 5 days after the initiation of daclizumab and etanercept. In cases of evolving thrombotic thrombocytopenic purpura (TTP) ($n=2$), treatment with calcineurin inhibitor was discontinued and treatment with MP, daclizumab and etanercept was continued. The diagnosis of TTP was established using standard criteria, that is, the presence of hemolysis in combination with fragmented red cells in the blood smear, thrombocytopenia as well as the need for red cell transfusions.[36] In patients with TTP, daclizumab was continued at a dose of 1 mg/kg i.v. weekly for at least 8 weeks with subsequent tapering in cases of complete remission of GVHD.

All patients received antifungal prophylaxis/treatment consisting of an agent active in infections with *Aspergillus* species (itraconazole, amphothericin B or voriconazole). All patients were monitored weekly for CMV reactivation (early antigen pp65) and preemptive treatment with ganciclovir was initiated when CMV reactivation was detected. If clinically indicated (thrombocytopenia and/or leukopenia), additional screening for HHV6 and parvovirus B19 by PCR was performed. In addition, all patients were monitored for invasive aspergillosis by PCR and serological antigen screening (galactomanan-test).

Single-nucleotide polymorphisms (SNPs) 8, 12 and 13 of the NOD2/CARD15 gene were analyzed by Taqman PCR in 18 of 21 donor–patient-pairs as previously published.[37]

The relationship between response to treatment and duration of aGVHD from diagnosis to the initiation of treatment with daclizumab and etanercept was evaluated using the Student's *t*-test. The association of response to treatment to the day of initiation of the study medication after transplantation was evaluated with the χ^2 test.

Patients

In total, 21 patients were included in the study. Diagnoses included AML 1st CR ($n=3$), AML $>$1st CR ($n=2$), CLL Binet C ($n=1$), CML chronic phase ($n=2$), ALL $>$1st CR ($n=4$), NHL ($n=2$), aplastic anemia ($n=1$), MDS-RAEB ($n=1$), and multiple myeloma ($n=4$). The median age at time of transplantation was 44 (range 15–61) years. Donor types were HLA-matched sibling (mVRD) ($n=5$), HLA-matched mother ($n=1$), HLA-matched unrelated (mVUD) ($n=14$), and HLA-mismatched unrelated (mmVUD) ($n=1$). Results of SNP-typing of the NOD2/CARD15 gene of the donor and recipient were available for 18 of 21 patients. In all, 11 donor-patient pairs had homozygous wild-type alleles, two patients (UPN 294 and 308) and three other donors (donors of patients UPN 340, 383, and 478) had heterozygous variants at SNP 8, one donor patient pair had a heterozygous variant at SNP 12 (UPN 306), and patient UPN 412 had a heterozygous variant at SNP 13. All patients with SNP 8 and 12 variants had intestinal GVHD $\geqslant 2$ (patient UPN 340 progressed to intestinal aGVHD grade 3 on daclizumab and etanercept), while the patient with the SNP 13 variant (UPN 412) developed steroid

refractory aGVHD of skin without intestinal involvement. The conditioning regimen was toxicity reduced but myeloablative (treosulfan and fludarabine) in 12 patients whereas nine patients received a conventional myeloablative conditioning regimen.[38] GVHD-prophylaxis consisted of CsA alone ($n=8$), CsA and methotrexate ($n=8$), or CsA and mycophenolat mofetil (MMF) ($n=5$). The graft source was peripheral blood stem cells in 17 patients, and four patients received bone marrow stem cells. The median time of onset of aGVHD was 21 (range 9–105) days. Late onset aGVHD (>day 100) was defined as aGVHD in the presence of the typical clinical and histological signs of aGVHD occurring after donor lymphocyte infusion ($n=1$, UPN 318) or withdrawal of immunosuppression ($n=2$, UPN 306, 368). The median time from first diagnosis of aGVHD to the initiation of antibody treatment was 17 (range 3–66) days. The overall clinical grade of aGVHD at the time of initiation of second-line treatment of aGVHD was grade IV ($n=3$), grade III ($n=17$) and refractory skin GVHD with diffuse erythroderma (skin grade III, clinical grade II) ($n=1$). Patient characteristics are summarized in Table 1.

