Treatment of graft failure with TNI-based reconditioning and haploidentical stem cells in paediatric patients

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Summary
Graft failure is a life-threatening complication after allogeneic haematopoietic stem cell transplantation (HSCT). We report a cohort of 19 consecutive patients (median age: 8·5 years) with acute leukaemias (n = 14) and non-malignant diseases (n = 5) who experienced graft failure after previous HSCT from matched (n = 3) or haploidentical donors (n = 16) between 2003 and 2012. After total nodal irradiation (TNI)-based reconditioning combined with fludarabine, thiotepa and anti-T cell serotherapy, all patients received T cell-depleted peripheral blood stem cell grafts from a second, haploidentical donor. Median time between graft failure and retransplantation was 14 d (range 7–40). Sustained engraftment (median: 10 d, range 9–32) and complete donor chimerism was observed in all evaluable patients. 5 patients additionally received donor lymphocyte infusions. Graft-versus-host disease (GvHD) grade II and III occurred in 1 patient each (22%); no GvHD grade IV was observed. 2 patients had transient chronic GvHD. The regimen was well tolerated with transient interstitial pneumonitis in one patient. Treatment-related mortality after one year was 11%. Event-free survival and overall survival 3 years after retransplantation were 63% and 68%. Thus, a TNI-based reconditioning regimen followed by transplantation of haploidentical stem cells is an option to rescue patients with graft failure within a short time span and with low toxicity.

Keywords: Haploidentical, stem cell transplantation, children, T cell depletion, graft failure.

Graft failure after allogeneic haematopoietic stem cell transplantation (HSCT) is a rare but life-threatening complication and randomized trials or standardized treatment protocols addressing the type of reconditioning regimen and donor selection are missing to date (Wolff, 2002). Myeloablative reconditioning regimens were associated with intolerable organ toxicity and so non-myeloablative but highly immunosuppressive regimens including total nodal irradiation (TNI) were evaluated by several groups (Grandage et al, 1998), (Guardiola et al, 2000), (Gaziev et al, 1999). Here we report a cohort of 19 consecutive paediatric patients who experienced graft failure after allogeneic HSCT with matched unrelated donors (MUD), or mismatched related donors (MMRD). All patients received a TNI-based reconditioning regimen combined with fludarabine or cyclophosphamide, thiotepa and anti-T cell serotherapy followed by a T cell-depleted stem cell graft from a haploidentical donor. Our aim was to evaluate the toxicity and effectiveness of this approach.

Patients and methods

Patients
This retrospective analysis included all patients who received TNI-based reconditioning and a second stem cell donation from haploidentical donors with T cell-depleted peripheral
blood stem cell grafts (PBSC) due to primary non-engraftment or graft rejection between 2003 and 2012. No other reconditioning protocols were investigated concurrently. Patients with appropriate indications (haemoglobinopathies or other non-malignant diseases without bone marrow involvement), for whom cryopreserved stem cells were available, could alternatively receive an autologous graft to reconstitute haematopoiesis, according to the decision of the treating physician. These patients usually underwent another myeloablative SCT after a minimum interval of at least 6 months and were not included in our analysis. Informed consent was obtained from legal guardians. All patients were treated within German protocols, which were approved by the local ethical committees. A total of 19 patients received this reconditioning approach during persistent pancytopenia after previous allogeneic HSCT with matched unrelated donors \((n = 3)\) or with fully haplotype mismatched family donors \((n = 16)\). Patient characteristics are shown in Table I. Nine patients had acute lymphocytic leukaemia \([\text{complete remission (CR1}: n = 4; \text{CR2}: n = 3, \text{CR3}: n = 1] \), non-remission (NR): \(n = 1\)], 5 had myeloid disease \([\text{relapsed acute myeloid leukaemia (AML), NR:} n = 1, \text{chronic myeloid leukaemia (CML, 2nd chronic phase):} n = 1, \text{myelodysplastic syndrome, refractory anaemia with excess blasts in transformation (MDS-RA):} n = 1, \text{myelodysplastic syndrome, refractory anaemia (MDS-RA):} n = 1, \text{Kostmann disease:} n = 1, \text{and major histocompatibility complex (MHC) class-II deficiency:} n = 1\] \((\text{Table I})\).

Eighteen of the 19 patients received total body irradiation \((\text{TBI})\): \(n = 1\), busulfan- or melphalan-based myeloablative conditioning regimens \((n = 17)\) at first transplantation. The patient with MDS-RA received a non-myeloablative preparative regimen with fludarabine, thiotepa and antithymocyte globulin \((\text{ATG})\) according to the European Working Group on MDS in childhood \((\text{EWOG-MDS})\) SCT with reduced intensity conditioning study \((\text{Strahm et al, 2007})\) \((\text{Table I})\).

**Methods**

**Assessment of engraftment, chimerism and immune reconstitution**

The day of engraftment was defined as the first of 3 consecutive days on which the absolute neutrophil count \((\text{ANC})\) was >0.5 x 10⁹/l. Platelet recovery was defined as independence from platelet transfusion for at least 7 d with a platelet count >20 x 10⁹/l.

Graft rejection \((\text{secondary graft failure})\) was defined as recurrence of pancytopenia with persisting neutrophil counts <0.5 x 10⁹/l and was confirmed by chimerism analysis of residual mononuclear cells in blood and bone marrow aspirate. Residual cells of recipient origin were documented by polymerase chain reaction \((\text{PCR})\) analysis of VNTR regions as previously described \((\text{Bader et al, 2000})\). Flow cytometry of lymphocyte subsets with monoclonal antibodies against human leucocyte antigen \((\text{HLA})\) antigens of recipient and donor was additionally carried out in patients with haploidentical donors \((\text{One Lambda Inc., Canoga Park, USA})\) \((\text{Schumm et al, 2007})\).

Non-engraftment \((\text{primary graft failure})\) was considered if an absolute neutrophil count \((\text{ANC})\) >0.5 x 10⁹/l was not reached for 3 consecutive days within 28 d after stem cell donation and was confirmed by a bone marrow aspirate. Time point of diagnosis of graft failure was defined as the day of bone marrow puncture. After successful engraftment, chimerism was assessed by PCR and, if possible, by weekly flow cytometry in peripheral blood samples until day 100 and then assessed every 3 months. Reconstitution of CD3+ , CD4+ , CD8+ , CD19+ and CD16+56+ lymphocytes was monitored weekly by fluorescence-activated cell sorting analysis until day +100 and was subsequently assessed every 3 months.

**Stem cell mobilization and graft manipulation for retransplantation**

All 3 patients who received stem cells from unrelated donors at 1st HSCT were retransplanted from haploidentical donors. Thirteen of the 16 patients initially undergoing haploidentical transplantation received stem cells from a second haploidentical donor for retransplantation. 3 patients received grafts from the same haploidentical donor because a second donor \((\text{other than the first})\) was not available. Thus, in all cases peripheral-blood stem cells \((\text{PBSC})\) from fully haplotype mismatched family donors \((\text{parents} n = 17; \text{siblings} n = 2)\) were mobilized by administration of 10 µg/kg of granulocyte colony-stimulating factor \((\text{G-CSF})\) daily for 5 d and were harvested by 1-2 leukapheresis procedures.

Three different immunomagnetic separation procedures were employed for retransplantation:

1) positive selection of CD34+ stem cells \((n = 1)\)
2) depletion of T and B cells \((\text{CD3/CD19 depletion}) \) \((n = 15)\)
3) depletion of alpha/beta-T cell receptor \((\text{TCR})\) T cells and B cells \((\alpha \beta \text{TCR/CD19 depletion}) \) \((n = 3)\)

Graft processing was done according to the manufacturers instructions with a CliniMACS®Plus device using the respective selection program \((\text{Milenyi-BioTec, Bergisch-Gladbach, Germany})\) \((\text{Lang et al, 2005})\), \((\text{Schumm et al, 2006})\). Graft compositions are shown in Table II.

**Treatment protocol**

All patients received total nodal irradiation \((\text{TNI, 1 x 7 Gray (Gy)})\) as described previously \((\text{Heinzelmann et al, 2008})\) and anti T cell serotherapy. Seven patients received ATG only \((\text{ATG-Fresenius} \times n = 1; \text{Thymoglobulin} \times n = 6)\). Two
Table I. Patient characteristics and conditioning regimens.