Results

Response

Of the 21 patients included in the study, 20 were eligible for response evaluation. Patient UPN 282 died 5 days after the start of treatment due to rapid progression of pre-existing invasive aspergillosis, preventing evaluation of response. Eight patients achieved complete response of aGVHD. Five patients showed partial remission of aGVHD. One patient (UPN 383) died 7 days after initiation of antibody treatment in partial remission of aGVHD due to progression of pre-existing invasive aspergillosis. Six patients failed treatment with daclizumab and etancercept. Early onset of aGVHD (before day 50, $n=16$) was associated with eight complete and three partial remissions and five treatment failures. Patients with onset of aGVHD after day 50 ($n=5$) achieved three partial remissions with one treatment failure and one early death. Neither the time point of initiation of second-line treatment of aGVHD after transplantation (<day 50 and >day 50, $P=0.573$) nor length of the interval between onset of aGVHD and initiation of treatment with daclizumab and etanercept influenced response rate (<14 days: CR $n=3$, PR $n=2$, NR $n=4$, NA $n=1$, >14 days CR $n=5$, PR $n=4$, NR $n=2$) ($P=0.74$). Treatment results are summarized in Table 2.

Survival

Four of 21 patients are currently alive with a median follow-up of 586 days post transplantation (range 185–1155). Three patients died of relapse of their underlying hematologic malignancies (ALL $n=2$, AML $n=1$).

Patients not responding to treatment with daclizumab and etanercept ($n=6$) had 100% mortality with a median survival of 60 (15–94) days after initiation of second-line treatment of aGVHD. Causes of death were liver aGVHD in combination with invasive aspergillosis (UPN 426), relapse of AML in combination with invasive aspergillosis (UPN 340), sepsis with candida (UPN 376), invasive aspergillosis ($n=2$), and bacterial sepsis ($n=1$).

Of six patients with partial remissions, two responded to a third-line treatment (Pentostatin UPN 478, Rapamycine + FK 506 UPN 318). Patient UPN 318 died 253 days after initiation of second-line treatment due to relapse of aGVHD resulting from withdrawal of immunosuppression due to noncompliance. While patient UPN 480 is currently alive with a follow-up of 185 days, the remaining four patients died a median of 26 days (range 8–246 days) after initiation of second-line treatment, due to invasive aspergillosis ($n=2$), Candida sepsis ($n=1$), and bacterial sepsis ($n=1$). Patient UPN 383 died of rapid progression of pre-existing invasive aspergillosis.

Three of eight patients achieving complete remission are long-term survivors with a median follow-up of 804 days post transplantation (368–1155). Two patients died of relapsed ALL. Two patients died due to infectious complications associated with chronic GVHD on day 713 (UPN 281) and day 330 (UPN 308).

Infectious complications

Bacterial infections. Two patients died due to bacterial sepsis during treatment with daclizumab and etanercept (patient UPN 410 sepsis, patient UPN 296 pneumonia). Two additional patients developed bacterial infections WHO grade III during treatment with daclizumab and etanercept but responded to antibiotic treatment.

Fungal infections. Four patients developed invasive aspergillosis during treatment with daclizumab and etanercept (UPN 282, 294, 383, 426). Patients UPN 282 and 383 had active invasive aspergillosis at the time of initiation of treatment and died on days 5 and 8 after start of daclizumab and etanercept due to rapid progression of invasive aspergillosis. The remaining two patients died due to invasive aspergillosis ($n=1$) or refractory GVHD ($n=1$). Four patients (UPN 308, 368, 340, 412) developed invasive aspergillosis after completion of treatment with daclizumab and etanercept, in association with chronic GVHD ($n=3$) or relapse of AML ($n=1$).

Three patients developed systemic infections with Candida species during treatment with daclizumab and etanercept (patients UPN 281, 296, 376). Patient UPN 376 died of Candida sepsis in association with refractory aGVHD and patient UPN 296 died of bacterial pneumonia. Patient UPN 348 died on day 139 (95 days after initiation of treatment with daclizumab and etanercept), of Candida sepsis.