| Patient | Sex | Age (years) | Diagnosis       | Stage | Graft failure | 1st Donor | HLA match | Conditioning regimen for 1st SCT | 2nd Donor | 2nd SCT | Conditioning regimen |
|---------|-----|-------------|-----------------|-------|---------------|-----------|-----------|----------------------------------|-----------|---------|---------------------|
| 1       | m   | 9-0         | c-ALL           | CR1   | 2             | father    | haplo     | TBI, Flu, VP16, ATG             | mother    | TNI     | ATG-F, Flu          |
| 2       | m   | 4-6         | MHC-II defic    | 2      | father        | haplo     | Mel       | Flu, TT, OKT3                   | father    | TNI     | ATG-T, Flu         |
| 3       | m   | 9-8         | MDS, RAEB-T     | NR    | 2             | father    | haplo     | Bu, Mel, Cy, OKT3               | mother    | TNI     | ATG-T, Flu          |
| 4       | f   | 15-3        | c-ALL           | CR1   | 2             | mother    | haplo     | Mel, Flu, TT, ATG               | brother   | TNI     | ATG-T, Flu          |
| 5       | f   | 21-0        | PNH             | 2      | MUD           | 9/10      | Mel       | Flu, TT, ATG, Camp              | mother    | TNI     | OKT3, Flu          |
| 6       | f   | 8-5         | CML             | CP2   | MUD           | 8/10      | Bu, Mel   | Cy, ATG                         | mother    | TNI     | OKT3, Flu          |
| 7       | f   | 18-8        | c-ALL           | CR2   | 2             | mother    | haplo     | Mel, Flu, TT, OKT3              | mother    | TNI     | ATG-T, OKT3, Flu   |
| 8       | f   | 3-0         | SAA             | 2      | father        | haplo     | Mel       | Flu, TT, OKT3                   | mother    | TNI     | ATG-T, OKT3, Flu   |
| 9       | m   | 5-7         | AML             | NR3   | 2             | father    | haplo     | Mel, AraC, Clo, ATG              | mother    | TNI     | ATG-T, OKT3, Flu   |
| 10      | f   | 9-1         | T-ALL           | CR2   | 2             | father    | haplo     | Mel, Flu, TT, OKT3              | father    | TNI     | ATG-T, OKT3, Flu   |
| 11      | f   | 15-9        | c-ALL           | CR1   | 2             | mother    | haplo     | Mel, Flu, TT, OKT3              | mother    | TNI     | ATG-T, OKT3, Flu   |
| 12      | m   | 3-1         | Kostmann        | 2      | father        | haplo     | Mel       | Flu, TT, OKT3                   | mother    | TNI     | ATG-T, OKT3, Flu   |
| 13      | m   | 6-3         | JMML            | NR    | 2             | father    | haplo     | Bu, Mel, Cy, OKT3               | mother    | TNI     | ATG-T, OKT3, Flu   |
| 14      | m   | 14-3        | MDS, RC         | 2      | MUD           | 9/10      | Flu       | TT, ATG                         | mother    | TNI     | ATG-T, OKT3, Flu   |
| 15      | m   | 1-4         | pre-B ALL       | CR1   | 2             | father    | haplo     | Mel, Flu, TT, OKT3              | mother    | TNI     | ATG-T, OKT3, Flu   |
| 16      | m   | 11-8        | T-ALL           | NR1   | 2             | father    | haplo     | Mel, Clo, TT, OKT3              | mother    | TNI     | ATG-T, OKT3, Cy   |
| 17      | m   | 4-6         | c-ALL           | CR2   | 1             | mother    | haplo     | Mel, Flu, TT, ATG               | mother    | TNI     | ATG-T, Flu         |
| 18      | m   | 4-9         | JMML            | NR    | 1             | mother    | haplo     | Mel, Flu, TT, ATG               | mother    | TNI     | ATG-T, Flu         |
| 19      | m   | 7-2         | c-ALL, 2nd SCT  | CR3   | 2             | mother    | haplo     | Mel, Flu, TT, ATG               | mother    | TNI     | ATG-T, Flu         |

m, male; f, female; MHC-II defic, major histocompatibility complex II deficiency; PNH, paroxysmal nocturnal haemoglobinuria; MDS, RAEB-T, myelodysplastic syndrome with excess blasts in transformation; MDS, RC, refractory cytopenia; ALL, acute lymphoblastic leukaemia; c-ALL, common acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; SAA, severe aplastic anaemia; SCT, stem cell transplantation; CR1, first complete remission; CR2, second CR; CR3, third CR; NR, non remission; graft failure: 1, primary failure (non-engraftment); 2, secondary failure (rejection); 1st donor, donor used for initial SCT; 2nd donor, donor used for retransplantation; MUD, matched unrelated donor; HLA, human leucocyte antigen; haplo, haploidentical (fully haplotype mismatched donors); TBI, total body irradiation; Mel, melphalan; Bu, busulfan; ATG, antithymocyte globulin; Camp, Campath; Flu, fludarabine; VP-16, etoposide; TT, thiotepa; Cy, cyclophosphamide; AraC, cytarabine; Clo, cladribine; Clo, clofarabine; TNI, total nodal irradiation; ATG, antithymocyte globulin; ATG-F Fresenius; ATG-T, Thymoglobuline.

*Patient 2 received VP-16 instead of TT because of macrophage activating syndrome.

**Patient 17 received Cy 120mg/kg instead of 60 mg/kg.

†this patient had relapsed after 1st SCT and experienced graft failure after 2nd SCT.
Table II. Graft composition, outcome and toxicities.

| Pathogen | n | % |
|----------|---|---|
| CMV viraemia | 4 |
| Adv viraemia | 1 |
| + haemorrhagic enterocolitis | 1 |
| + eosopagitis | 1 |
| BKV haemorrhagic cystitis | 4 |
| Epstein–Barr viraemia | 2 |
| EBV PTLD | 1 |
| HHV6 viraemia | 1 |
| + hepatitis | 1 |
| + encephalitis | 1 |
| HSV esophagitis | 1 |
| RSV pneumonia | 1 |
| Rota gastroenteritis | 1 |

Table II. (Continued)

| Infections with proof of pathogen | n | % |
|----------------------------------|---|---|
| Viral |  | |
| CMV viraemia | 4 |
| Adv viraemia | 1 |
| + haemorrhagic enterocolitis | 1 |
| + eosopagitis | 1 |
| BKV haemorrhagic cystitis | 4 |
| Epstein–Barr viraemia | 2 |
| EBV PTLD | 1 |
| HHV6 viraemia | 1 |
| + hepatitis | 1 |
| + encephalitis | 1 |
| HSV esophagitis | 1 |
| RSV pneumonia | 1 |
| Rota gastroenteritis | 1 |

Cell numbers are given in cells/kg recipient’s body weight; acute and chronic graft-versus-host disease (GvHD) were graded according to the Glucksberg criteria (Glucksberg et al, 1974). 2 patients died due to treatment-related mortality [Adv infection and multi organ failure (MOF)/macrophage activating syndrome (MAS)]; Toxicity was defined according to modified Common Terminology Criteria for adverse events of the National Cancer Institute Version 3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcav3.pdf). All pulmonary toxicities [3 patients needed oxygen supply, one patient with MOF needed mechanical ventilation, one patient had steroid sensitive interstitial pneumonitis/bronchiolitis obliterans organizing pneumonia (BOOP)], gastrointestinal, neurological and haematological toxicities were transient and resolved completely; no renal toxicity > grade 2 occurred. All described infections were manageable and resolved except one case of Adv. ANC, absolute neutrophil count; TCR, T cell receptor; VOD, veno occlusive disease; BO, bronchiolitis obliterans; Adv, adenovirus; CMV, cytomegalovirus; BKV, BK virus; EBV, Epstein–Barr virus; PTLD, post-transplant lymphoproliferative disease; HHV6, human herpes virus 6; HSV, herpes simplex virus; RSV, respiratory syncytial virus.

patients received OKT3 only; a combination of both was given in 10 patients (Thymoglobulin® and OKT3, n = 10). Fludarabine (120 mg/m²) and thiopeta (5–10 mg/kg) were added as additional immunosuppressive agents in most patients. As OKT3 was no longer available, cyclophosphamide was introduced instead. Thus, the current reconditioning protocol consists of fludarabine 40 mg/m² (day –6 to –4), thiopeta 5 mg/kg (day –6), TNI 7 Gy (day –1), ATG (Thymoglobulin® 4.5 mg/kg, divided in 0.5–2–2 mg/kg, days –6 to –4) and cyclophosphamide (1 × 60 mg/kg, day –2) in combination with either
CD3/CD19-depleted or α/βTCR/CD19-depleted grafts (Table I). G-CSF was started routinely on day +4 until leucocyte recovery.

Recipients of grafts containing more than 2.5 × 10⁸ T cells/kg bodyweight additionally received mycophenolate mofetil (MMF) 1200 mg/m²/day. Immunosuppression was stopped if autologous T cells became detectable again after retransplantation. In case of persisting autologous signals, donor lymphocyte infusions (DLI) with 25 000 T cells/kg were administered. Supportive care was as described previously (Lang et al, 2014).