Viral infections/reactivation

In total, 13 patients developed CMV reactivation requiring preemptive treatment with ganciclovir, and, in the event of rising numbers of pp65 positive cells, with foscarnet. One patient (UPN 281) developed diarrhea possibly associated with CMV reactivation. Nine of 10 patients

Table 1 Patient characteristics

UPN	*Diagnosis*	*Donor type*	*Age*	*Sex-match*	*GVHD prophylaxis*	*Stem cell source*	*Conditioning regimen*	*aGVHD grade (onset)*	*Skin max grade*	*Gut max grade*	*Liver max grade*	*Day start AB-treatment*
277	MM primary refr.	mVRD	48	Male to male	CsA	PBSCT	Treo, Flud	III (d21)	0	4	1	d39
281	MM 1st relapse	mVUD	50	Female to male	CsA	BM	Treo, Flud, ATG	IV (d49)	2	1	4	d75
282	ALL 2nd PR	mVUD	57	Female to female	CsA	PBSCT	Treo, Flud, ATG	III (d95)	2	4	1	d109
288	ALL 2nd relapse	mVRD	49	Male to male	CsA	PBSCT	TBI, VP16	III (d13)	3	4	3	d30
294	MDS RAEB	mVUD	48	Male to male	CsA	PBSCT	Treo, Flud, ATG	III (d44)	0	3	3	d52
296	T-NHL 2nd PR	mVRD	43	Male to male	CsA	PBSCT	Treo, Flud	IV (d27)	2	2	4	d48
306	ALL 1st PR	mVRD	44	Female to male	CsA, MTX	PBSCT	Treo, Cyclo	II (d13) III (d86)	2	3	0	d149
308	foll. NHL 1st PR	mVUD	40	Female to male	CsA	PBSCT	Treo, Flud, ATG	III (d14)	1	3	0	d24
318	T-ALL primary refr.	mVUD	22	Male to male	CsA	PBSCT	TBI, VP16, Cyclo	III (d105)	2	4	1	d117
334	sAML 1st CR	mVUD	57	Male to female	CsA, MTX	PBSCT	Treo, Flud, ATG	III (d14)	3	2	1	d36
340	AML 1st PR	mmVUD	34	Female to male	CsA, MMF	PBSCT	TBI, VP16	III (d9)	0	2	3	d12
344	CML CP	mVUD	44	Female to female	CsA, MTX	PBSCT	Treo, Flud, ATG	III (d21)	3	2	0	d35
348	AML 1st CR	mVRD	54	Female to male	CsA, MMF	PBSCT	TBI, AraC	III d15	4	4	1	d44
368	CLL Binet C	mVUD	61	Male to male	CsA, MTX	PBSCT	Treo, Flud, ATG	III (d172)	1	2	2	d201
376	AML 2nd CR	mVUD	26	Female to female	CsA, MMF	BM	TBI, AraC	IV (d33)	0	4	4	d65 (TTP)
383	sAML 1st CR	mVUD	32	Male to female	CsA, MMF	PBSCT	TBI, AraC	III (d91)	1	3	0	d116
408	MM 1st CR	mVUD	33	Female to male	CsA, MTX	BM	Treo, Flud, ATG	III (d37)	1	3	1	d48
410	AML 2nd relapse	mVUD	60	Female to male	CsA, MTX	PBSCT	Treo, Flud, ATG	III (d18)	3	4	2	d22
412	CML CP	mVUD	36	Male to female	CsA, MMF	BM	TBI, AraC	II (refr. skin) (d23)	3	0	0	d82
426	MM 2nd relapse	mVUD	50	Male to male	CsA, MTX	PBSCT	Treo, Flud, ATG	III (d86)	2	1	3	d94 (TTP)
478	Aplastic anaemia	mVRD	15	(mother) Female to female	CsA, MTX	PBSCT	Cy, ATG	III (d14)	3	2	0	d17

AB = antibody; AraC = cytosinarabinosid; BM = bone marrow; CsA = cyclosporin; Cyclo = cyclophosphamide; foll. NHL = follicular lymphoma; Flud = fludarabine; MM = multiple myeloma; MTX = methotrexate; MMF = mycophenolat mofetil; mVUD = matched voluntary unrelated donor; mVRD = matched voluntary related donor; mmVUD = mismatched voluntary unrelated donor; refr. = refractory; sAML = secondary AML; Treo = treosulfan; TBI = total body irradiation; TTP = thrombotic thrombocytopenic purpura; UPN = unique patient number.