Statistical analysis
Overall survival (OS) was defined as the number of days after HSCT to death from any cause. Event-free survival (EFS) was defined as the time interval from the date of HSCT to the date of relapse/progression of the underlying disease or death from any cause. EFS and OS were calculated using the Kaplan-Meier approach with lognormal approximation. All tests were performed with GraphPad Prism Software (GraphPad Software Inc., La Jolla, CA, USA).

Results
Graft failure
Seventeen out of 19 patients experienced graft rejection (secondary graft failure) after a median time of 25 (range, 15–86) days. This process was typically associated with high fever, persistence of autologous CD3+ /CD8+ T cells in blood and bone marrow, elevation of C-reactive protein, ferritin and lactate dehydrogenase. Non-engraftment (primary graft failure) was diagnosed in two patients presenting with persisting pancytopenia and recipient chimerism in bone marrow aspirates. Median time from diagnosis of graft failure to retransplantation was 14 days (range 7–40).

Haematopoietic engraftment after retransplantation
Engraftment after retransplantation was achieved in 18 out of 18 evaluable patients with a median time of 10 days (range 9–32 days). One patient died at day +3. All evaluable patients became independent from platelet transfusions after a median of 10 days (range 6–45) (Table II.).

Due to persisting or increasing levels of autologous T cells after retransplantation immunosuppression was stopped in 8 patients after a median time of 22 days. Five patients additionally received DLI because of increasing autologous T cells despite cessation of immunosuppression. One child received another course of ATG. CD34+ selected stem cell boosts were utilized in 3 patients who exhibited decreasing neutrophil counts (<1 × 10⁹/l) within 34 – 247 (56, median) days post-transplant despite complete donor chimerism.

A median 1.8 × 10⁸/kg CD34+ progenitors was infused per boost. With these measures a sustained tri-lineage engraftment was achieved in all evaluable patients.

Incidence of graft-versus-host disease (GvHD)
Acute and chronic GvHD were graded according to the Glucksberg criteria (Glucksberg et al, 1974).

Nine out of 18 evaluable patients (50%) showed no symptoms of acute GvHD (aGvHD). Five patients (28%) experienced grade I aGvHD, 2 patients developed grade II aGvHD (11%) and 2 patients presented with grade III aGvHD (11%) (Table II.).

Chronic GvHD (cGvHD) was evaluated in 17 out of 19 patients surviving more than 100 days. 1 patient experienced limited cGvHD and 1 patient developed extensive cGvHD (Table II.).

Survival
As of October 2013, 13 of 19 patients are alive with a median follow-up of 6.5 years (range 2.1 to 10.4). Five of the 14 patients with leukaemia relapsed a median of 58 days after retransplantation (range 27–444 days); 4 of these patients subsequently died. One patient with JMML was salvaged with an additional cord blood transplantation (Table II.). Day 100 and overall TRM was 11% (Fig 1A). OS and EFS estimates of the total cohort were 68% and 63% at 3 years (Fig 1B). OS and EFS rates for patients with leukaemia (n = 14) were 64% and 57% (Fig 1C). The EFS for the 5 patients with non-malignant disorders was 80% at 3 years (Fig 1D).

Treatment-related toxicity
Toxicity was defined according to modified Common Terminology Criteria for adverse events of the National Cancer Institute Version 3-0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf; Table II., Table SI.). Five patients experienced temporary pulmonary toxicities and transient grade III-IV elevation of liver enzymes were observed in 8 patients. No de-novo veno-occlusive disease was detected. Cardiac toxicity > grade II was observed in only one patient with multi-organ failure. 3 patients presented with neurotoxicity grade III-IV [leucoencephalopathy, human herpes virus 6 (HHV6) encephalitis, seizure].

Several infections occurred but were not life-threatening in all but one patient. Four patients had cytomegalovirus DNAemia without organ involvement. 4 patients had transient BK virus associated grade IV haemorrhagic cystitis. Adenovirus DNAemia was detected in 3 patients with haemorrhagic enterocolitis or esophagitis. Epstein–Barr viraemia was detected in 3 patients with one case of post-transplant lymphoproliferative disease, which was successfully treated by rituximab. HHV6 viraemia was observed in 3
patients (symptomless viraemia or hepatitis, or HHV6 encephalitis, successfully treated by foscavir or ganciclovir).

Immune reconstitution

Fig 1E–F shows the immune reconstitution. At day 90, the mean CD3⁺ cell count was 0.435 × 10⁹/l (range, 0–2.203). T cell counts normalized within one year post-transplant. In contrast, the recovery of CD56⁺ NK cells was rapid. The mean number of B cell count at day 90 was 0.066 × 10⁹/l.