Table 2 Results of treatment with daclizumab and etanercept

UPN	*Results*	*cGVHD*	*Follow-up*	*Current status/cause of death*
277	CR	ext	d1155	Alive, extensive chronic GVHD
281	CR	ext	d713+	Death due to bacterial sepsis extensive chronic GVHD
282	NR	NA	d114+	Death due to preexisting invasive aspergillosis
288	CR	ext	d299+	Death due to relapse of ALL
294	NR	NA	d146+	Death due to invasive aspergillosis
296	PR	NA	d75+	Death due to bacterial sepsis
306	CR	lim	d649+	Death due to relapse of ALL, limited chronic GVHD
308	CR	ext	d330+	Death due to invasive aspergillosis and extensive chronic GVHD
318	PR	ext	d363+	Death due to acute GVHD after withdrawal of immunosuppression, progressive onset chronic GVHD
334	CR	lim	d804	Alive limited chronic GVHD
340	NR	NA	d76+	Death due to relapse of AML
344	CR	ext	d187+	Death due to acute GVHD after withdrawal of immunosuppression
348	PR	NA	d139+	Death due to candida sepsis
368	PR	ext	d277+	Death due to invasive aspergillosis progressive onset chronic GVHD
376	NR	NA	d148+	Death due to candida sepsis
383	PR	NA	d124+	Death due to invasive aspergillosis
408	CR	ext	d368	Alive, current no GVHD despite the absence of immunosuppression
410	NR	NA	d37+	Death due to bacterial sepsis
412	NR	ext	d148+	Death due to invasive aspergillosis, refractory cutaneous GVHD
426	NR	NA	d150+	Death due to acute GVHD (liver), premature withdraw of etanercept due to invasive aspergillosis
478	PR	ext	d185	Alive, CR on Pentostatin

ext = extensive disease; lim = limited disease.

with positive donor and recipient CMV serology developed CMV reactivation. Four of six patients with positive CMV serology of the donor or the recipient developed CMV reactivation. None of the five patients with negative donor and recipient CMV markers developed CMV infection. No major morbidity or mortality due to CMV reactivation was observed although three patients required prolonged treatment with ganciclovir or foscarnet for more than 1 year (UPN 281, 308, 306).

Five patients (UPN 349, 410, 478, 348, 340) developed HHV6 reactivation, which was treated with preemptive ganciclovir. Except for thrombocytopenia and neutropenia, no additional clinical signs were attributable to the HHV6 reactivation.

Two patients (UPN 281 and 480) developed hemorrhagic cystitis due to a polyoma virus (BK virus) infection requiring prolonged red cell transfusions in patient UPN 281.

Toxicity

Except for infectious complications no adverse effects due to daclizumab and etanercept were observed.

TTP

Four patients developed TTP in association with aGVHD. TTP was diagnosed day 120 (UPN 348), day 65 (UPN 376), day 123 (UPN 408), and day 106 (UPN 426). Patient UPN 376 developed TTP at the time of diagnosis of steroid refractory GVHD, and patient UPN 426 developed TTP on day 12 of treatment with daclizumab and etanercept. In both patients CsA was stopped and treatment with daclizumab was continued. Since patient UPN 426 showed no response to treatment with daclizumab and etanercept, pentostatin 4 mg/m^2 (single dose) was added. While the TTP did not progress in either patient after withdrawal of CsA, GVHD did not respond to this approach.

Patients UPN 348 and 408 developed TTP 76 and 75 days after initiation of treatment with daclizumab and etanercept. While patient UPN 348 showed complete response of TTP after substitution of CsA with daclizumab and remained in remission of GVHD, patient UPN 348 died due to Candida sepsis and continuing active GVHD.

Chronic GVHD

In total, 12 patients developed chronic GVHD (all patients who responded to treatment and survived beyond day 100), which was staged as extensive disease in 10 patients and limited disease in two patients (UPN 334, 306). One patient with limited disease is currently alive with a follow-up of 804 days after transplantation. The second patient with limited disease (UPN 306) died of relapsed ALL. Chronic GVHD in complete responders developed typically after withdrawal of steroids ($n = 5$) or reduction of MP below 0.5 mg/kg/day ($n = 3$). Three of 10 patients with extensive chronic GVHD (UPN 334, 368, and 478) are currently alive, while four died of infectious complications associated with chronic GVHD, two died of reappearance of symptoms of aGVHD after withdrawal of immunosuppression due to noncompliance ($n = 1$), and reduction of immunosuppression due to severe side effects ($n = 1$). One patient with extensive chronic GVHD died due to relapse of ALL.