Discussion

The optimal reconditioning regimen after graft failure still needs to be defined because standardized protocols are
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lacking. In our patient cohort, we used a non-myeloablative but immunosuppressive conditioning regimen followed by infusion of T- and B-cell depleted haploidentical stem cells. Although the influence of exchange of donors remains unclear (Guardiola et al., 2000) we replaced the initial donor by a second donor other than the first in 16/19 patients, because residual host T cells sensitized against HLA antigens or minor antigens of the initial donor might increase the risk of a second graft failure. However, our regimen allowed successful retransplantation of 3 patients in whom a second donor was not available, also with another infusion of stem cells from the initial donor. Compared to matched unrelated donors, haploidentical donors can be identified within a shorter time, thus minimizing the period of severe pancytopenia and reducing the risk of fatal infections. In our patients, second stem cell grafts were infused within a median of 14 days from diagnosis of graft failure.

Our backbone of TNI, fludarabine/cyclophosphamide and serotherapy was well tolerated and sufficiently immunosuppressive to allow a sustained engraftment in all evaluable patients. The fast haematopoietic recovery, probably facilitated by large stem cell doses, substantially reduced the period of severe pancytopenia. Moreover, the TRM rate of 11% at one year after retransplantation compares favourably with recently published trials using chemotherapy-only based protocols, which reported TRM rates of 30–45% (Kedmi et al., 2009), (Remberger et al., 2011). Several attempts have been made to design optimized chemotherapy-only based reconditioning regimens in order to avoid late-developing tumours at sites that are most sensitive to radiation exposure, such as the brain, thyroid, salivary gland, bone and connective tissue (Keil et al., 1996), (Socie et al., 2000), (Pommier et al., 2009). To reduce radiation portals, a single dose of 7 Gy TNI was administered in our approach to cervical, supraclavicular, infraclavicular, axillar and mediastinal lymph nodes, thymus, spleen, paraaortic, iliacal and inguinal lymph nodes while shielding non-lymphoid tissues in the head, chest, abdomen and pelvis. No secondary malignancy has been detected after a median follow-up of 6-5 years in our group of patients.

The incidence of GvHD was low due to intensive depletion of T cells. In a similar trial by Park et al. (2014), a higher incidence of acute GvHD was documented despite more intensive post-transplant immunosuppression because these patients received a larger number of contaminating T cells (median 1-1x10⁶/kg). Thus, we hypothesize that the presence of donor T cells in the grafts is not absolutely mandatory to achieve a reliable haematopoietic engraftment if the conditioning regimen exerts sufficient immunoablation and that a rigorous depletion strategy leading to very low residual T cells may be of advantage in order to avoid GvHD. On the other hand, a weakness of our approach is that T cell recovery post-transplant was delayed, leading to some viral reactivations. However, such reactivations were manageable by consequent antiviral prophylaxis, virus screening and aggressive preemptive antiviral treatment. Another important component of our approach is the strict surveillance of the chimerism status. In case of increasing portions of autologous T cells two measures were taken: 1), immunosuppressive drugs were stopped, and 2), if the first measure was not sufficient, low doses of DLI were administered.

Interestingly, a low relapse rate of 36% was observed despite the high risk profile of our patients. Given that antitumour effects in a mouse allograft model were recently attributed to rejection of haematopoietic grafts, one could speculate that the rejection process itself may have contributed to the favourable relapse rate, probably via release of cytokines and cell-mediated cytolytic activity (Rubio et al., 2003).

In conclusion, TNI-based reconditioning regimens followed by infusion of haploidentical stem cells represent a promising option to rescue patients with graft failure after allogeneic HSCT.

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Author contributions

H.M.T., F.H. and P.L. provided patients, collected and analysed data, performed statistical analysis and wrote the paper. T.F., I.M., B.K., B.G. and R.H. provided patients, collected data and contributed to writing the manuscript; M.S. and P.S. provided immune recovery data; M.E., H.O. and D.Z. provided patients and critically reviewed the manuscript; M.D. for reviewing the manuscript and R.H. We thank Barbara Lang, M.D. and David Martin, M.D. for reviewing the manuscript and the Stiftung fuer krebskranke Kinder Tuebingen e.V. for continuous support.

Conflict of interest

None of the authors has a conflict of interest to declare.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Toxicity grading scales
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