Discussion

Data from this phase II trial demonstrate activity of the combination of daclizumab and etanercept in the treatment of steroid-refractory aGVHD. The overall response rate was 67%, which was superior to the response rate observed by Willenbacher *et al* (50%).[20] Another study by Przepiorka *et al* evaluated daclizumab as a single agent in the treatment of steroid-refractory aGVHD. The overall response rate in their study was comparable to our overall response rate. However, a significant proportion of patients (40%) in this study received additional ATG making comparison of results more difficult.[19] About one-third of

patients did not respond to treatment with daclizumab and etanercept. One reason for incomplete response and treatment failure may have been late initiation of second-line treatment for aGVHD. Although we were not able to detect a significant association of response rate with the time of initiation of second-line treatment, late initiation of second-line treatment has been associated with lower response rates in patients treated with ATG for steroid-refractory GVHD.[6] Additional reasons for the high mortality may have been advanced age and disease status of the majority of patients compared to a phase II trial combining daclizumab and infliximab in patients with steroid-refractory aGVHD after dose reduced allogeneic hematopoietic stem cell transplantation (alloHSCT) in which a significant proportion of patients received treatment for solid tumors.[39]

All patients or donors with NOD2/CARD15 variants at SNP 8 and 12 had moderate to severe intestinal involvement by aGVHD, further supporting a role for these polymorphisms in the pathophysiology of gut involvement.[37]

Infectious complications were the leading cause of death in the present study and overall survival was 19%. A significant portion of the patients in this study were of an advanced age and had an unfavorable disease status, factors that are known to contribute to higher mortality rates. However, mortality from infection (alone or in combination with aGVHD) must be addressed.

Mortality due to invasive aspergillosis possibly related to immunosuppressive treatment was observed in four of 21 patients. Fatal complications due to invasive aspergillosis may be separated into two groups. Two patients died of rapid progression of preexisting invasive aspergillosis shortly after initiation of treatment with daclizumab and etanercept, despite response of aGVHD, indicating an association with the antibody treatment. The remaining two patients did not achieve complete remission of aGVHD, and mortality due to invasive aspergillosis occurred after completion of treatment of daclizumab and etanercept.

The use of infliximab after HSCT has been associated with an increased frequency of invasive aspergillosis.[26,29] The rapid progression of invasive aspergillosis in two patients with pre-existing aspergillosis and case reports on infections with toxoplasmosis after HSCT as well as an increased infection rate with *Listeria monocytogenes* in association with the use of etanercept suggest an increased risk of infectious complications.[40,41] Moreover, a randomized phase III trial on first-line treatment of aGVHD with daclizumab revealed an inferior survival rate in the arm containing steroids and daclizumab due to an increased rate of infections and relapse rate, suggesting that daclizumab may contribute to treatment-associated infectious mortality.[42] However, it should be noted that steroid-refractory GVHD itself is associated with an increased risk for invasive aspergillosis, partly explaining the associated mortality in four patients after completion of treatment with daclizumab and etanercept.[43,44]

The use of daclizumab in primary treatment of aGVHD was associated with an increased relapse rate.[42] It is unlikely that the three relapses of underlying malignancy observed in the present study are due to the use of daclizumab since relapses occurred despite chronic GVHD ($n=2$) or no response to daclizumab and etanercept ($n=1$).

It should be emphasized that all patients at risk developed chronic GVHD, which was associated with a high mortality. Prior aGVHD is the most important risk factor for chronic GVHD.[45] An additional cause for the high rate of chronic GVHD may have been the high proportion (17 of 21 patients) of peripheral blood stem cell grafts, which has been associated with an increased rate of chronic GVHD.[46,47] In addition, use of cytokine blocking agents instead of T-cell cytotoxic strategies might favor progression to chronic GVHD. Here, randomized studies are urgently needed.

In conclusion, the present study demonstrates that the combination of daclizumab and etanercept increases response rate compared to single agent daclizumab, in steroid-refractory aGVHD. However, this did not translate into improved overall survival due to a significant rate of infectious complications and long-term mortality due to chronic GVHD.

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